Horticulture Australia Limited

Final report

Integrated systems for managing nematodes on bananas

Project FR99011 (January 2000 to December 2002)

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HAL FR99011: Integrated systems for managing nematodes on bananas

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Objective of project:
To integrate current and new nematode practices for managing burrowing nematodes into a strategy for Australian banana growers. This includes investigation of the refined use of nematicides applied to the soil and the potential for pseudostem injection for systemic nematicides, the use of biological controls to suppress burrowing nematode, investigation of the resistance in banana cultivars to endoparasitic nematodes, investigation of methods alternative to the current method of treating vegetative banana planting material and extension of results and outcomes to the banana industry.

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Media summary

Burrowing nematode is a major constraint to banana production world wide. In Australia, routine use of nematicides has been the most common control strategy. However, these chemicals are expensive, toxic and declining in efficacy due to enhanced biodegradation. This project aimed at developing nematicide application strategies to prolong the usefulness of currently registered nematicides, determining the economics of nematicide application in the subtropics, the efficacy of biological control, the resistance of banana cultivars to nematodes and alternative methods of treating vegetative banana planting material.

A nematicide rotation strategy where nematicides were changed after each application was able to significantly improve the control of nematodes compared to continual application of the same product and an untreated control. The rotation of nematicides was able to reduce nematode damage below the economic threshold after four applications, which meant that treatments could be omitted. The rotation strategy was able to slow the development of enhanced biodegradation of soil applied nematicides and allowed nematicides that were being degraded to recover. Severe degradation of one organophosphate nematicide was found to increase the likelihood that other organophosphate nematicides would also develop enhanced biodegradation.

Nematicides were not cost effective in sub-tropical banana production. Improved crop management such as irrigation, regular fertiliser application, weed control and deleafing were able to increase the bunch weights of bananas, whereas, the application of nematicides was not.

The injection of the systemic nematicides Vydate 240 L® and Nemacur 400® into the pseudostem of the following sucker was found to be as efficacious as soil application of nematicides. However, there was an increased risk of phytotoxicity damage, such as stem splitting, particularly when using Nemacur 400®.

Compost was able to suppress the number of nematodes in the roots of banana plants and maintain high levels of plant growth compared to sterile potting mix. This appeared to be due to an increase in the suppressive microorganisms around the roots of the plants. Endophytic fungi and bacteria isolated from nematode suppressive soils were able to suppress the number of nematodes on the roots of banana plants when added into the potting mix of tissue cultured banana plants.

No alternative method of disinfesting banana bits from burrowing nematode was as successful as the currently registered practice using Nemacur 400®. Cold temperatures, bleach and Vydate 240 L® were all unsuccessful at reducing nematode numbers in banana bits. However, Nemacur 400® dipping did not eliminate all nematodes from the planting material.

A survey of current nematode management practices used by banana growers revealed a decrease in the use of nematicide. 61 % of growers in 1994 used a nematicide whereas only 39 % of growers in 2003 had used a nematicide in the past 12 months. The survey also revealed an increase in banana growers awareness of the nematode status of their crops and how to manage plant-parasitic nematodes in bananas.

A nematode management pyramid has been developed to combine all the information derived from this and previous nematode management projects for the banana industry.

Technical summary

Burrowing nematode (*Radopholus similis*) is a major constraint to banana production worldwide. In Australia, routine use of nematicides has been the most widely used control strategy. However, these chemicals are expensive, toxic and declining in efficacy due to enhanced biodegradation. This project aimed at developing an integrated approach to nematode management in bananas by combining nematicide application strategies, biological control, resistance of banana cultivars to nematodes and alternative methods of treating vegetative banana planting material. Findings from previous projects were combined to develop a nematode management pyramid (Figure 1).

Refined nematicide application regimes

- The rotation of nematicides after each application was able to significantly reduce the number of *R. similis* and the disease index on the roots of bananas compared to an untreated control and the continual use of each nematicide.
- The rotation of nematicides was able to slow the development of enhanced biodegradation and allowed the recovery of Rugby 100 G®, which was found to be have enhanced biodegradation prior to commencing the trial.
- In soil with enhanced biodegradation of Rugby 100 G®, cross degradation of organophosphate nematicides developed, even though they had not been applied to the soil for more than six years.

Nematicide injection

- The injection of Vydate 240 L® into the following banana sucker was found to be as efficacious as soil application of Vydate 240 L® and Rugby 100 G®.
- The injection of Vydate 240 L® into the following banana sucker was significantly better than injection into the harvested pseudostem.
- The injection of Nemacur 400® into the following sucker was able to reduce nematodes in the roots of bananas to the same level as soil application of this chemical.
- The injection of Nemacur 400® significantly increased the symptoms of stem splitting compared to Nemacur 240 CS® and the untreated control.

Biological control

- Growing tissue culture banana plants in compost either with soil amendments, chitin (1 % v/v), mill ash (33 %) or molasses (1 %) or without amendment was able to suppress the number of nematodes compared with potting mix.
- 70 bacterial isolates were tested for growth promotion and burrowing nematode suppression, with three isolates demonstrating consistent nematode suppression.
- The non-pathogenic isolate of *Fusarium oxysporum*, A3, was able to significantly suppress burrowing nematodes, 6 weeks after addition to potting mix.

Resistance of banana cultivars

- The banana cultivar Yangambi Km5 demonstrated the most consistent resistance to burrowing nematode in pot trials.
- Cultivars derived from Pisang jari buaya were not resistant to the Tully isolate of *R. similis*.

Alterative methods of treating banana planting material

- No alternative methods of treating banana planting material such as cold temperatures (<4°C), bleach (2 % NaOCl) or Vydate 240 L® were as successful as dipping in an equivalent 1 $mL.L^{-1}$ solution of Nemacur 400® for 10 minutes.
- Nemacur 400® was unable to eliminate all nematodes from the planting material.

Technology transfer

- There was a decrease in the number of banana growers using nematicide from 61 % in 1994 to only 39 % in 2003.
- There was an increase in banana growers awareness about the nematode status of their crop.
- The DPI was seen as the most important source of information about management of nematodes amongst banana growers.

A nematode management pyramid has been developed to combine all the information derived from this and previous nematode management projects for the banana industry.

1. Refined nematicide application regimes

Introduction

Burrowing nematode (*Radopholus similis*) is the most serious soil-borne disease of bananas in north Queensland. The nematode is an endoparasite of the roots where it feeds and reproduces. Burrowing nematode reduces plant vigour, bunch weight, increases the time between harvests and causes plants to topple. Burrowing nematode is commonly managed in bananas by routine use of non-volatile chemical nematicides applied to the soil surface.

There are currently four nematicides registered in Australia for control of burrowing nematode. Three of the nematicides belong to the organophosphate group of agricultural chemicals and one to the carbamate group. The nematicides are nematostatic in action; they do not kill nematodes immediately but cause starvation due to inability to feed. Bunt (1987) described the nematostatic effects of nematicides in general on nematodes with decreasing concentrations. Nematicides at 5-10 µg/mL inhibit egg hatch, at 2-5 µg/mL egg hatching is restored but movement of the nematode remains inhibited, at 1-2 µg/mL movement is restored but root penetration remains inhibited, and at 0-1 µg/mL ability to penetrate roots is restored and nematode activity may be stimulated. (Ibrahim and Haydock, 1999) found that a cadusafos (Rugby®) concentration of 0.05 µg/mL permanently inhibited egg hatch of the potato cyst nematode and that at a concentration of 0.0005-0.002 µg/mL nematode activity was reduced by 50 %. In field situations (Johnson *et al.*, 1981) found that fenamiphos (Nemacur®) at a concentration of 2.0-3.8 µg/g soil for nine days controlled root-knot nematodes in corn and pea crops. Similarly, Mojtahedi, *et al.* (1991) found that 7.2 µg/g soil of ethoprofos (Mocap®) protected tomato roots from *M. chitwoodi* for 5 weeks. Several studies have shown reduced nematode damage and increased yields of bananas, up to 50 %, with the use of non-volatile nematicides (Araya and Cheves, 1997; Broadley, 1979a; Queneherve, *et al.* 1991; Schipke and Ramsey, 1994).

Effective nematode control generally requires nematicide activity for 6-8 weeks in the soil. Most nematicides persist for a short time, with half lives in the soil between of 7 days for Vydate® and 45 days for Rugby (Tomlin, 1997). This short persistence in the soil makes their efficiency for nematode control in perennial crops poor, often requiring multiple applications each year (Stirling and Dullahide, 1987). The soil environment may impact on nematicide efficacy due to the redistribution of the nematicide in the soil (Stirling and Dulahide, 1987), leaching of nematicides (Leistra, *et al.* 1980; Rahi, *et a,.* 1992; Smelt, *et al.* 1977) and immobilisation due to organic matter (Bromilow, 1973). With continual use, several of these nematicides are becoming less effective because of enhanced biodegradation (Anderson, 1988).

One method of maintaining the efficacy of nematicides and reducing enhanced biodegradation is to rotate the products used (Anderson, 1988). The microorganisms responsible for biodegradation are specific to that product (Anderson, 1988). Also, the differences in water solubility of the nematicides (Tomlin, 1997) may mean that nematicides more soluble in water are better suited to drier periods of the year when they are less prone to leaching away from the root zone. Similarly, the less water

soluble nematicide formulations would be more suited to wet periods when they can be washed into the root zone.

It is the aim of the trials described to compare the efficacy of soil applied nematicides in a subtropical environment as described on the manufacturers label. Also, to compare the efficacy of nematicides when the products are used consecutively or in rotation and the subsequent effects on biodegradation of the chemical.

Materials and methods

Experiment 1 –Sub-tropical efficacy trial

Trial site

In-vitro-propagated (tissue-cultured) banana plants (*Musa* AAA Cavendish group cv. Williams) were deflasked into steam sterilised standard UC mix in 600 mL pots and acclimatised under plastic for 2 weeks. Twelve weeks after deflasking plants were repotted into 1.5 L pots. Fourteen weeks after deflasking the plants were inoculated with 300 burrowing nematodes/pot and maintained in the glasshouse for four months until planting in the field. The burrowing nematode inoculum was maintained at 26°C on monaxemic carrot cultures (Moody, *et al.* 1973).

The field trial was planted on December, 9th 1999 at Redlands Research Station (20 klm southeast of Brisbane CBD) on a red soil. The site had never previously grown bananas and had no history of nematicide application. There were six treatments that included four nematicides, Nemacur®, Vydate®, Counter®, Rugby® and two control treatments. One control treatment contained nematodes with no chemicals and the other control was no nematodes with no chemicals. Each block contained 12 plants in 4 rows and there were 4 replications of each treatment.

Nematicide application

Nematicide was applied according tho the manufacturers recommendations for sub-tropical banana production (Table 1). Nemacur® was applied twice a year in April and December. Vydate® was applied four times a year in February, April, September and December and Counter® and Rugby® were each applied three times a year in April, September and December. These applications were carried out for three years, beginning in April 2000.

Table 1. Characteristics and rates of nematicides applied to banana plants

	Nemacur 400®	Vydate 240 L®	Counter B®	Rugby 100 G®
Active ingredient	fenamiphos	oxamyl	terbufos	Cadusafos
Amount of active ingredient ($g.L^{-1}$)	400	240	150	100
Formulation	Liguid	Liguid	Granule	Granule
Application rate per plant	6 mL in 20 mL	12.5 mL	20 g	20 g
Solubility in water (Tomlin, 1997)	0.4 $g.L^{-1}$	280 $g.L^{-1}$	0.004 $g.L^{-1}$	0.248 $g.L^{-1}$

Trial management

The trial was split into two management regimes. Two replicate blocks received poor management conditions, where plants received irregular fertiliser, irregular weed control, irregular deleafing and irregular desuckering and no irrigation. A further two replicates received good management conditions with regular fertiliser applications, irrigation, deleafing, desuckering and weed control. Both management regimes received the same total fertiliser applied over the duration of the trial.

Nematode assessments

All treatments were sampled four times each year in February, April, September, and December for 3 years, commencing in April 2000 and finalised in September 2002. At each sampling a soil block 25 x 25 x 25 cm was cut with a spade next to the bunching pseudostem and lifted. All functional roots were removed from the soil block, washed and split lengthwise to determine cortical damage caused by burrowing nematode.

Cortical damage on the banana roots was rated according to the method used by (Broadley, 1979b) which quantifies damage by estimating the percentage of the root cortex occupied by lesions. Root ratings are: 0, no lesions;1, 1-25% of root cortex occupied by lesions; 3, 26-50%; 5, 51-75%; and 7, 76-100%. The ratings are then used to calculate the disease index using the equation:

$$\text{Disease index} = \frac{\sum \text{ratings} * 100}{\text{total number of roots} * 7}$$

Nematode populations were assessed by extracting from roots for 7 days in a misting chamber (Hooper, 1986).

Biodegradation assessment

Soil was sampled from the four chemical treatments and used to determine the extent of enhanced biodegradation of the nematicide, using the method described by (Pattison, *et al.* 2000). Vydate® and Nemacur® treated plots were sampled in February and the Counter® and Rugby® treatments were sampled in April each year. One kilogram of soil was sampled from around the pseudostems of banana plants. A 200 g sub-sample of soil from each plot was either treated with nematicide only, autoclaved then nematicide added or left untreated (control).

Nematicide was added to achieve a soil concentration of 10 μg of active ingredient per gram of soil. A 30 g sub-sample of the soil was added to a vial 7 cm high and 2 cm in diameter for each 200 g soil treatment. A pre-germinated sweet corn seed (cv. Honeysweet) was planted in the soil and inoculated with 500 burrowing nematodes per vial and grown for 7 days. Nematodes were extracted from the sweet corn roots for 7 days in a misting chamber and then counted. The soil samples were bioassayed every 2 weeks for 6 weeks (i.e. 0, 2, 4 and 6 weeks after the addition of nematicide). The number of nematodes recovered from the roots of the bioassay plants for the control and the nematicide-treated, sterilised and unsterilised soils were compared to determine the extent of microbial degradation of nematicides. The area under the curve (AUC) of each nematicide treatment was calculated for the six week period.

Bunch assessment

Emergence dates for new bunches was estimated monthly. Bunches were tagged and the number of fingers estimated using the method described by (Turner, Mulder et al. 1988).

Fingers/bunch = No. hands(Fingers on hand3 + Fingers on hand n-1)/2*

Bunches of the plant and ratoon crops were harvested at first colour and weighed and the date recorded.

Experiment 2 – Tropical nematicide rotation efficacy trial

Trial site

A trial was established on a commercial banana crop that had been in place for four years. During this time the banana plants had received four applications of Rugby®. Nemacur® had been applied to the previous banana crop, but not for six years. The trial site was located 20 km south-west of Tully, Queensland on a yellow-brown, silty loam. The soil temperature, rainfall and soil moisture were measured weekly at the trial site. Soil temperature was measured to a depth of 10 cm using a hand held thermometer. Soil moisture was determined using tensiometers at two depths, 20 and 40 cm below the soil surface (Soilspec, H&TS electronics, Healesville, Australia).

Seven treatments were imposed on the trial site consisting of four nematicides applied either consecutively or in rotation and compared to an untreated control (Table 2). Each treatment was replicated six times and each plot consisted of 30 plants. The trial was conducted over two years beginning on May 30, 2000.

Table 2. Nematicide application schedule to determine efficacy of nematicide rotations relative to continual use and an untreated control.

Treatment	Nematicide application date							
	30/5/00	30/8/00	13/12/00	14/3/01	20/6/01	5/9/01	5/12/01	6/3/02
Counter®	Counter	Counter	Counter	Counter	Counter	Counter	Counter	Counter
Nemacur®	Nemacur	Nemacur	Nemacur	Nemacur	Nemacur	Nemacur	Nemacur	Nemacur
Rugby®	Rugby	Rugby	Rugby	Rugby	Rugby	Rugby	Rugby	Rugby
Vydate®	Vydate	Vydate	Vydate	Nil	Vydate	Vydate	Vydate	Vydate
Rotation 1	Nemacur	Vydate	Rugby	Counter	Nil	Vydate	Nemacur	Counter
Rotation 2	Counter	Rugby	Nemacur	Vydate	Nil	Counter	Rugby	Vydate
Untreated	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Nematicide application

Nematicides were applied every three months if the disease index was determined to be above the economic threshold (10) (Pattison, *et al.* 2002). The nematicides were applied as recommended by the manufacturer (Table 1). The nematicides were applied to the soil in a semi circle around the base of the following sucker and incorporated either by irrigation or rainfall.

Nematode assessment

Before each application of nematicide and three months after the eighth application of nematicides five bunched plants from each plot were assessed as described in Experiment 1. A soil block 25 x 25 x 25 cm was taken from in front of the following sucker in the nematicide application zone. Banana roots were assessed for nematode damage and nematodes extracted as described in Experiment 1.

Yield assessment

The time when bunches emerged from the plant was recorded weekly. Two weeks after bunch emergence the number of fingers on each bunch was assessed as described in Experiment 1. The bunches were harvested according to commercial practices and weighed.

Nematicide biodegradation

Nematicide biodegradation bioassays were conducted in May each year while the trial was underway using the method described by (Pattison, *et al.* 2000). The sampling of soil for the biodegradation bioassay occurred, prior to the first nematicide application, prior to the fifth nematicide application and three months after the final nematicide application to the trial site.

Soil was collected from each plot by bulking together six 25 mm diameter soil cores taken in the nematicide application zone to a depth of 10 cm. The soil corer was washed free of all soil and sterilised with 70 % methanol between sampling treatments. A 250 g sub-sample that had passed through a 5 mm sieve was taken from each plot sampled. The soil remained unsterilised or was sterilised by autoclaving at 121 °C for 15 minutes. The soil moisture in the autoclaved sample was readjusted to field moisture following autoclaving.

Nematicide, corresponding to the field treatment, was applied to the sub-samples achieve a level of 10 µg of active ingredient per g of soil. Plots that received nematicide rotation treatments were treated with all four nematicides. The untreated soil was left untreated to determine if sterilisation had any effect on nematode recovery.

From each 250 g sub-sample a 40 g soil sample was weighed into a vial, 75 mm high and 25 mm in diameter. After placing the soil into the tube three mung bean (*Vigna mungo*) seeds were placed on the soil surface and covered with 5 g of sterile sand. The seeds were then watered with 5 mL of distilled water.

Five days after planting, the seeds were inoculated with 500 motile *R. similis* taken from monaxenic carrot cultures (Moody, *et al.* 1973). Four days later the plants were harvested and the nematodes extracted from the roots by placing in a misting chamber for five days (Hooper, 1986). Nematodes were then quantified using a compound microscope.

Each 250 g soil sample was sampled on four occasions, immediately after nematicide application and 2, 4 and 6 weeks after nematicide application to the sub-samples. The sub-samples were stored in 1 L plastic food containers with air holes. The plastic food containers were stored inside a Styrofoam box with 2 cm of water in the base

and stored at room temperature. Soil samples waiting to be processed were stored in plastic bags at 4°C.

Nematicide cross degradation

At the completion of the trial, in May 2002, a 15 kg soil sample was collected from each plot in the nematicide application zone. The soil was collected to a depth of 10 cm from six plants using a shovel. The shovel was washed and sterilised using 70 % methanol between sampling the different treatments.

From each sample five, 2 kg sub-samples were placed in 150 mm diameter pots. Into each pot a tissue cultured banana plant was transferred and then inoculated with 1000 *R. similis,* one week after planting. One week after inoculation the plants were treated with the four nematicides, (Table 1) so that 10 µg active ingredient per g of soil was applied to the soil surface of the pot. One pot from each plot sample was left untreated.

The banana plants were grown in the glasshouse, fertilised with 5 g of osmocote mini (Scotts International, Herleen, The Netherlands) and allowed to grow for six weeks before being harvested. At harvest the fresh root and shoot weight were recorded. The shoots were then dried and the weight of the dry shoots determined. The roots were cut into 20 mm lengths and placed in a misting chamber for seven days. Nematodes extracted from the roots of the plants were collected on a 25 µm sieve and counted under a compound microscope. Soil waiting to be tested was stored in sealed plastic buckets at 4 °C.

Statistics

Data were analysed using an analysis of variance and the means separated using the least significant differences method. Nematode population data were transformed using ln(x+1) prior to statistical analysis to normalise data and presented as back transformed means. The area under the curve (AUC) was calculated from the disease index and nematode populations measured over the length of the trials. The area under the curve allows the treatments to be compared over the length of the trial. Similarly, the area under the curve calculations was used to determine the differences between the treatments for enhanced biodegradation data. All statistical calculations were conducted using Genstat 4.2.

Results and discussion

Experiment 1 – Sub-tropical efficacy trial

Management of the banana crop had a larger impact on the productivity of the banana crop than nematicide treatments. Regular fertiliser, water, deleafing and weed management were able to increase the weight of bunches in the plant and first ratoon crops by 24 and 23 % respectively (Table 4). The good management of the crop also produced significantly more fingers on each bunch and was able to reduce time for the emergence and harvest of the first ratoon crop (Table 4). This trend would be expected to be maintained as the trial continued. Given an average price of bananas at the market of $13 per 13 kg box, good management would be expected to increase returns to growers by $98,000 / Ha over the two bunch cycles (32 months). However, this may be an under estimate of the improved returns since it does not account for the reduced cycling time of plants and the improved fruit quality.

The soil application of all nematicides, except Nemacur®, were able to significantly increase the weight bunches relative to the nematode infected untreated control in the plant crop (Table 5). This increase was not carried through to the ratoon crop. The time taken for the plant to produce a bunch was significantly less where plants had not been inoculated with burrowing nematodes. Bunch weights are less affected by nematicide management than other crop management techniques, such as fertiliser and irrigation. Nematicide application was not economically viable as the nematicides were unable to continue increased bunch weight relative to the untreated plants, and returns would not exceed the cost of the chemical application (Table 3). In sub-tropical banana production nematicide application according to the manufacturer's recommendations would not be a cost effective practice.

Table 3. Number and cost of nematicide applications following manufacturers recommendations.

Treatment	Number of applications	Cost of Chemical
No treatment	0	$0.00
Nemacur	5	$2,076.90
Vydate	10	$4,988.29
Rugby	8	$1,983.48
Counter	8	$2,631.89

Preventing burrowing nematode from becoming established in the early stages of the plantation establishment significantly suppressed the number of nematodes in the roots of the banana plants (Table 6). However, as the trial progressed nematode infection was transferred from neighbouring plots via soil and water movement. At the last of the three sampling periods, the treatment that was not inoculated with nematodes was not significantly different from untreated nematode controls. In the analysis of the area under the curve the use of nematicides, except Nemacur® was able suppress nematode populations to a similar level as the uninoculated nematode treatment.

The use of Vydate® at four applications per year was able to significantly reduce the number of burrowing nematodes extracted from the roots and the amount of damage

on the roots of banana plants (Table 6). The use of four applications per year of Vydate® over the length of the trial also displayed no signs of enhanced biodegradation. Efficacy was maintained in both the sterilised and unsterilised soil relative to the untreated control (Table 7). The maintenance of the efficacy of the Vydate® in the soil contributed to reduced burrowing nematode populations. However, the cost of four nematicide applications, recommended by the manufacturer, was $4,988.29, which was 1.9 to 2.5 times more expensive than other nematicide treatments (Table 3). This cost would not be recouped by increased bunch weights.

Counter® and Rugby® were both able to reduce nematode populations and root damage when applied three times a year, as recommended by the manufacturer. The reduction in nematode populations was equal to using Vydate® and was significantly better than the untreated controls with nematodes (Table 6). Both Rugby® and Counter® were able to suppress nematodes in the unsterile soil relative to the untreated control in 2000 and 2002. The results from the 2001 biodegradation assay should be discounted as they suggest a problem with the procedure; as nematodes recovered in the sterile nematicide treated soil were higher or equal to the control with no nematicide added (Table 7). The cost of nematicide again would not be recouped by improved bunch weights where nematodes are a problem in sub-tropical banana production.

The application of Nemacur® was not able to significantly reduce the number of burrowing nematodes or the amount of damage measured by the disease index. The reduced efficacy was largely due to the development of enhanced biodegradation. Before nematicides were applied, Nemacur® was able to suppress nematode recovery relative to the untreated soil and there was no significant difference in the recovery of nematodes from sterile and unsterile treated soil (Table 7). However, in 2001 and 2002, the recovery of nematodes from the sterile treated soil treated with Nemacur® was significantly less than the unsterile Nemacur® treated soil (Table 7). This suggested that biodegradation was reducing the efficacy. However, some nematode control would be expected, as nematode recovery from the untreated bioassay plants was significantly higher than from Nemacur® treated plants (Table 7). Again the cost of Nemacur® could not be justified as there was no reduction in nematode populations and no increase in production.

Table 4. Crop management effects on bunch parameters of sub-tropical bananas infected with burrowing nematode for two cropping cycles.

Management	Estimated return ($. Ha^{-1})*	Yield (tonnes/Ha)	Bunch weight (kg)	Finger number	Bunch emergence time (days)	Bunch harvest time (days)
Plant crop						
Good	$279,630	34.99 a	23.9 b	133 b	362 a	474 a
Bad	$224,640	33.17 a	19.2 a	119 a	352 a	475 a
Ratoon crop						
Good	$237,510	25.31 a	20.3 a	141 b	732 a	840 a
Bad	$194,220	19.17 a	16.6 b	119 a	796 b	927 b

*Assumes $13 per 13 kg carton, 10 % wastage per bunch and a plant density of 1000 plants per Ha.
Means in columns with the same subscript are not statistically different at the 5 % level.

Table 5. Nematicide application effects on bunch parameters of sub-tropical bananas infected with burrowing nematode compared to an untreated control and an uninfected untreated control for two cropping cycles.

Treatment	Yield (t/ha)	Bunch weight (kg)	Finger number	Bunch emergence time (days)	Bunch harvest time (days)
Plant crop					
No nematodes no treatment	39.4 a	22.3 b	125 a	314 a	438 a
Nematodes, no treatment	30.0 a	19.6 a	120 a	349 b	466 ab
Nematodes, no treatment	33.0 a	21.4 ab	124 a	362 bc	473 b
Nemacur®	29.3 a	19.7 a	127 a	370 bc	484 b
Vydate®	32.9 a	22.2 b	125 a	365 bc	478 b
Rugby®	35.6 a	22.4 b	135 a	372 c	489 b
Counter®	38.4 a	22.9 b	128 a	370 bc	493 b
Ratoon crop					
No nematodes no treatment	25.7 a	20.1	135 a	735 a	862 a
Nematodes, no treatment	17.6 a	18.1 a	122 a	770 a	862 a
Nematodes, no treatment	19.6 a	16.6 a	136 a	753 a	881 a
Nemacur®	20.7 a	18.0 a	138 a	744 a	894 a
Vydate®	25.9 a	17.9 a	117 a	784 a	899 a
Rugby®	17.2 a	18.4 a	124 a	782 a	877 a
Counter®	29.0 a	20.0 a	140 a	781 a	910 a

Means in columns with the same subscript are not statistically different at the 5 % level.

Table 6. Nematicide effects on disease index caused by burrowing nematode and number of nematodes extracted from the roots of bananas compared to an untreated control and an uninoculated-untreated control.

Treatment	Sampling date										
Disease index	**Apr 2000**	**Sep 2000**	**Dec 2000**	**Feb 2001**	**Apr 2001**	**Sep 2001**	**Dec 2001**	**Feb 2002**	**Apr 2002**	**Sep 2002**	***AUC***
Nematodes, no treatment	8.8 a	6.0 a	2.6 a	7.9 bc	7.6 a	8.4 a	7.2 a	5.5 a	6.9 a	15.4 c	5322 a
Nematodes, no treatment	3.3 a	13.1 a	4.7 a	11.0 c	9.6 a	22.6 a	22.6 a	10.5 a	7.6 a	13.7 bc	8640 a
Nemacur®	4.0 a	14.0 a	0.3 a	3.0 ab	4.8 a	12.7 a	12.7 a	5.8 a	12.9 a	5.0 ab	6707 a
Vydate®	0.4 a	0.2 a	0.1 a	0.1 a	0.2 a	1.1 a	1.1 a	0 a	1.7 a	0.7 a	248 a
Rugby®	4.2 a	15.9 a	0.6 a	2.6 ab	3.0 a	4.5 a	4.5 a	1.6 a	3.5 a	2.9 a	5322 a
Counter®	2.7 a	11.5 a	0.8 a	1.6 ab	2.4 a	1.5 a	1.5 a	0.2 a	7.1 a	0.8 a	3537 a
No nematodes no treatment	0.4 a	5.1 a	0.4 a	0.0 a	3.7 a	6.5 a	6.5 a	1.8 a	5.8 a	5.0 ab	2489 a
***R. similis* in 100 g of root**	**Apr 2000**	**Sep 2000**	**Dec 2000**	**Feb 2001**	**Apr 2001**	**Sep 2001**	**Dec 2001**	**Feb 2002**	**Apr 2002**	**Sep 2002**	***AUC***
Nematodes, no treatment	124 b	20 c	26 bc	595 cd	795 a	28 bc	105 b	477 a	178 a	34 a	3241 c
Nematodes, no treatment	336 b	15 c	236 c	236 d	1393 a	106 c	100 b	339 a	1603 a	32 a	3368 c
Nemacur®	153 b	10 bc	45 bc	80 bc	3040 a	94 c	26 ab	191 a	113 a	8 a	2905 c
Vydate®	16 a	0 a	1 a	6 ab	18 a	0 a	2 a	17 a	25 a	1 a	930 a
Rugby®	664 b	7 bc	52 bc	52 ab	150 a	4 ab	2 a	3 a	14 a	2 a	1579 ab
Counter®	316 b	5 abc	2 a	2 ab	472 a	0 a	1 a	98 a	33 a	5 a	1970 b
No nematodes no treatment	2 a	2 ab	7 ab	7 a	95 a	4 ab	10 ab	18 a	12 a	11 a	1268 ab

Numbers of nematodes are back transformed means of 12 plants. Means in columns with the same subscript are not statistically different at the 5 % level.

Table 7. Average recovery of nematodes from the roots of bioassay plants in sterile-nematicde treated soil, unsterile-nematicide treated soil and untreated soil for a 6-week period on three successive years.

Chemical	Treatment	Year		
		2000	2001	2002
Counter®	Unsterile	7 a	14 a	4 a
	Sterile	24 b	55 b	7 a
	Untreated	109 c	21 a	49 b
Nemacur®	Unsterile	0 a	45 b	13 b
	Sterile	1 a	3 a	5 a
	Untreated	71 b	117 c	43 c
Rugby®	Unsterile	2 a	4 a	0 a
	Sterile	6 b	10 b	4 b
	Untreated	107 c	12 b	14 c
Vydate®	Unsterile	0 a	1 a	1 a
	Sterile	1 a	0 a	1 a
	Untreated	107 b	38 b	45 b

Means in columns with the same subscript are not statistically different at the 5 % level.

Experiment 2 - tropical nematicide rotation efficacy trial

The rotation of the nematicides was an efficacious management strategy, significantly increasing the efficacy of the nematicides relative to continual application of the same product, except for Vydate®. The disease index and the number of nematodes extracted from the roots was significantly higher in the untreated control relative to the two rotation treatments and Vydate® (Table 8). The use of the chemical rotation and soil application of Vydate® was able to reduce the disease index below the economic threshold (10) (Pattison, *et al.* 2002), which enabled a treatment to be missed in June and May, 2001 respectively. At the last assessment in May 2002, the disease index of both the rotation and the continual Vydate® treatment were below the economic threshold (Pattison, *et al.* 2002). Nematicide rotation is able to sustain the efficacy of the chemicals and hence reduce the impact that burrowing nematode has on banana production. The rotation of nematicides was a more cost effective than continual use of Vydate®, Nemacur® and Counter® (Table 10).

The rotation of the nematicides was able to delay the onset of enhanced biodegradation of Counter® and Nemacur® and allowed the recovery of Rugby® in the first year of the trial, relative to continual application (Table 12). Nemacur®, Rugby® and Counter® applied to unsterile soil that had been treated consecutively at three month intervals, had significantly higher nematode recovery than when Nemacur®, Rugby® and Counter® were applied to unsterile soil from the rotation treatments (Table 12). Rotation of nematicides meant that when cross degradation was tested all chemicals, except Vydate®, were unable to significantly reduce nematode numbers in the two rotation treatments (Table 13).

Although there was no significant increase in bunch weights or reduction in toppling (Table 10), the rotation 1 treatment was able to significantly increase the number of

fingers per bunch, relative to the untreated control between August and November, 2001 (Table 9). The lack of bunch weight response from nematode reduction relative to the untreated control could be due to bunches being cut down due to an outbreak of black sigatoka (*Mycospherlla fijiensis*) in the vicinity of the trial area. Also the environmental conditions between November 2001 and May 2002, were drier with higher soil temperatures than the previous year (Table 11). This corresponded to a reduction in finger numbers per bunch (Table 9). The harsh environmental conditions may have masked the effect of burrowing nematode on bunch weights. There was a significant correlation ($P<0.05$) between the disease index in May 2001 (Table 8) and the number of fingers per bunch between August and November, 2001 (Table 9) ($y = -1.31x + 135$. $R^2=0.69$). This suggested that the improvement in finger number per bunch occurred three to six months after a significant reduction in root damage relative to the untreated control.

The environmental conditions did not significantly reduce the efficacy of the nematicide rotation treatments from one another. This suggested that using a chemical rotation to delay enhanced biodegreadation was more important than applying chemicals depending on expected weather conditions. However, caution is still recommended before applying nematicides in very wet weather.

Enhanced biodegradation of Rugby® was observed before the treatments were imposed on the trial site. This was due to four consecutive applications of Rugby® in the two years prior to the trial commencing in May 2000. However, the rotation of nematicides allowed an improvement in the efficacy of Rugby® to control burrowing nematodes after 1 year. This did not continue to the second year, although no further applications of Rugby® were applied to the rotation 1 treatment. Continuous Rugby® application failed to consistently reduce the disease index and the number of nematodes recovered from the roots of banana plants relative to the untreated control throughout the trial (Table 8). This was due to enhance biodegradation of Rugby® as sterilisation of the soil significantly improved the efficacy of Rugby® (Table 12). There was no difference in nematode recovery between the untreated soil and Rugby® applied to unsterile soil for the three sampling times. This result confirmed that Rugby® had undergone enhanced biodegradation (Table 12).

The soil that received continuous Rugby® rapidly degraded the other organophosphate nematicides, Nemacur® and Counter®. This is evident from the rapid development of enhanced degradation to Counter®, which had not previously been applied at the site (Table 12 and 13). Counter® and Nemacur® applied to bananas grown in pots of soil from the continual Rugby® plots did not reduce the recovery of burrowing nematode relative to the untreated control and were significantly less efficacious than Vydate® (Table 13). This result suggested that cross degradation of organophosphate nematicides might occur when there is severe enhanced biodegradation of an organophosphate chemical, such as has developed with Counter® and Nemacur® when applied to the soil that had enhanced biodegradation of Rugby®.

Vydate® in continual application or rotation with other nematicides did not undergo enhanced biodegradation (Table 12) and remained efficacious, reducing the disease index and nematode numbers (Table 8). Therefore, Vydate® is an important chemical part of any nematicide rotation program. Continuous Vydate® application was able to

reduce the disease index below the economic threshold after three applications (Table 8). This corresponded with an increase in the number of fingers per bunch between May and August 2001. However, when the application of the chemical stopped in March, 2001, the number of nematodes in the roots of the plants increased rapidly (Table 8).

Counter® was able to suppress the number of nematodes and the disease index throughout the trial (Table 8). The suppression of the nematode indices was intermediate, as it was significantly better then the untreated control but was not as efficacious as the nematicide rotation 1 treatment (Table 8). There was evidence that enhanced biodegradation of Counter® was developing with a significant reduction in the recovery of nematodes from sterile treated soil relative to the untreated soil treated with Counter®. Also, in the cross degradation pot trial, Counter® applied to continuous Counter® soil was unable to reduce nematode numbers relative to the untreated soil (Table 13).

Nemacur® applied continuously failed to reduce nematode indices on bananas (Table 8). Evidence of enhanced biodegradation was evident after the first year (Table 13). However, when Nemacur® had not been applied for at least 12 months, the efficacy could be preserved (Table 13).

There was no significant difference in the number of plants that toppled due to nematode damage of the roots. This is in contrast with previous trial work where nematicide application had significantly reduced the toppling of banana plants in the tropics (Stanton and Pattison, 2000). The nematicide rotation treatment 1 had 1.1% of the plants toppling compared to the untreated plots 7.5 % of the plants toppled due to nematode damage. The non-significant difference between the treatments in this trial may be due the plants being tied to one another after bagging. This is a standard commercial practice to reduce the number of plants toppling and the kinking of the pseudostem due to the weight of the banana bunch.

Table 8. Disease index and nematode numbers extracted from the roots of banana plants comparing nematicide rotation with continual use of nematicide products and an untreated control.

Treatment	Sampling date									
Disease Index	**May 00**	**Aug 00**	**Nov 00**	**Feb 01**	**May 01**	**Aug 01**	**Nov 01**	**Feb 02**	**May 02**	**AUC**
Untreated	14.4 a	16.2 a	27.7 c	26.0 e	23.9 e	36.6 d	26.3	23.3 b	26.1 d	597 d
Rotation 1	11.4 a	15.6 a	15.5 a	13.3 a	7.3	15.2 a	20.2	14.4 a	6.6 a	335 a
Rotation 2	14.9 a	20.8 a	16.4 a	13.5 b	8.7 b	19.7 ab	16.0	14.8 a	11.2 ab	376 ab
Counter®	18.1 a	21.5 a	18.6 ab	18.0 bc	13.6 bcd	26.6 bc	18.8	15.0 a	11.9 ab	447 bc
Nemacur®	13.7 a	18.7 a	17.3 ab	28.5 e	16.6 cd	32.2 cd	24.6	22.4 b	18.6 c	539 cd
Rugby®	17.4 a	19.7 a	23.7 bc	22.2 d	18.0 d	31.0 cd	20.2	14.4 a	15.0 bc	497 cd
Vydate®	19.1 a	17.4 a	16.7 a	7.3 abc	11.8 abc	20.6 ab	15.6	14.1 a	7.2 a	352 ab
***R. similis* in 100 g of root**										
Untreated	897 a	607 a	1635 d	2275 d	1668 b	1509 c	1152 cd	298 d	1042 d	166 d
Rotation 1	415 a	333 a	102 a	383 ab	80 a	625 ab	218 ab	8 a	122 b	119 a
Rotation 2	577 a	644 a	346 bc	436 ab	193 a	924 abc	632 cd	30 ab	63 ab	136 ab
Counter®	2185 a	619 a	160 ab	651 bc	705 b	1063 bc	544 bc	40 b	168 bc	143 bc
Nemacur®	897 a	836 a	712 cd	1668 cd	1235 b	1299 bc	1524 d	269 cd	1352 d	164 d
Rugby®	1754 a	658 a	1152 d	1152 bcd	972 a	1248 bc	1555 d	75 bc	512 cd	156 cd
Vydate®	1736 a	601 a	104 a	193 a	1140 b	427 a	87 a	29 ab	18 a	128 ab

Means in columns with the same subscript are not statistically different at the 5 % level.

Table 9. Finger numbers per bunch of banana plants comparing nematicide rotation with continual use of nematicide products and an untreated control.

Treatment	Measurement interval								
Average finger number per bunch	May –Aug 2000	Aug - Nov 2000	Nov 2000 - Feb 2001	Feb – May 2001	May - Aug 2001	Aug – Nov 2001	Nov 2001– Feb 2002	Feb – May 2002	Mean
Untreated	137 b	142 a	132 a	121 a	116 ab	107 ab	112 a	107 a	125 a
Rotation 1	138 b	147 a	128 a	136 a	127 bc	130 d	114 a	96 a	131 a
Rotation 2	134 b	154 a	136 a	124 a	103 a	117 bc	119 a	103 a	127 a
Counter®	164 b	152 a	133 a	133 a	123 abc	121 cd	109 a	103 a	133 a
Nemacur®	100 a	148 a	136 a	119 a	127 bc	113 abc	107 a	107 a	127 a
Rugby®	136 b	149 a	135 a	133 a	117 ab	104 a	107 a	104 a	126 a
Vydate®	139 b	148 a	131 a	124 a	139 c	122 cd	117 a	104 a	130 a

Means in columns with the same subscript are not statistically different at the 5 % level.

Table 10. Yield, plant toppling and chemical cost comparing nematicide rotation with continual use of nematicide products and an untreated control.

Treatment	Cumulative fruit yield (t/Ha)	Plant toppling (%)	Chemical treatment cost ($/Ha)
Untreated	66.6 a	7.5 a	$ 0.00
Rotation 1	67.2 a	1.1 a	$ 2,566.88
Rotation 2	74.0 a	6.9 a	$ 2,734.33
Counter®	73.6 a	2.2 a	$ 2,631.89
Nemacur®	65.7 a	7.4 a	$ 3,323.04
Rugby®	70.0 a	5.7 a	$ 1,983.48
Vydate®	71.2 a	5.1 a	$ 3,491.80

Means in columns with the same subscript are not statistically different at the 5 % level

Table 11. Soil temperature, soil moisture and rainfall over two years at the trial site comparing the efficacy of nematicide rotation with continual use of the nematicide products and an untreated control.

Nematicide application period	Temperature (°C)		Mean soil moisture (kPa)		Total rainfall (mm)
	Mean	Range	20 cm	40 cm	
1 (30/05/00-30/08/00)	19.3	16.6 - 20.3	24.7	16.1	254
2 (31/08/00-13/12/00)	23.3	19.2 - 27.2	20.4	29.9	517
3 (14/12/00-14/03/01)	25.5	23.7 - 27.4	15.2	11.8	1724
4 (15/03/01-20/06/01)	21.4	18.0 - 24.2	22.2	34.1	416
5 (21/06/01-05/09/01)	19.7	16.8 - 22.4	44.3	35.3	68
6 (06/09/01-05/12/01)	24.4	20.9 - 27.4	31.4	36.6	132
7 (06/12/01-06/03/02)	27.6	25.8 - 30.8	18.7	33.8	566
8 (07/03/02-15/05/02)	24.6	22.8 - 26.8	13.7	10	574

Table 12. Recovery of nematodes from bioassay plants to determine the biodegradation of nematicides used in rotation and continual use compared to an untreated control.

Treatment	Sterilisation	Pre-treatment	Year 1	Year 2
Nemacur®	Sterile	3.0 a	7.4 a	5.1 a
	Unsterile	15.1 b	25.1 c	22.1 b
Nemacur® rotation 1	Sterile	2.7 a	3.6 a	4.9 a
	Unsterile	13.5 b	18.2 b	22.2 b
Nemacur® rotation 2	Sterile	2.0 a	7.2 a	7.8 a
	Unsterile	16.2 bc	17.2 b	19.0 b
Untreated	Sterile	21.8 d	26.2 c	23.7 b
	Unsterile	18.8 cd	26.1 c	20.6 b
Treatment	**Sterilisation**	**Pre-treatment**	**Year 1**	**Year 2**
Counter®	Sterile	2.8 a	2.3 a	4.7 a
	Unsterile	8.5 b	18.8 c	20.1 bc
Counter® rotation 1	Sterile	2.0 a	2.7 a	2.7 a
	Unsterile	13.9 cd	17.9 c	22.1 c
Counter® rotation 2	Sterile	3.2 a	5.7 ab	2.7 a
	Unsterile	10.0 bc	7.8 b	17.1 b
Untreated	Sterile	21.8 e	26.2 d	23.7 c
	Unsterile	18.8 de	26.1 d	20.6 bc
Treatment	**Sterilisation**	**Pre-treatment**	**Year 1**	**Year 2**
Rugby®	Sterile	2.0 a	2.7 a	3.8 a
	Unsterile	17.3 c	23.6 d	20.3 b
Rugby® rotation 1	Sterile	2.9 a	5.3 ab	6.6 a
	Unsterile	19.3 bc	16.2 c	22.8 b
Rugby® rotation 2	Sterile	2.6 a	7.9 b	5.8 a
	Unsterile	18.6 bc	9.8 b	20.0 b
Untreated	Sterile	21.8 c	26.2 d	23.7 b
	Unsterile	18.8 bc	26.1 d	20.6 b
Treatment	**Sterilisation**	**Pre-treatment**	**Year 1**	**Year 2**
Vydate®	Sterile	0.9 a	0.5 a	3.9 a
	Unsterile	0.8 a	0.8 a	9.3 a
Vydate® rotation 1	Sterile	0.3 a	1.2 a	2.2 a
	Unsterile	1.1 a	1.7 a	3.4 a
Vydate® rotation 2	Sterile	0.2 a	1.2 a	1.9 a
	Unsterile	3.3 a	1.6 a	7.4 a
Untreated	Sterile	21.8 b	26.2 b	23.7 b
	Unsterile	18.8 b	26.1 b	20.6 b

Means in columns with the same subscript are not statistically different at the 5 % level.

Table 13. Recovery of nematodes from banana plants treated with nematicides grown in soil taken from plots where nematicides were used in rotation or continually compared to an untreated control.

Field treatment	Nematicide treatment	Nematodes recovered in 100 g of root
Counter®	Counter®	2087 klm
	Nemacur®	341 cdefgh
	Rugby®	612 efghijk
	Untreated	1535 ijklm
	Vydate®	12 a
Nemacur®	Counter®	428 cdefghij
	Nemacur®	934 fghijklm
	Rugby®	218 cdef
	Untreated	4100 m
	Vydate®	121 bcd
Rotation 1	Counter®	1278 ghijklm
	Nemacur®	584 efghijk
	Rugby®	759 efghijkl
	Untreated	1748 jklm
	Vydate®	44 ab
Rotation 2	Counter	520 defghijk
	Nemacur	226 cdef
	Rugby	606 efghijk
	Untreated	1359 hijklm
	Vydate	299 cdefg
Rugby®	Counter®	1428 hijklm
	Nemacur®	629 efghijk
	Rugby®	2898 lm
	Untreated	1803 jklm
	Vydate®	184 bcde
Untreated	Counter®	867 fghijkl
	Nemacur®	239 cdef
	Rugby®	346 cdefghi
	Untreated	2031 klm
	Vydate®	111 bc
Vydate®	Counter®	715 efghijkl
	Nemacur®	705 efghijkl
	Rugby®	365 cdefghi
	Untreated	1491 hijklm
	Vydate®	42 ab

Means in columns with the same subscript are not statistically different at the 5 % level.

Conclusion

Nematicides in the sub-tropics were able to significantly reduce the number of nematodes in the roots of banana when used according to the manufacturer's recommendations, but failed to significantly reduce the disease index or improve bunch weights. The uninoculated untreated plots had better productivity of bananas than plants that were treated with nematicides. The cost of nematicides was so high in the sub-tropics that it would not be recovered with increased productivity. Improved management such as timely irrigation, fertiliser application, weed control, deleafing and desuckering would be more beneficial for improving crop productivity than the application of nematicides.

The rotation of nematicides in the tropics was able to significantly reduce the number of nematodes and the disease index caused by nematodes in the roots of bananas. Continual use of Vydate® was equal to chemical rotations in reducing burrowing nematode numbers and root damage and there was no evidence of the development of enhanced biodegradation after eight applications over two years. The continual use of nematicides other than Vydate® developed enhanced biodegradation as determined by a bioassay procedure. The severe enhanced biodegradation of Rugby® appeared to allow the development of enhanced biodegradation of the other two organophosphate nematicides, Counter® and Nemacur®. Nematicide rotations were able to delay the development of enhanced biodegradation and allowed partial recovery of nematicides that were being degraded rapidly. However, there was no significant improvement in the productivity with the use nematicides, except finger number per bunch. This may have been due to a loss of bunches as a result of black sigatoka quarantine measures and unfavourable climatic conditions masking the effects of nematode damage.

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2. Nematicide injection

Introduction

Burrowing nematode (*Radopholus similis*) is a major constraint to the production of bananas world wide (Gowen and Queneherve, 1990). The nematode feeds on the cortical cells of the roots of plants causing necrosis in the root. Nematode affected banana plants have a reduced bunch weight and longer period between harvests; plants topple due to the root damage caused by burrowing nematodes (Gowen and Queneherve, 1990). Nematodes have been managed with the use of organophosphate and carbamate nematicides. However, continual use of nematicide on some soils the can lead to enhanced biodegradation of the chemical (Pattison, *et al.* 2000; Stanton and Pattison, 2000). Also, concerns about the soil environment and possible movement of nematicide off-target mean that the use of nematicides in the banana industry is being re-evaluated.

The use of systemic nematicides as a pseudostem injection offers many advantages over soil application of nematicides if efficacy can be demonstrated. The injection of nematicides into the plant removes the chemical from the soil environment, which reduces the chances of off-site contamination and loss of efficacy due to enhanced biodegradation. The injection of 1 mL of abamectin into tissue culture banana plants was able to significantly reduce the number of nematodes in the roots of banana plants relative to untreated plants (Jansson and Rabatin, 1997; Jansson and Rabatin, 1998). In the field the harvested pseudostem was the preferred site for nematicide injection, as the chance of phytotoxicity was less than if it was injected into the developing pseudostems. This method was able to increase the bunch weight of bananas but failed to reduce the number of nematodes in the roots of banana plants (Araya, 1999).

The developing pseudostem also offers a site for oxamyl injection if phytotoxicity damage can be avoided. The injection of 5 or 10 mL of oxamyl into the leaf axil of banana plants was able to reduce nematode numbers on the roots and increase the weight of bunches.

The aim of the trials in this report was to determine the efficacy of application of nematicides as pseudostem injection into the harvested pseudostem shortly after harvest and as a pseudostem injection into the developing sucker for the management of burrowing nematodes in bananas.

Materials and methods

A number of trials were conducted to determine the best method and efficacy of nematicide injection. However, only those trials, which produced a significant difference between the treatments is included in this report. Three field trials of a total of five field trials, failed to produce a significant difference. These field trials involved injection into the harvested pseudostem only.

Trial 1 - Glasshouse trial

Tissue cultured banana plantlets (*Musa* AAA, Cavendish subgroup, cv. Williams) were grown in 150 mm diameter pots in 1.5 kg of steam pasteurised soil and sand mixture and inoculated with 1,000 motile *Radopholus similis* one week after planting. One week after inoculation with nematodes the plants were treated with nematicides; Vydate 240 L®, Nemacur 400®, Rugby 100 G® on the soil or abamectin as an injection, equivalent to 10 $\mu g.g^{-1}$ of soil. Nematicide was applied either to the soil surface or injected into the pseudostem 25 mm above the soil surface, and compared to an untreated control. Each treatment was replicated five times. The height of the plants was recorded at the time of nematicide application and again at harvest. Banana plants were harvested six weeks after nematicides were applied. At harvest, the height, shoot dry weight and root fresh weight were determined. The root system was then cut into 20 mm lengths and the nematodes extracted in a misting chamber for seven days before being counted (Hooper 1986).

Trial 2 – Injection of Vydate 240 L® - Field trial

Trial site selection

A trial site was established in a 10 year banana crop (*Musa* AAA, Cavendish subgroup, cv. Williams), 10 km north-west of Tully, north Queensland. The banana crop was established on an alluvial soil, irrigated using drip irrigation and commercial fertiliser and leaf disease management.

Banana plants were selected at harvest and the bunch weights of the plants determined. 72 plants were selected and assigned to six treatments (Table 14). The trial was conducted in a completely randomised design with 12 replicate plants per treatment. Plants were selected at bunch harvest and assigned a treatment.

Treatment application methods

Vydate 240 L® was applied to banana plant as described in Table 14. The injection treatments were applied using an injection gun with a 20 mL graduated cylinder and a perforated needle at the end of a 1 m lance. The nematicide was applied 15 cm from the base of the harvested pseudostem at a downward angle (Figure 1). Treatments that received Vydate 240 L® into following sucker, had the nematicide injected into plants that were greater than 1.5 m high but were less than 2.5 m and did not have a bunch. Plants this size were selected to reduce the possibility of phytotoxicity, which may occur

in very small plants. Plants with bunches were avoided to reduce any potential residues of oxamyl in fruit. Soil application of Vydate 240 L® was applied to the soil in a band around the following sucker.

Rugby 100 G® applications were made according to the label instructions by spreading 20 g of granules around the base of the following sucker.

Table 14. Treatments applied to evaluate the injection of Vydate into the following sucker compared to injection into the harvested pseudostem, soil application and an untreated control for the control of nematodes in banana roots.

Treatment	Formulation	Rate	Method
1. Vydate 240 L® 2 x 12 mL follower pseudostem	240 g/L oxamyl	24 mL / plant	12 mL of Vydate injected into the following sucker at bunch harvest and a follow up treatment of 12 mL injected into the following sucker 3 months later.
2. Vydate 240 L® 12 mL harvested + 12 mL follower pseudostem	240 g/L oxamyl	24 mL / plant	12 mL of Vydate injected into the harvested pseudostem at bunch harvest and a follow up treatment of 12 mL injected into the following sucker 3 months later.
3. Vydate 240 L® 24 mL harvested pseudostem	240 g/L oxamyl	24 mL / plant	24 mL of Vydate injected into the harvested pseudostem at bunch harvest.
4. Vydate 240 L® 2 x 12 mL soil	240 g/L oxamyl	24 mL / plant	12 mL of Vydate applied to the soil at bunch harvest and a follow up treatment of 12 mL applied to the soil around the following sucker 3 months later.
5. Rugby 100 G® 2 x 20 g soil	100 g/L cadusafos	40 g / plant	20 g of Rugby applied to the soil at bunch harvest. This treatment was used as a standard commercial soil application. 20 g was applied 3 months later
6. Untreated control	nil	nil	nil

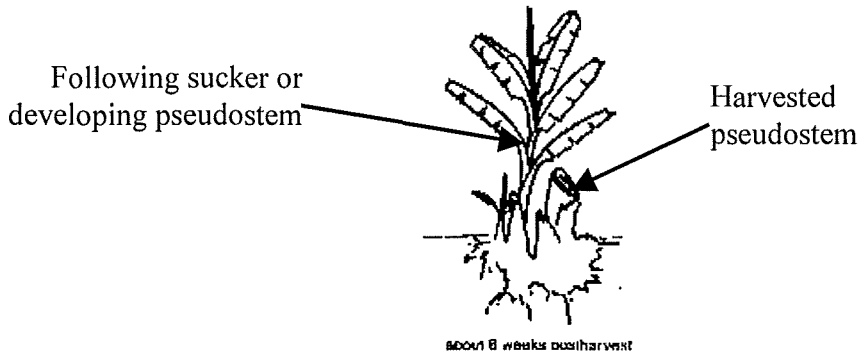

Figure 1. Placement of chemical when injecting nematicides into bananas

Nematode assessment methods

Nematode damage was assessed by determining the disease index and the number of burrowing nematodes in 100 g of banana root. Banana roots were sampled by digging a soil block 25 x 25 x 25 cm from the base of the banana plant to one side of the following sucker. All roots were separated from the soil and washed to remove adhering soil. Ten roots were selected from each sample and split length ways (Broadley, 1979). The amount of the root cortex containing lesions was determined and the disease index calculated as described below (Table 15).

Table 15. Root rating descriptions used to evaluate damage in banana roots caused by burrowing nematode.

Root rating	Description
0	Healthy root, no visible lesions
1	1-25 % of root cortex occupied by lesions
3	26-50 % of root cortex occupied by lesions
5	51-75 % of root cortex occupied by lesions
7	76-100 % of root cortex occupied by lesions

The disease index is then calculated from the root ratings using the following equation;

$$\textbf{Disease Index} = \frac{\textbf{Sum of all root ratings x 100}}{\textbf{Total number of roots x 7}}$$

Following disease index assessments nematodes were extracted from roots by cutting the roots into 1-2 cm pieces and placing on a screen in a misting cabinet for 7 days (Hooper, 1986). The nematodes were then collected from the water which had passed over the root surface by pouring the solution through a 25 μm screen and backwashing into a vial for counting.

Nematode assessment timing

The number of nematodes present in the roots was determined before treatment application and again every three months as outlined below (Table 16). The final

nematode assessment corresponded with bunch harvest and was therefore conducted over a number of weeks.

Table 16. Dates of assessment of nematode indices following injection of banana plants with Vydate 240 L® compared with ground application of Vydate 240 L® and Rugby 100 G compared to an untreated control.

Assessment Date	Description
June, 2000	Pre-treatment
October, 2000	First post treatment assessment
January, 2001	Second post treatment assessment
March, 2001	Final assessment

Plant growth assessments

Bunch weights of plants were determined prior to treatment applications and at the conclusion of the following plant cycle. Prior to harvest the number of fingers of fruit on each bunch was estimated using the method described by (Turner, *et al.* 1988) The growth of the following sucker was determined by measuring leaf emergence rate and the increase in the height of the suckers at monthly intervals for four months following the initial nematicide treatment.

Trial 3. Fenamiphos formulation injection – Field trial.

Trial site selection

A trial site was established on a 3 year old banana field (*Musa* AAA, Cavendish subgroup, cv. Williams). The site was located at East Palmerston, 30 km west of Innisfail on a krasnozem soil. The plants were irrigated using drip irrigation when needed. Leaf disease and nutrition were applied as normal commercial practice.

Treatment list

Two formulations of fenamiphos, Nemacur 400 and Nemacur 240 CS, supplied by Bayer Australia Pty Ltd, were compared to soil application of Nemacur 400, the registered commercial application method and an untreated control (Table 17). Nemacur was injected into the following sucker to achieve a rate of 4.8 g of fenamiphos per plant at each treatment time. The soil application received only 2.4 g of fenamiphos per plant per application.

Two injected applications four months apart were compared to a single injection (Table 17). The trial was conducted as a randomised block with 6 replicate plots of each treatment. Within each plot there were 12 data plants.

Treatment application methods

Injection treatments were applied using an injection gun with a 20 mL graduated cylinder and a perforated needle at the end of a 1 m lance. The nematicide was applied into the following sucker 10 cm from the base at a downward angle. Two, 10 mL applications of the nematicide solutions were applied on opposite sides of each sucker treated. Suckers treated were above 1m in height at the time of treatment and without a bunch.

The Nemacur 400® soil applications were applied in a band around the following sucker.

Table 17. Treatments applied to evaluate the injection of two formulations of Nemacur into the following sucker compared to soil application and an untreated control

Formulation	Treatment	Rate	Method
1. Nemacur 240 CS®	Injection	20 mL / plant undiluted	One injection into the following sucker at trial commencement.
2. Nemacur 240 CS®	Injection	20 mL / plant undiluted	Two injections into the following sucker 4 months apart following trial commencement.
3. Nemacur 400®	Injection	12 mL + 8 mL of water per plant	One injection into the following sucker at trial commencement.
4. Nemacur 400®	Injection	12 mL + 8 mL of water per plant	Two injections into the following sucker 4 months apart following trial commencement.
5. Nemacur 400®	Soil application	6 mL per plant + 14 mL of water	Two applications in front of the following sucker 4 months apart following trial commencement.
6. Untreated control	Nil	Nil	Nil

Treatment application details

All treatments were applied on October 25, 2001. The follow up 4 month treatments were applied on February 27, 2002.

Nematode assessment methods

Nematode damage was assessed by sampling three plants from each plot, at each sampling time as described previously (Trial 2). The percentage of roots with symptoms of burrowing nematode damage, the disease index and number of number of burrowing

nematodes in 100 g of banana root were determined from each plant sampled as described previously.

Nematode assessment timing

The number of nematodes present in the roots was determined before treatment application and again every three months as outlined below (Table 18). The trial was terminated one year after the initial treatment applications.

Table 18. Dates of assessment of nematode and plant growth indices following injection of suckers with two formulations of fenamiphos compared with soil application of fenamiphos and an untreated control.

Description	Assessment Date
Pre-treatment	17, 19 and 25 October, 2001
3 month post-treatment	14, 16 and 21 January, 2002
6 month post-treatment	8, 10 and 15 April, 2002
9 month post-treatment	8, 10 and 16 July, 2002
12 month post-treatment	22, 23 and 23 October, 2002

Plant growth assessment

The growth of plants was determined by measuring the height and the emergence of leaves of the suckers at each assessment date, three months apart. The difference in plant height and the number of new leaves was determined and used to calculate the increase in plant height per week, the leaf emergence rate per week and the height increase per leaf. All plants in the trial were measured at each assessment period and the plot mean determined.

Phytotoxicity

Phytotoxicity was determined by assessing the level of stem splitting on the following sucker on November 14, 2001, 20 days after the first nematicide treatment.

Statistics

The number of nematodes extracted from banana roots was normalised using a ln(x+1) transformation before statistical analysis. All analysis of variance were determined using Genstat 4. The data was then presented as back transformed means (e^x-1). The progression of the plant and disease parameters was determined by calculating the area under the curve for each treatment over the twelve month trial period using Genstat 4.

Results and discussion

Trial 1 – Glasshouse trial

All nematicides applied to banana plants significantly reduced the number of nematodes in the roots relative to the untreated control, except injection of Vydate 240 L®. However, the efficacy of injecting Vydate 240 L® was not significantly different from other nematicides (Table 19). This suggested that the nematicides Nemacur 400®, Vydate 240 L® and abamectin all had some systemic activity, and were translocated to the roots of the banana plant where they reduced burrowing nematode numbers. However, the injection was not significantly better than soil application at reducing nematode numbers (Table 19).

Injection of Nemacur 400® and abamectin had a phytotoxic effect when injected into the pseudostem on the banana plants. The injection of these two nematicides reduced the height of plant at harvest below the height of the plants when treatments were applied six weeks earlier (Table 19). Similarly, the dry weight of plants injected with Nemacur 400® and abamectin was significantly reduced relative to the untreated plants (Table 6). The injection of Vydate 240 L did not significantly reduce the plant dry weight or the fresh root weight relative to the untreated plants (Table 19).

Vydate 240 L® shows potential for use as a pseudostem injection due to low phytotoxicity and its ability to reduce the number of burrowing nematodes to a level compared to other nematicides.

Table 19. Efficacy and growth of tissue cultured banana plants following injection of nematicides into the pseudostem compared to soil application.

Treatment	Method of application	Nematodes in 100 g root	Dry Wt. (g)	Fresh root wt. (g)	Plant height increase (cm)
Untreated	untreated	153 b	9.2 c	50.7 bc	4.8 b
Vydate®L	injection	17 ab	8.0 bc	41.0 ab	4.4 b
Vydate®L	soil	1 a	6.8 ab	42.8 abc	4.4 b
Nemacur®	injection	9 a	4.9 a	38.0 a	-6.0 a
Nemacur®	soil	8 a	8.3 bc	50.1 bc	5.1 b
Rugby®	soil	7 a	8.3 bc	52.0 c	4.8 b
Vertimec®	injection	7 a	5.1 a	33.7 a	-3.8 a

Means in columns followed by the same letter are not significantly different from one another ($P = 0.05$).

Trial 2 – Injection of Vydate 240 L® - Field Trial

Nematode control

The injection of Vydate 240 L® into the following sucker significantly reduced the number of nematodes 3 and 6 months after treatment relative to the untreated control (Table 20). However, the injection of Vydate 240 L® into the following sucker was similar to the soil application of Vydate 240 L® and Rugby 100 G at suppressing nematodes (Table 20). The injection of Vydate 240 L® into the following sucker after injecting into the harvested pseudostem was able to significantly reduce nematode numbers at the 6 month assessment relative to the untreated control. This is further evidence that injection of Vydate 240 L® into the following sucker is able to reduce nematode numbers in the roots of banana plants.

The application of Vydate 240 L® into the harvested pseudostem alone had no effect on nematode numbers. This suggested poor movement of the chemical from the harvested pseudostem to the roots of the developing sucker. (Araya, 1999) was able to increase the weight of bananas, but was unable to reduce nematode numbers when injecting nematicide into the harvested pseudostem.

There was no significant difference in the disease index calculated at each sampling period between the different treatments (Table 20).

Table 20. Disease index on banana roots prior to treatment and 3, 6 and 8 months after treatment with Vydate 240 L® injected into the harvested or following sucker compared to Vydate 240 L® and Rugby 100 G® applied to the soil and an untreated control.

Formulation	Rate per stool	Method	Nematode numbers in 100 g of banana root				Disease index			
			Jun, 2000	Oct, 2000	Jan, 2001	Mar, 2001	Jun, 2000	Oct, 2000	Jan, 2001	Mar, 2001
1. Vydate 240 L	24 mL / plant	2x12 mL following sucker	1129 a	78 a	201 a	851 a	20 a	22 a	15 a	12 a
2. Vydate 240 L	24 mL / plant	12 mL harvested pseudostem + 12ml following sucker	196 a	465 b	357 ab	181 a	19 a	21 a	15 a	10 a
3. Vydate 240 L	24 mL / plant	24 ml harvested pseudostem	772 a	461 b	1336 b	947 a	21 a	23 a	25 a	13 a
4. Vydate 240 L	24 mL / plant	2x12 ml soil	1036 a	93 a	345 a	1133 a	20 a	16 a	13 a	13 a
5. Rugby 100 G	40 g / plant	2x20 g soil	223 a	318 ab	406 ab	689 a	18 a	22 a	6 a	14 a
6. Untreated control	Nil	Nil	858 a	666 b	1974 b	2095 a	20 a	16 a	19 a	18 a

Numbers in columns followed by the same letter are not significantly different from one another (P=0.05).

By calculating the area under the nematode number curve for the eight months of the trial, it was found that the injection of Vydate 240 L into the following pseudostem was able to significantly suppress the number of nematodes in the roots compared to the untreated control (Figure 2). The injection of Vydate 240 L into the following sucker was not significantly different in efficacy to the application of nematicide to the soil. However, this result confirms that Vydate 240 L is translocated to the roots of the banana plant, when injected into the following sucker in sufficient quantities to significantly reduce the number of nematodes; the efficacy of the product is not reduced using the injection method of application.

Treatments where Vydate 240 L® injected into the harvested pseudostem exhibited nematode recovery similar to the untreated control (Figure 2). Again, the injection of chemical into the harvested pseudostem does not allow sufficient translocation of the chemical to reduce nematode numbers and is not be a viable treatment.

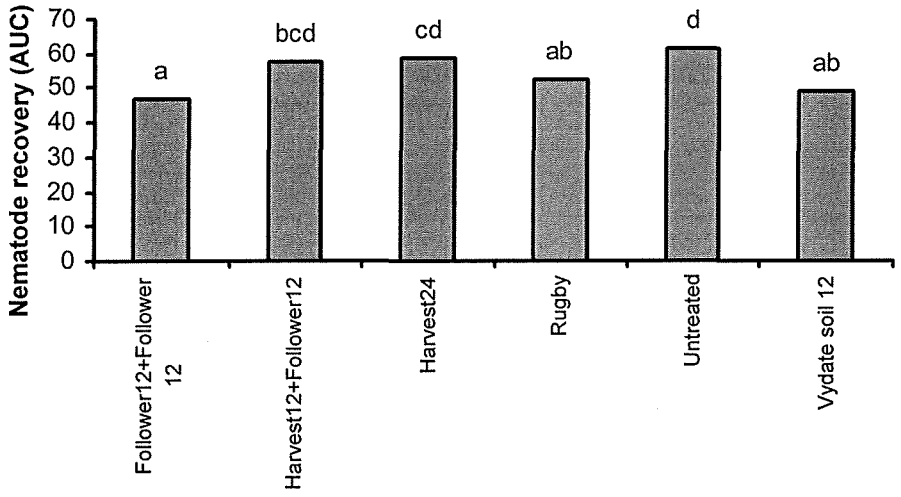

Figure 2. Area under the nematode number curve from June 2000 to March 2001 for banana plants treated with Vydate 240 L® as a pseudostem injection into the harvested pseudostem or following sucker compared to Vydate 240 L® and Rugby 100 G® applied to the soil and an untreated control. (Columns with by the same letter above are not significantly different from one another (P=0.05).)

Bunch weights and finger number

There was no significant difference in bunch weight between treatments at the commencement of the trial (Table 21). However, at the final assessment of bunch weight the untreated plants had significantly higher bunch weight than all Vydate 240 L® treatments. Due to a disease outbreak not all fruit was harvested, which meant not all bunches were weighed. This may have biased bunch weights in favour of plants that bunched earlier.

The number of fingers was determined on all plants that produced a bunch. The finger number per bunch was significantly higher on plants receiving two injections of Vydate 240 L® into the following sucker compared to treatments where Vydate 240 L® was injected into the harvested pseudostem (Table 21). However, the finger number on plants with Vydate 240 L® injected into the following sucker was not significantly different from the untreated control or the soil application of nematicides (Table 21).

Table 21. Bunch weight prior to treatment and bunch weight and finger number after treatment with Vydate 240 L® as a pseudostem injection into the harvested following sucker compared to Vydate 240 L®and Rugby 100 G® applied to the soil and an untreated control.

Formulation	Rate per stool for trial	Method	Pre-treatment bunch weight (kg)	Post treatment bunch weight (kg)	Post treatment bunch finger number
1. Vydate 240 L®	24 mL / plant	2x12 mL following sucker	25.2 a	26.9 a	118 c
2. Vydate 240 L®	24 mL / plant	12 mL harvested pseudostem + 12mL following sucker	27.1 a	23.7 a	100 ab
3. Vydate 240 L®	24 mL / plant	24 mL harvested pseudostem	23.6 a	25.0 a	95 a
4. Vydate 240 L®	24 mL / plant	2x12 mL soil	26.4 a	26.1 a	113 bc
5. Rugby 100 G®	40 g / plant	2x20 g soil	24.5 a	28.3 ab	112 bc
Untreated control	Nil	Nil	25.2 a	31.9 b	111 bc

Numbers in columns followed by the same letter are not significantly different from one another (P=0.05).

Plant growth

There were no significant differences in plant growth following nematicide applications into the harvested pseudostem or onto the soil relative to the untreated control (data not shown). This demonstrates that there were no phytotoxic effects from the injection of Vydate 240 L® into the pseudostem of the following sucker.

Trial 3. Nemacur formulation injection

Nematode and root damage

The injection of Nemacur 400® into the following sucker of bananas was able to significantly reduce the amount of damage caused by burrowing nematode 6 months after treatment (Table 22). Similarly, the injection of Nemacur 400® was also able to reduce the percentage of nematode infected roots six months after treatment relative to the untreated plants (Table 22). Only the treatment consisting of two injections of Nemacur

240 CS® did not significantly reduce the amount of nematode damage relative to the untreated control (Table 22). The injection of Nemacur 400® into the following sucker was equally as effective as the current practice of applying Nemacur 400® onto the soil. However, twice as much chemical was applied as the two pseudostem injection treatments relative to the soil treatment.

Injection of Nemacur 400® gave better control of nematode damage on the roots of banana plants than the use of Nemacur 240 CS® (Table 22). Two injections of Nemacur 240 CS® consistently gave a higher disease index than a single injection of Nemacur 240® CS® (Table 22).

There was no difference in the reduction of nematode damage between a single application of Nemacur 400® and two applications of Nemacur 400® 4 months apart. The reduction in nematode damage from the first application in October, 2001 appeared to be sufficient to protect throughout the production cycle of that banana sucker.

There was no significant effect of fenamiphos treatments on the number of nematodes in the roots of banana plants until twelve months after the first treatment (Table 22). At the twelve month nematode assessment the number of nematodes in the single Nemacur 240 CS® injection treatment had significantly lower nematodes than the untreated control. The variability in nematode numbers within the plots may be the cause of the non-significant difference between treatments.

Plant growth

There was significantly more leaf production 6 and 12 months after the first nematicide treatment in plants that received two applications of Nemacur 400® relative to the untreated control (Table 23). This suggested that although there was a high level of stem splitting incurred with the injection of Nemacur 400® it did not affect the growth of plants. Instead, improved plant growth was observed which may be due to improved root conditions from the control of nematodes or due secondary chemicals in Nemacur 400® resulting in growth promotion when injected into the following sucker.

Table 22. Nematode and root damage ratings of banana plants injected with Nemacur 240 CS or Nemacur 400 into the pseudostem of the following sucker for control of burrowing nematodes on bananas for a 12 month period compared with soil application of Nemacur 400 and an untreated control.

Treatment	Number and rate of application	Assessment Time										Area under disease curve	
		Pre-treatment		Post treatment									
Disease index				3 months		6 months		9 months		12 months			
Nemacur 240 CS	1x20 mL	13.6	a	10.7	a	21.1	bc	14.3	a	9.4	a	162	a
Nemacur 240 CS	2x20 mL	12.2	a	18.6	a	20.3	abc	18.2	a	11.2	a	211	a
Nemacur 400	1x12 mL	13.5	a	15.3	a	16.0	ab	14.6	a	10.4	a	176	a
Nemacur 400	2x12 mL	6.1	a	17.7	a	14.4	ab	17.1	a	11.8	a	188	a
Soil Nemacur 400	2x6 mL	10.7	a	12.7	a	12.2	a	17.9	a	13.9	a	173	a
Untreated	0	13.8	a	17.7	a	27.3	c	19.8	a	11.2	a	227	a
Burrowing nematodes in 100 g of root													
Nemacur 240 CS	1x20 mL	298	a	275	a	283	a	458	a	191	a	69.4	a
Nemacur 240 CS	2x20 mL	289	a	712	a	379	a	406	a	803	c	74.7	a
Nemacur 400	1x12 mL	259	a	632	a	472	a	350	a	566	bc	73.5	a
Nemacur 400	2x12 mL	136	a	698	a	170	a	316	a	264	ab	70.5	a
Soil Nemacur 400	2x6 mL	129	a	423	a	214	a	741	a	477	bc	72.9	a
Untreated	0	88	a	870	a	915	a	749	a	468	bc	78.0	a
Percentage of roots with nematode damage													
Nemacur 240 CS	1x20 mL	30.4	a	35.8	a	57.7	bc	55.8	a	38.3	a	547	a
Nemacur 240 CS	2x20 mL	34.6	a	56.9	bcd	53.8	abc	62.2	a	41.9	a	666	bc
Nemacur 400	1x12 mL	41.8	a	49.6	abc	50.8	ab	54.8	a	43.3	a	606	ab
Nemacur 400	2x12 mL	19.7	a	63.9	cd	45.9	ab	55.8	a	38.9	a	639	ab
Soil Nemacur 400	2x6 mL	32.6	a	41.9	ab	38.4	a	59.2	a	40.7	a	562	a
Untreated	0	37.7	a	70.2	d	68.2	c	66.2	a	44.3	a	768	c

Numbers are the means of 18 plants sampled. Means in columns with the same letter following are not significantly different from one another ($P<0.05$).

Table 23. Weekly leaf emergence ratings of banana plants injected with Nemacur 240 CS® or Nemacur 400® into the pseudostem of the following sucker for control of burrowing nematodes on bananas over a 12 month period compared with soil application of Nemacur 400® and an untreated control.

Treatment	Number and rate of application	Assessment Time				
		Post treatment				Area under LER curve
7 day leaf emergent rate		3 months	6 months	9 months	12 months	
Nemacur 240 CS	1x20 mL	0.80 a	0.69 a	0.42 a	0.59 a	0.58 a
Nemacur 240 CS	2x20 mL	0.78 a	0.70 ab	0.42 a	0.60 ab	0.60 a
Nemacur 400	1x12 mL	0.80 a	0.68 a	0.40 a	0.58 a	0.58 a
Nemacur 400	2x12 mL	0.81 a	0.73 b	0.44 a	0.62 b	0.62 a
Soil Nemacur 400	2x6 mL	0.81 a	0.68 a	0.41 a	0.58 a	0.58 a
Untreated	0	0.83 a	0.68 a	0.38 a	0.58 a	0.58 a

Numbers are the means of 72 plants sampled. Means in the column with the same letter following are not significantly different from one another ($P<0.05$).

Phytotoxicity

There was significantly more splitting of the pseudostem when Nemacur 400® was injected into the following sucker relative to Nemacur 240 CS® injection and the untreated control (Figure 3). The injection of Nemacur 240 CS® also significantly increased the amount of stem splitting relative to the untreated control (Figure 3). However, there was only approximately 50 % splitting when Nemacur 240 CS® was injected compared to 80 % when suckers were treated with Nemacur 400® (Figure 3). The size of the splitting varied in length from 10 cm in length to 60 cm in length. Also, some brown necrotic areas around the splitting was observed where Nemacur 400® had been injected. Three suckers that were treated with Nemacur 400® died. No other plant deaths were recorded.

A large quantity of Nemacur 400® (12 mL of product) was injected into each plant. This is equivalent to two soil applications being applied in the one treatment. The symptoms of phytotoxicity may be reduced by smaller applications. Similarly, if Nemacur 240 CS® is used, 10 mL application at three to four month intervals would cause less phytotoxicty.

The splitting tended to be worse in smaller plants, so it may be necessary to impose height and restrictions when injecting banana plants avoiding following suckers under 1.5 m tall.

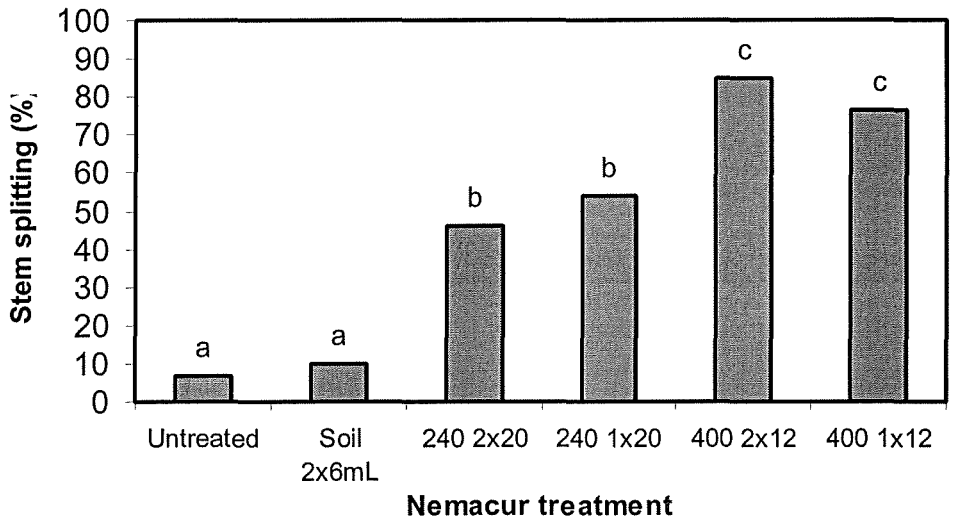

Figure 3. Splitting of the following sucker in an evaluation of the efficacy of injection of two Nemacur® formulations for control of burrowing nematode relative to soil application and an untreated control. **(Bars with by the same letter above are not significantly different from one another (*P*=0.05).)**

Conclusion

Injection of systemic nematicides Vydate 240 L® and Nemacur 400® into the pseudostem of the following sucker was able to reduce burrowing nematode numbers as effective as soil application of the nematicides, but no better. The injection of the nematicide into the following sucker increased the risk of phytotoxic damage to plants. However, plants were able to recover from mild symptoms of stem splitting in the field.

Two 12 mL injections of Vydate 240 L®, three months apart, into the following sucker effectively reduced nematode populations equal to the application of Vydate 240 L® to the soil. Similarly, a single injection of 12 mL of Nemacur 400® into the following pseudostem in October appeared sufficient to significantly reduce the symptoms of burrowing nematode on bananas. However, the injection of Nemacur 400® increased the risk of plant death in small suckers and superficial stem splitting.

Nemacur 400® caused significantly more stem splitting than injection of Nemacur 240 CS®. The stem splitting appeared to be superficial as there was no reduction in leaf emergence relative to the untreated control and conversely two injections of Nemacur 400®, four month apart significantly increased leaf emergence rate relative to the untreated control.

Low phytotoxicity of Vydate 240 L® was demonstrated in a pot trial where the nematicides Vydate 240 L®, Nemacur 400® and abamectin were injected into the pseudostem of young tissue culture plants in the glasshouse. Injection of Vydate 240 L® into the following sucker in the field trial had no phytotoxic effects on the plants. The growth of the following sucker was similar to untreated plants and where nematicides were applied to the soil. Similarly, the bunch size of banana plants injected with Vydate 240 L® had the highest number of fingers although not significantly better than the untreated control.

Injection of Vydate 240 L® into the harvested pseudostem shortly after harvest had no effect on nematode numbers in the roots. There appeared to be poor translocation of Vydate 240 L® from the harvested pseudostem to the roots of the developing pseudostem.

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3. Biological control

Introduction

Tissue culture banana plantlets are free of burrowing nematode and other diseases, but have been found to be more susceptible to attack by soil borne pathogens such as *Fusarium oxysporum* f. sp. *cubense* (*Foc*) (Smith, *et al.* 1998). The susceptibility of tissue culture plants to soil borne diseases is thought to be due to the lack of microflora associated with the plants when planted in the field. There has been an increasing amount of work investigating the introduction of biological agents to tissue culture plants to protect the plant when planted in the field (Jonathan, *et al.* 2000; Sikora and Schuster 1999; Sutra, *et al.* 2000). Microorganisms that offer potential protection to tissue culture plants may be found in soils that naturally suppress burrowing nematode.

Soils that suppress nematodes usually contain a range of natural enemies that are able to attack their nematode host at different stages of the life cycles; a combination of their activities may prevent nematode numbers from building up (Kerry, 1990). However, one or two species usually dominate as antagonists to prevent or reduce the damage caused by nematodes. Antagonists with the best potential as biocontrol agents of nematodes are: plant-health promoting rhizobacteria; obligate bacterial parasites; fungal egg pathogens/parasites; predacious or trapping fungi; endoparasitic fungi; fungal pathogens/parasites of females and endomycorrizal fungi (Sikora, 1992). (Sutra, *et al.* 2000) found that the rhizosphere of banana plants constitute a favourable microenvironment for soil biota and therefore could contain potential antagonists to burrowing nematode.

The rhizosphere of the plant is a zone of intense microbial activity (Zehnder, *et al.* 2001). There are a number of organisms that persist in the rhizosphere but bacteria species are amongst the most common. Rhizobacteria that exert beneficial effects on plants are referred as plant growth-promoting rhizobacteria (PGPR) (Zehnder, *et al.* 2001). Endophytic fungi such as Fusarium oxysporum have been found to be antagonistic to nematodes on bananas (Sikora and Schuster, 1999). The endophytic nature of some microorganisms makes them suitable for use in vegetatively propagated crops because of their capability to colonise and persist in the intracellular spaces. Endophytic microorganisms have been used as biocontrol agents for the suppression of soil-borne diseases by competing with pathogens for resources, such as nutrients, producing antibiotics or activating host defence mechanisms (Ramamoorthy, *et al.* 2001; Zehnder, *et al.* 2001; Zehnder, *et al.* 2000).

Addition of amendments to the soil may also be able to enhance microorganisms that suppress soil-borne pathogens. The use of chitin was found to enhance general suppression to soil pathogens and nematodes through alterations to the microbial community structure (Kloepper, *et al.* 1999). Chitin amendments stimulate microorganisms producing the enzyme chitinase. Chitin can constitute up to 30 % of the eggshell of nematodes (Bird and Bird 1998). It was thought that additions of chitin would increase egg destroying microorganisms (Davies, *et al.* 1991). An addition of 1 % (w/w) chitin was reported to have eliminated *Meloidogyne incognita* in the first planting of cotton and significantly reduced the population in the second year (Hallmann, *et al.* 1999). The reduction in nematode numbers was attributed to

an increase in endophytic bacteria specifically promoted by the chitin amendment. (Benhamou, *et al.* 1998) found that chitin amendment not only had an inhibitory affect on pathogens but also had the ability to elicit plant defence, physiological and biochemical reactions at the site of attempted pathogen penetration. Similarly, Dann and Muir (2002) found that the addition of silicon to pea seedlings was able to induce systemic resistance to foliar pathogens. Mill ash is composed of approximately 60 % silicon oxide, which may be able to induce resistance to burrowing nematode.

The aim of this trial work was to isolate microorganisms from soils thought to suppress burrowing nematode, to determine if isolates were antagonistic to burrowing nematode in banana plants.

Materials and Methods

Rhizobacteria

Suppressive soils

Two soils that had long term banana cultivation, greater than 10 years, were compared with potting mix and a municipal solid waste (MSW) compost for their ability to suppress burrowing nematode and enhance the growth of bananas. The four soils were left untreated, autoclaved for 15 minutes at 121 °C, amended with 1 % (w/w) chitin, 33 % (v/v) mill ash or 1 % (w/w) molasses.

Tissue culture banana plants, *Musa* cultivar Williams (AAA genomic group, Cavendish sub-group), were deflasked into soils with the different amendments. The plants were fertilised fortnightly with soluble fertiliser (Thrive, Arthur Yates and Co. Ltd, Homebush, Australia). The plant shoots were measured for height increase and leaf emergence rate 66 days for after deflasking and repotted into 150 mm pots that had mung beans (*Vigna mungo*) growing that had been infected with burrowing nematode. At the time of replanting there was an average of 4,500 burrowing nematode in each pot.

The plants were allowed to grow for a further 60 days before harvesting. At harvest the plant height, leaf emergence, last fully emerged leaf area and the shoot weight were determined. The roots were washed free of soil and scored for the appearance of lesions (Netscher and Sikora, 1990). The roots were then cut into 20 mm lengths and the nematodes extracted in a misting chamber for seven days before identification and counting (Hooper, 1986).

Rhizobacteria isolation

Root samples were taken from eight treatments that were able to suppress the number of nematodes in the roots of bananas relative to unsterile potting mix. Root samples were taken from the two farm soils amended with chitin and ash, and from the unsterile compost and compost amended with molasses, chitin and ash.

Bacteria were isolated from the rhizosphere using dilution plating spread on Nutrient Agar (NA). Endophytic bacteria were isolated from root pieces transversely sectioned and plated onto NA after soaking in NaOCl (10 %) plus Tween 80 for 5 minutes,

followed by 2 washes in sterile distilled water. The bacterial isolates were maintained on nutrient agar slopes in 10 mL McCartney bottles until used

Rhizobacteria suppression of nematodes

70 bacterial isolates were screened for growth promotion and suppression of burrowing nematodes compared to an untreated control and a fluorescent *Pseudomonas* species identified as giving growth promotion and nematode suppression in previous trial work (Pattison, A.B. unpublished data). Three trials were conducted to screen the 70 bacterial isolates for growth promotion and suppression of *R. similis* on bananas.

A screening protocol was established in FR98016 and used throughout this trial (Figure 4). The isolates were retrieved from the slopes using a sterile loop and streaked out on nutrient agar. Two days later the bacteria were scraped from the surface of the agar plate and rinsed with sterile water.

Tissue cultured banana plants were deflasked into a 10^8-10^9 bacterial isolate suspension for 15 minutes and then potted into 50 x 50 mm square pots, 150 mm deep, containing a peat, sand and perlite (2:1:1) potting mix. 5 mL of a 10^8-10^9 suspension of the same bacterial isolate was applied to the pot at planting and again 21 days after planting. 42 days after deflasking the banana plants were repotted into 150 mm diameter pots with 2.0 kg of a soil and sand mix. Measurements of plant growth were made on plant height and leaf emergence of the banana plant at the time of repotting. Again, 5 mL of a 10^8-10^9 suspension of the bacterial isolate was applied to the soil surface of the pot and incorporated in with 20 mL of water at repotting. Each treatment was replicated four times in a completely randomised design and maintained in the glasshouse at temperatures between 21 and 30 °C.

Prior to replanting the banana, mung beans (*Vigna mungo*) were grown in the pots (Figure 4). The mung beans were inoculated with approximately 500 motile burrowing nematode five days after planting seeds. The tops of the mung bean plants were removed two weeks later to kill the plants and enhance the movement of nematodes from the mung bean roots into the roots of banana plants (Figure 4).

The banana plants were allowed to grow for six weeks after repotting, before being harvested. At harvest, the height, leaf emergence, size of the last fully emerged leaf and the dry weight of the shoots were determined. The soil was washed from the roots and the appearance of lesions on the root surface was determined using a 0 to 10 scale, 0 for healthy roots with no lesions and 10 for a dead root system (Netscher and Sikora, 1990). The roots were cut into 20 mm lengths and the nematodes extracted in a misting cabinet for seven days (Hooper, 1986).

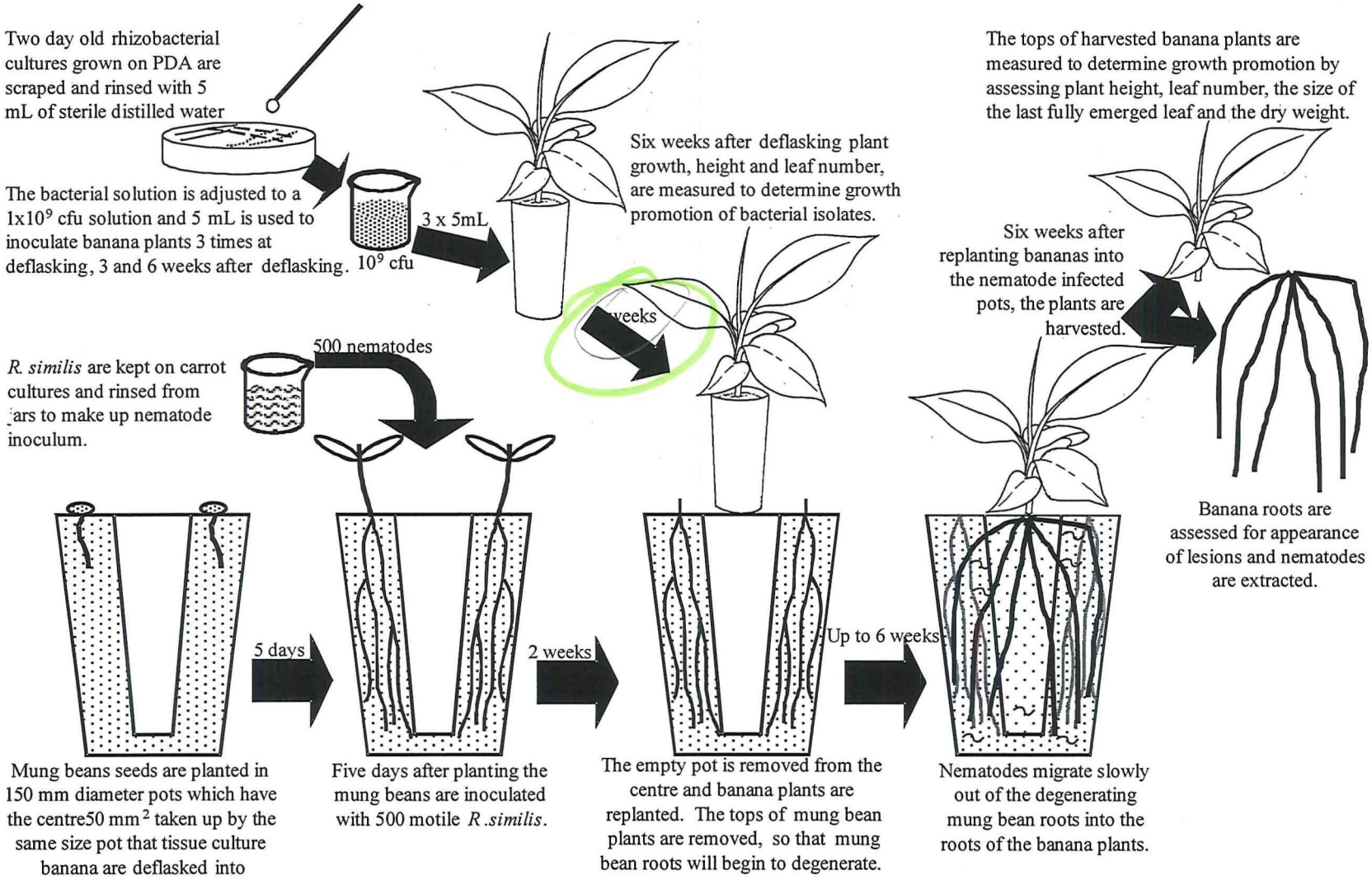

Figure 4. Screening of bacterial isolates for growth promotion and suppression on bananas.

Fungal Endophytes

Isolate experiment

Several fungal isolates had been identified for biological control of burrowing nematode in project FR96016 (Stanton and Pattison 2000). Other trials had been conducted on the most efficacious isolate and methods to improve efficacy without success (data not shown). Endophytic fungi (*Fusarium oxysporum*) were isolated from the cortex and stele of surface sterilised banana roots and were screened for the suppression of burrowing nematode and the enhancement of the growth of banana plants. The fungal isolates were grown on sterile grain sorghum for 7 days. Fungal isolates included A3, AM6 and AM7. The banana cv. Williams were inoculated with fungi by placing 80 g of inoculated grain sorghum under the roots at repotting. The growth of the banana plants and suppression of nematodes was compared with both an untreated and sterile sorghum control. At 3 and 6 weeks after repotting, plants were inoculated with 1000 nematodes each. Treatments were replicated four times and pots were maintained in the glasshouse. Ten weeks after inoculation, nematodes were extracted from the roots for 7 days in a misting chamber and counted (Hooper, 1986).

Penetration and mortality experiment.

80 g of grain sorghum inoculated with *Fusarium* isolate A3 was placed under the roots of 13 week old banana plants when repotted into 175 cm diameter pot with standard UC mix. 3 and 6 weeks after inoculating the soil with the *Fusarium* isolate, the pots were inoculated with 4000 nematodes per pot and compared with untreated and sterile sorghum controls. Treatments were replicated four times and pots were maintained in a glasshouse. One week after inoculation, nematodes were extracted from the roots for 7 days in a misting chamber and counted to determine nematode penetration into the roots. Simultaneously, a 200 g sub-sample of soil was set up in a Whitehead tray (Whitehead and Hemming, 1965) to extract nematodes over 3 days to determine nematode mortality in the soil.

Combination experiment

Three different potential biocontrol organisms were added to 14 month old tissue culture plants in combination or alone. The soil was treated with an endophytic fungi (A3), a fluorescent pseudomonas sp. (83) and a nematode trapping fungi (*Arthrobotrys dactyloides*).

An isolate of non-pathogenic *Fusarium oxysporum* A3 was grown on sterile sorghum seed and 80 g of inoculated sorghum added to the pots. The fluorescent pseudomonas sp., 83, was grown on nutrient agar plates and a 10^8-10^9 suspension was made in sterile water so that the pots could be drenched with 25ml of suspension. This was repeated twice at 3 weekly intervals before inoculating with nematodes. Nematode trapping fungi, NTF, were prepared and 5 g of algenate pellets containing the NTF was added to the pots. The treatments were compared with a sterile sorghum and an untreated control. In the sterile sorghum control, 80 grams of sterile sorghum was added to the pots.

Plants were harvested 10 weeks after inoculation and nematodes were extracted in a misting chamber over 7 days (Hooper, 1986). Three weeks after treatments were imposed, banana plants were inoculated with 1000 burrowing nematodes per pot. Each treatment was replicated five times.

Results and discussion
Rhizobacteria

Suppressive soils

Due to difficulties with the in vitro propagation of the banana plants there were insufficient banana plants to test all four soils. Only three amendments were applied to Farm B soil, which included 33 % mill ash, 1 % chitin and 1 % molasses. The trial was analysed as an unbalanced factorial trial using linear mixed models. Fixed effects were tested using the Wald test statistic and average LSDs calculated to separate means.

Banana plants grown in Farm A and B soil amended with 33 % mill ash had significantly less nematodes than the unsterile potting mix, the conventional method of propagating *in vitro* banana plants (Table 24). Similarly, banana plants grown in unsterile compost and compost amended with chitin and molasses were able to significantly suppress the number of nematodes in the roots (Table 24). Additionally, treatments where compost was amended with molassess and ash were able to reduce the appearance of lesions on the roots (Table 24). These reduction in nematode numbers and improvement in root health appear to be biologically derived as sterilising the Farm A soil and compost significantly increased the number of nematodes in the roots and increased the development of lesions relative to the unsterile soil. This effect was not seen in the potting mix treatment, which suggested that there was no biological suppression in the potting mix.

The use of farm soil penalised early growth of the banana plants with significantly shorter plants in the Farm A and B soil relative to the unsterile potting mix (Table 24). Only the addition of molasses to Farm A soil was able to produce early plant growth that was not significantly different from the growth of the unsterile potting mix (Table 24). This penalty in early growth possibly occurs through decreased drainage and aeration, making it difficult for *in vitro* banana plantlets to establish early growth.

Soil amendments had a significant effect on time of leaf emergence, shoot dry weight and root weight of banana plants. However, no amendment significantly improved plant growth relative to the untreated plants. Banana plants grown with 1 % chitin had significantly fewer leaves after 66 days growth than plants grown in ash. Similarly, plants grown in 1 % chitin were significantly lighter than the untreated plants. However, when chitin and ash were added to the Farm A soil there was a suppression in nematode numbers. The suppression of burrowing nematode in amended Farm A soil appears to be due from a biological origin and is therefore a potential source of antagonistic microorganisms.

The sterilisation of the soil had an adverse effect on root growth (Table 25). This was possibly due to greater nematode damage on the plants grown in sterile soil where biological nematode antagonists were removed (Table 24).

The potting mix or soil used in the treatments had a significant effect on the growth of banana plants. Dry shoot weight, fresh root weight and the leaf emergence after 66 days were all significantly less in the two farm soils relative to the compost and potting mix treatments (Table 26). The leaf area of the last fully emerged leaf was significantly larger in the compost treated plants relative to the farm soil (Table 26). However, compost did not produce a plant with a significantly bigger leaf than the potting mix (Table 26). The slow initial growth and height disadvantage when using farm soil highlights the unsuitability of the farm soil as a growth medium for deflasking *in vitro* bananas.

Table 24. Nematode damage, numbers and plant height after 66 days on banana plants grown in four soils with different amendments relative to sterile and unsterile soil.

Soil	Amendment	66 day plant height (cm)	Root health (0-10)	Burrowing nematode in 100 g of root
Potting mix	Unsterile	19.3 hi	5.4 bcde	11,824 cdefg
	Ash	16.5 fgh	5.4 bcde	21,141 fg
	Chitin	12.1 bcde	5.3 bcde	18,508 efg
	Molasses	17.2 fgh	4.9 abcde	11,613 cdefg
	Sterile	17.7 ghi	4.4 abcde	10,259 cdefg
Compost	Unsterile	21.6 i	4.1 abcd	3,995 bc
	Ash	19.5 hi	3.0 a	6,008 bcde
	Chitin	17.5 fghi	4.4 abcde	3,884 b
	Molasses	16.5 fgh	3.6 abc	4,328 bc
	Sterile	13.6 cdefg	6.2 de	19,593 fg
Farm A	Unsterile	10.1 abc	4.2 abcd	9,320 cdefg
	Ash	13.3 cdef	3.9 abc	2,630 ab
	Chitin	8.3 ab	6.2 de	6,856 bcdef
	Molasses	14.0 cdefgh	5.7 cde	12,015 cdefg
	Sterile	10.2 abc	6.4 e	24,293 g
Farm B	Ash	11.8 bcd	3.5 ab	1,086 a
	Chitin	7.20 a	4.7 abcde	14,086 defg
	Molasses	10.20 abc	5.0 abcde	5,297 bcd

Means in columns with the same subscript are not significantly different from one another ($P<0.05$).

Table 25. Leaf emergence, shoot dry weight and fresh root weight of banana plants grown in three different amendments compared to untreated and sterile potting substrates.

Amendment	Leaf emergence (66 days)	Shoot dry weight (g)	Root weight (g)
Untreated	6.7 ab	12.53 b	62.24 b
Ash (33%)	6.8 b	12.06 ab	57.89 ab
Chitin (1%)	5.8 a	9.63 a	44.72 ab
Molasses (1%)	6.3 ab	11.38 ab	56.84 ab
Sterile	6.1 ab	10.16 ab	42.24 a

Means in columns with the same subscript are not significantly different from one another ($P<0.05$).

Table 26. Leaf emergence, plant height, leaf area, root and shoot weight of banana plants grown in four different potting substrates.

Soil	Leaf emergence		Plant height (cm)	Root weight (g)	Leaf area (cm^2)	Shoot dry weight (g)
	66 days	121 days	121 days			
Potting mix	7.1 b	13.9 a	27.4 b	63.99 b	5003 bc	14.61 b
Compost	6.7 b	13.3 a	28.0 b	79.98 b	5258 c	15.26 b
Farm A soil	5.6 a	12.3 a	22.1 a	32.28 a	3996 b	7.57 a
Farm B soil	6.0 a	20.0 b	30.8 b	34.88 a	1333 a	7.17 a

Means in columns with the same subscript are not significantly different from one another ($P<0.05$).

Rhizobacteria isolation

70 isolates of rhizobacteria were isolated from the roots of banana plants from eight different treatments in the previous trial. It was possible to increase plant growth and suppress nematodes on bananas by adding the organisms back to the potting mix.

In the first screening trial, five bacterial isolates were able to significantly suppress the number of nematodes within the roots of bananas relative to the untreated control (Table 27). Three of these isolates, 4-1, 5-3 and 8-1 had significantly fewer nematodes in the roots of bananas relative to the fluorescent pseudomonas treatment, 83. Two of the isolates, 5-3 and 8-1, had a significantly larger leaf area relative to the untreated control. There were no significant differences in the dry weight of the shoots or the appearance of lesions on the roots (Table 27).

In the second screening trial there was no significant reduction in the number of nematodes recovered or the lesions on the roots of banana plants inoculated with bacterial isolates relative to the untreated plants. The bacterial isolates 8SS-3 and 4SS-9 had a lower number of nematodes on the roots, although the difference was not significant relative to the untreated control. Similarly, no isolate was able to increase the area of the last fully emerged leaf or the dry weight of shoots relative to the untreated plants. However, the isolates 5SS-8 and 7SS-7 were able to significantly increase the dry weight of the shoots relative to the fluorescent pseudomonas isolate 83 (Table 28).

In the third screening trial only the isolate 7-4, was able to suppress nematode numbers on the roots of banana plants relative to the untreated plants (Table 29). However, 18 isolates were able to reduce the appearance of lesions on the roots relative to the untreated control (Table 29). Only one isolate, 4SS-5, was able to significantly increase the weight of shoots relative to the untreated control.

The origin of bacterial isolates able to suppress nematode numbers on the roots of banana plants came mostly from the compost treatments. 62 % of the bacterial isolates that were able to reduce the number of nematodes below the number recovered from the untreated control, originated from the compost amended with chitin (Table 30). Similarly, 75 % of the bacterial isolates from the compost amended with chitin were able to increase the dry weight of the shoots above the untreated control (Table 30). The addition of carbohydrate substrates to the potting mix appears to be able to stimulate an increase in the number of bacteria with favourable

characteristics for suppressing nematodes and promoting plant growth. An understanding of the mechanisms of nematode suppression by microorganisms would improve the screening and selection of potentially useful microorganisms.

Table 27. First screening of bacterial isolates from soil and compost treatments to promote the growth and suppress burrowing nematodes on in vitro bananas.

Isolate	Origin	Leaf area (cm^2)		Shoot dry weight (g)	Root health (0-10)	Nematodes in 100 g of root	
Untreated		119	a	1.13	1.7	3071	f
83		229	abcd	2.47	2.7	1199	def
1-1	Farm B - chitin	327	bcdef	5.13	2.0	1129	def
1-2	Farm B - chitin	249	abcde	5.03	2.0	625	bcdef
1-3	Farm B - chitin	306	bcdef	3.90	3.7	1074	def
2-1	Farm A - ash	305	bcdef	5.13	2.0	915	cdef
2-2	Farm A - ash	386	def	4.65	1.5	684	bcdef
2-3	Farm A - ash	322	bcdef	5.27	3.0	638	bcdef
3-1	Farm B - ash	308	bcdef	3.95	1.5	368	bcd
3-2	Farm B - ash	227	abcd	2.84	1.0	764	bcdef
3-3	Farm B - ash	364	cdef	5.48	1.7	566	bcde
4-1	Compost - molasses	196	ab	3.50	1.5	27	a
4-2	Compost - molasses	310	bcdef	3.78	1.2	2321	ef
4-3	Compost - molasses	284	bcdef	4.95	2.2	862	bcdef
5-3	Compost - chitin	402	ef	6.05	1.0	188	bc
5-4	Compost - chitin	407	ef	6.47	2.0	1085	def
6-1	Farm A - chitin	342	bcdef	4.25	1.2	943	cdef
6-2	Farm A - chitin	349	bcdef	3.68	3.0	1096	def
7-1	Compost - ash	223	abc	3.80	3.3	897	cdef
7-2	Compost - ash	321	bcdef	5.23	2.0	1140	def
8-1	Compost - unsterile	429	f	7.30	2.5	173	b
8-2	Compost - unsterile	403	ef	7.60	2.5	571	bcde

Means in columns with the same subscript are not significantly different from one another ($P<0.05$).

Table 28. Second screening of bananas and change in nematode numbers on roots when inoculated with bacteria isolated from the roots of bananas grown in compost.

Isolate	Origin	Leaf area (cm^2)	Shoot dry weight (g)	Root health (0-10)	Nematodes in 100 g of root
Untreated		378	5.15 cdef	1.7	2643
83		343	3.85 abcd	2.2	8021
1-5	Farm B - chitin	252	2.85 abc	3.5	10198
1SS-8	Farm B - chitin	289	3.90 abcd	3.0	8518
2-4	Farm A - ash	307	4.25 abcde	2.5	5323
2-7	Farm A - ash	291	3.83 abcd	2.2	7942
3-10	Farm B - ash	393	4.95 bcdef	1.5	7554
3-5	Farm B - ash	294	3.73 abcd	1.5	8865
3SS-6	Farm B - ash	233	2.23 a	2.7	11848
4-10	Compost - molasses	350	5.12 cdef	1.0	3261
4-11	Compost - molasses	358	4.62 abcde	1.5	3943
4SS-9	Compost - molasses	285	4.25 abcde	3.0	353
5-9	Compost - chitin	329	4.48 abcde	2.5	11848
5SS-14	Compost - chitin	355	4.53 abcde	1.2	2464
5SS-8	Compost - chitin	411	6.45 ef	3.0	3010
6-8	Farm A - chitin	384	5.00 bcdef	2.2	15834
6-9	Farm A - chitin	340	4.30 abcde	2.0	7630
6SS-12	Farm A - chitin	353	4.50 abcde	2.0	3827
6SS-14	Farm A - chitin	291	3.08 abc	2.2	7784
7-3	Compost - ash	293	3.88 abcd	3.0	5013
7-9	Compost - ash	284	3.03 abc	1.5	15521
7SS-6	Compost - ash	272	3.25 abcd	2.7	9044
7SS-7	Compost - ash	387	7.08 f	1.0	6904
8-11	Compost - unsterile	373	5.62 def	1.5	6835
8-8	Compost - unsterile	329	4.03 abcd	1.7	8518
8SS-3	Compost - unsterile	246	2.70 ab	2.0	1789
8SS-4	Compost - unsterile	284	3.62 abcd	2.0	3261

Means in columns with the same subscript are not significantly different from one another ($P<0.05$).

Table 29. Third screening of bananas and change in nematode numbers on roots when inoculated with bacteria isolated from the roots of bananas grown in compost

Isolate	Origin	Leaf area (cm^2)	Shoot dry weight (g)		Root health (0-10)		Nematodes in 100 g of root	
Untreated		201	5.81	abcdef	5.2	e	4242	bcdefgh
83		181	5.03	abcde	3.0	abcd	1999	abc
1SS-2	Farm B - chitin	232	3.89	abcd	3.0	abcd	16106	i
1SS-9	Farm B - chitin	258	6.02	abcdef	3.0	abcd	7412	efghi
2SS-10	Farm A - ash	163	3.61	a	4.2	de	7886	fghi
2SS-14	Farm A - ash	193	4.45	abcde	2.2	abcd	8690	fghi
2SS-6	Farm A - ash	249	6.35	abcdef	3.0	abcd	3781	abcdefgh
3SS-2	Farm B - ash	209	5.15	abcde	3.2	bcde	2007	abcd
3SS-5	Farm B - ash	224	4.16	abcd	2.7	abcd	10066	hi
4SS-4	Compost - molasses	179	5.88	abcdef	2.5	abcd	4486	cdefgh
4SS-5	Compost - molasses	199	11.05	g	2.2	abcd	1311	ab
5-5	Compost - chitin	209	8.16	defg	3.0	abcd	2591	abcdef
5SS-4	Compost - chitin	271	9.80	fg	4.2	de	5807	cdefghi
5SS-6	Compost - chitin	242	7.62	abcdefg	1.5	ab	2906	abcdefg
6SS-1	Farm A - chitin	218	7.99	abcdefg	2.7	abcd	3031	abcdefgh
6SS-16	Farm A - chitin	209	6.41	abcdef	1.0	a	2243	abcde
7-4	Compost - ash	245	8.68	efg	2.2	abcd	1182	a
7SS-1	Compost - ash	259	7.00	abcdefg	4.0	cde	7434	efghi
7SS-8	Compost - ash	247	8.01	bcdefg	2.0	abc	4208	bcdefgh
7SS-9	Compost - ash	268	8.07	cdefg	2.0	abc	2947	abcdefg
8-3	Compost - unsterile	230	3.66	ab	3.0	abcd	6720	defghi
8-7	Compost - unsterile	161	3.73	abc	3.7	cde	9181	ghi
8SS-1	Compost - unsterile	221	4.88	abcde	2.2	abcd	4683	cdefgh
8SS-2	Compost - unsterile	251	4.60	abcde	2.2	abcd	9255	ghi

Means in columns with the same subscript are not significantly different from one another ($P<0.05$).

Table 30. Proportion of bacterial isolates originating from three soils amended with 1 % chitin, 33% mill ash or 1% molasses, able to reduce the number of nematodes below the untreated control and increase banana dry shoot weight above the untreated control.

Treatment	Nematode suppression below unsterile potting mix (%)	Dry weight improvement greater than unsterile potting mix (%)
Farm B - chitin	42	57
Farm A - ash	50	57
Farm B - ash	50	37
Compost - molasses	62	62
Compost - chitin	62	75
Farm A - chitin	50	50
Compost - ash	40	70
Compost - unsterile	30	30

Twelve bacterial isolates were selected for re-screening for banana growth promotion and nematode suppression. Three isolates were selected from the first screening, four isolates from the second screening trial and five isolates from the third screening trial. Four bacteria originated from the compost amended with 1% chitin. There was also one isolate, 6SS-16 that originated from the Farm A soil amended with chitin. The success of bacterial isolates selected from chitin amended soil suggested that chitinase activity may play an important role in suppressing nematodes in the roots of banana plants. Also, nine of the organisms re-screened originated from surface sterilise roots, which suggested that these organisms were endophytic and able to colonise the root tissue. Therefore, organisms that are most likely to be successful at reducing burrowing nematode numbers in the roots of banana plants would be chitinolyic endophytes. Nematode egg shells have been estimated to be composed of up to 30 % chitin (Bird and Bird 1998). The female burrowing nematode lay their eggs within infected root tissue, with an average of four to five eggs per day (Gowen and Queneherve 1990). Therefore, it is likely that chitinolytic endophytic microorganisms may be affecting the hatching of eggs amongst other modes of action such as induced systemic resistance.

In the re-screening of the bacterial isolates, three isolates 2SS- 10, 4-1 and 5SS-6 were able to significantly reduce the number of nematodes on the roots relative to the untreated plants (Table 31). Two of the bacterial isolates, 4-1 and 5SS-6 originated from compost treatments.

There was no significant difference in plant growth parameters relative to the untreated plants. However, due to the significant nematode suppression, isolates 2SS-10, 4-1 and 5SS-6 warrant further screening in field trials. These three bacterial isolates have shown consistency in reducing nematode numbers in the roots and need to be tested in field trials for their ability to reduce nematodes and persistence on the roots of banana plants.

Table 31. Screening of bacterial isolates with the most potential to reduce nematode damage in the roots of bananas

Isolate	Origin	Leaf area (cm^2)	Shoot dry weight (g)	Root health (0-10)	Nematodes in 100 g of root
Untreated		159	3.72	2.6	4145 d
83		153	4.81	2.6	4104 d
2SS-10	Farm A -ash	155	3.40	2.0	507 ab
4-1	Compost -molasses	134	3.44	2.7	644 abc
4SS-4	Compost -molasses	153	3.97	3.0	2863 cd
4SS-9	Compost -molasses	176	4.96	3.1	1032 abcd
5-3	Compost - chitin	200	4.37	2.1	1977 bcd
5SS-4	Compost - chitin	156	4.11	2.8	2591 bcd
5SS-6	Compost - chitin	190	4.52	2.0	272 a
5SS-8	Compost - chitin	171	4.75	2.2	2344 bcd
6SS-16	Farm A- chitin	169	4.10	2.0	981 abcd
7SS-7	Compost - ash	172	4.32	2.0	1651 bcd
8-1	Compost- unsterile	152	3.74	2.7	1494 bcd
8SS-3	Compost- unsterile	179	4.11	2.9	1118 abcd

Means in columns with the same subscript are not significantly different from one another ($P<0.05$).

Fungal endophytes

All treatments that contained sterile sorghum were able to increase the growth of the banana plants compared to the growth of the plants that were untreatment (Table 32). However, only the A3 treatment produced significantly better plant growth than the sterile sorghum (Table 32). There was no significant control of nematodes by any of the treatments.

Table 32. Effect of fungal isolates on banana plants and control of nematodes 10 weeks after inoculation with nematodes.

Treatment	Top weight	Shoot:root ratio	Plant height (cm)	*R. similis* per 100g roots
Untreated	50.48 a	0.456 a	10.00 a	448 a
Sterile sorghum	89.07 b	0.939 b	17.50 b	1004 a
A3	114.78 c	1.264 c	19.25 c	969 a
AM6	103.80 bc	1.169 bc	17.37 b	152 a
AM7	108.05 bc	1.216 bc	17.87 bc	895 a

Means in columns followed by the same letter are not significantly different ($P<0.05$)

Treatments containing sterile sorghum were able to suppress the number of nematodes, relative to the untreated soil, when nematodes were added to the soil three and six weeks after bananas were planted (Table 33). However, only plants with A3 in the soil and inoculated with nematodes after six weeks were able to suppress the number nematodes in the roots (Table 33). This suggested that the addition of the organic matter was having an effect on nematodes in the soil but once the A3 fungus was established on the roots of banana plants, which took between three and six weeks, the fungus was able to suppress nematodes with in the root system.

Table 33. *Radopholus similis* in 100g of banana roots and 200 g of soil extracted 1 week after inoculation with nematodes, 3 and 6 weeks after inoculation with A3.

Treatment	Week 3		Week 6	
	Roots	Soil	Roots	Soil
A3	170 a	37 a	9 a	40 a
sterile sorghum	566 a	57 a	52 b	7 a
nil	395 a	128 b	82 b	217 b

Means are back transformed from an ln (x+1) transformation. Means in columns followed by the same subscript are not significantly different ($P<0.05$).

Combination experiment

Fusarium oxysporum (A3) was the only isolate to significantly reduce the number of nematodes recovered from the roots of banana plants. No other combinations of treatments reduced the number of nematodes recovered relative to the untreated control (Table 34).

Many of the treatments increased the plant growth but this could be the effect of the organic content of sterile sorghum on which the A3 isolate was grown. The addition of sterile sorghum may have also increased the water holding capacity of the UC soil mix, which promoted better plant growth. Treatments that did not contain sterile sorghum did not have increased plant growth.

All treatments containing sterile sorghum were able to significantly increase the growth of the banana plants relative to the untreated control.

The addition of A3 as an amendment to potting mix of tissue culture bananas requires further investigation as it has consistently reduce nematode numbers in the roots. However, at least six weeks is required before challenging the plant with nematodes. This allows the fungus to colonise the roots and possibly activate plant defence mechanisms.

The fluorescent pseudomonad isolate 83 was found to have no effect on nematode suppression or growth promotion. This was consistent with comparisons of the bacterial isolate in screening of bacterial endophytes from suppressive soil. The fluorescent pseudomonad 83 was found to have an inconsistent affect on plant growth and nematode suppression (Tables 27, 28, 29 and 31).

Table 34. Growth of banana plants and recovery of *Radopholus similis* recovered from the roots of banana plants 10 weeks after treating the soil with combinations of nematode trapping fungi (NTF), bacteria (83) and an isolate (A3) of non-pathogenic *Fusarium oxysporum*

Treatment	Nematodes in 100 g of roots	Root wt (g)	Height (cm)	Leaf area (cm^2)	Top wt (g)
Untreated	1987 b	66.10 ab	17.40 b	167 ab	49.20 b
Sterile sorghum	1215 b	77.26 bc	25.50 e	423 c	108.26 c
A3	257 a	90.12 cd	24.00 de	398 c	115.42 c
83	3550 b	53.60 a	15.60 ab	127 a	35.28 a
NTF	1973 b	65.74 ab	16.80 b	195 b	49.66 b
NTF, A3	901 ab	86.10 cd	24.20 de	418 c	104.70 c
NTF, 83	3244 b	60.22 ab	14.20 a	133 a	36.80 ab
83, A3	1394 b	99.76 d	20.70 c	380 c	115.68 c
NTF, A3, 83	1393 b	92.34 cd	22.50 cd	350 c	111.70 c

These values are log means ln(x+1) with back transformed means in parenthesis. Means in columns followed by the same subscript are not significantly different ($P<0.05$)

Conclusion

Endophytic microorganisms are able to significantly suppress burrowing nematodes when added to bananas. However, a rigorous screening process is necessary for consistent results. Soils containing potential nematode antagonists may have their antagonistic properties enhanced by the addition of amendments such as chitin. Endophytic bacteria which derived from chitin amended soils most consistently enhanced the growth of tissue culture banana plants and suppressed burrowing nematodes.

The endophytic *Fusarium oxysporum* isolate A3, was able to reduce the penetration of burrowing nematodes into the roots of banana plants. The fungal isolate gave better suppression of nematodes when added by itself. However, sterile sorghum, the media on which the fungus was cultured, also had growth promoting properties.

Field trials of three bacterial isolates, 2SS-10, 4-1 and 5SS-6 and the fungal endophyte A3 are warranted. These four microorganisms most consistently promoted the growth of tissue culture banana plants and may suppress burrowing nematodes when bananas are planted in the field.

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4. Resistance of banana cultivars

Introduction

Radopholus similis, the burrowing nematode, is the most important plant-parasitic nematode on bananas (Gowen and Queneherve, 1990). The nematode feeds on the cortical cells of plant roots resulting in lesions that reduce the function of the root system. *R. similis* is found throughout most banana producing areas in the world (Gowen and Queneherve, 1990). The reddish-brown lesions reduce the plant's ability to take up nutrients. Plants appear stunted and the bunching pseudostems are more likely to topple. Nematode damage leads to lower bunch weights and longer intervals between bunch harvests. *R. similis* is endemic in the tropical Queensland production area and commonly found in the subtropical banana producing areas of Queensland and New South Wales (Schipke and Ramsey, 1994).

Cavendish type (AAA group) bananas, mostly cv. Williams with some mons Mari and Grande Naine, are the most widely cultivated in Australia and are very susceptible to *R. similis* (Stanton 1999). Ladyfinger (AAB), are the next most important after Cavendish and are more common in the subtropics of south-east Queensland and northern NSW. Ladyfinger have reported partial resistance to *R. similis* (Stanton, 1999).

The development of nematode-resistant banana cultivars is extremely difficult because of the genetic complexity of the crop, its low fertility and the long periods required for the evaluation of progeny (Pinochet, 1988). Pisang jari buaya is a diploid AA, with confirmed resistance to *R. similis* (De Waele and Elsen, 2002; Elsen, *et al.* 2002). Pisang jari buaya has been utiliised in the Fundación Hondureña de Investigación Agrícola (FHIA) breeding program which has resulted in the *R. similis* resistant diploid AA hybrid, SH-3142 (De Waele and Elsen, 2002). SH-3142 has been crossed with the triploid AAB cultivar Prata Aña to produce the tetraploid AAAB hybrid FHIA-01 (Goldfinger) (Rowe and Rosales, 1993), which was partially resistant to *R. similis* when 28 week old plants were tested (Stanton, 1999; De Waele and Elsen,2002). The genetic resistance to *R. similis* in Pisang jari buaya is controlled by one or more dominant genes (De Waele and Elsen, 2002).

Yangambi Km5 is another confirmed source of resistance to burrowing nematode (De Waele and Elsen, 2002; Nguyet, *et al.* 2002; Sarah, *et al.* 1992) and lesion nematode (van den Berg, *et al.* 2000; van den Berg, *et al.* 2002). This variety is not currently used in *Musa* breeding because all progenies have so far produced abnormal leaves and erect bunches (De Waele and Elsen, 2002). Resistance to burrowing nematode has also been found in the *Musa balbisiana* group but these types are also not currently exploited (De Waele and Elsen, 2002)

Resistance of the banana plant to burrowing nematode appears to be a function of both reduction in the amount of root necrosis and inhibition of the nematode multiplication (Nguyet, *et al.* 2002). The production of phenols, condensed tannins and flavan-3,4-diols have been linked with resistance of *Musa* varieties to burrowing nematode (Collingborn, *et al.* 2000; Valette, *et al.* 1995).

A wide diversity of *R. similis* has been reported throughout the world in tail shape, optimum temperature, multiplication rate and pathogenicity (Fallas, *et al.* 1995; Fogain and Gowen, 1994) and RAPD analysis (Hahn, *et al.* 1993; (Elbadri, *et al.* 2002). Furthermore, Elbadri, *et al.* (2002), found two distinct groups of *R. similis* with differing pathogenicity on banana cv. Grande Naine. This suggests that pathotypes of the nematode may exist. The investigation of possible pathotypes has led to the description of a new species of *Radopholus, R. musicola* from Darwin (Stanton, *et al.* 2001). If pathotypes or new species exist in the Australian population of *R. similis,* it will become more difficult to recommend resistant banana cultivars and rotations to control this nematode.

It was the aim of this study to determine if there were any differences in the resistance of banana varieties to endoparasitic nematodes isolated from the roots of banana plants grown in Australia.

Materials and methods

Nematodes isolated and cultured from four sites.

Isolates of burrowing nematode (*Radopholus* spp.) and lesion nematode (*Pratylenchus goodeyi*) from throughout the major banana growing areas of Australia were sampled from the roots of separate banana crops (Table 35). There was one isolate of *R. similis* from north Queensland (Tully), one isolate from south-east Queensland (Pimpama) and also an isolate of *P. goodeyi* from south-east Queensland (Tallebudgera). Additionally, there was an isolate of *R. musicola* from the Northern Territory (Darwin).

Table 35. Nematode species and location where isolates were originally sampled.

Species	Location	Longitude (°E)	Latitude (°S)
R. musicola	Darwin, Northern Territory	130.84006	12.46105
R. similis	Tully, north Queensland	145.92172	17.93107
R. similis	Pimpama, south-east Queensland	153.29891	27.81265
P. goodeyi	Tallebudgera, south-east Queensland	153.41764	28.15987

The roots were washed thoroughly and placed in a misting chamber for several days to collect the nematodes (Hooper, 1986). After sieving, the nematodes were picked from the solution under the microscope and a single mature female placed on a sterile carrot. After the single nematode had reproduced, inoculum of up to 20 females for each carrot was washed repeatedly in sterile water and used to inoculate further carrots. Each month all nematode isolates were renewed onto new carrots and maintained at 26°C in monaxenic cultures (Moody *et al.* 1973).

Experiment 1 – Pisang jari buaya progeny resistance to *R. similis*.

Tissue culture plants of the banana varieties of Goldfinger, Ladyfinger, SH-3142 and Williams were tested with the nematode populations from Pimpama (SEQ) and Tully (NQ).

Thirteen weeks after deflasking, the banana plants were repotted into 175 mm pot of standard UC mix. A total of 8 months after deflasking the pots were inoculated with 300 nematodes per pot. Treatments were replicated five times and pots were kept in a glasshouse between 16 and 30 °C. Ten weeks after inoculation the plants were harvested and the nematodes were extracted from the roots over 7 days in a misting chamber and counted (Hooper, 1986).

Experiment 2 – In vitro banana cultivar resistance to *Radopholus* spp.

Tissue culture plants of the banana cultivars Pisang Ceylan, Cachaco Enano, Yangambi Km5, FHIA-25, FHIA-18, Saba, Pisang Mas, and Williams were tested with the nematode populations from Pimpama (SEQ), Tully (NQ) and Darwin (NT)

Plants were grown and nematodes extracted as described in Experiment 1.

Experiment 3 – Hybrid resistance to Radopholus spp.

Tissue culture plants of the banana varieties FHIA-03, TMB 5295-1, Yangambi Km5 and Williams were tested with the nematode populations from Pimpama (SEQ) and Tully (NQ) and Darwin (NT)

Plants were grown and nematodes extracted as described in Experiment 1.

Experiment 4 – Resistance of banana varieties grown from vegetative material

Suckers from 20 different banana varieties (Table 36) were removed from parent plants grown in a variety collection at South Johnstone Research Station (145° 42′ E and 17° 31′ S). The suckers collected weighed between one and two kilograms. The vegetative planting material was pared to remove all roots and any symptoms of burrowing nematode (*R. similis*) and banana weevil borer (*Cosmopolites sordidus*) damage. Planting material was then dipped into a solution containing 10 mL of Nemacur 400® in 10 L of water for 10 minutes and then allowed to dry for one day before being planted into 10 L plastic bags filled with river sand.

The banana varieties were allowed to grow for four weeks and then inoculated with 1000 motile burrowing nematode (*R. similis*). The plants were grown on raised benches outdoors and received additional irrigation daily. The banana varieties were fertilised with 5 g of Osmocote mini (Scotts International, Herleen, The Netherlands) on three occasions. All varieties were replicated five times.

The plants were harvested 24 weeks after inoculating with nematodes. Variability in growth occurred due to the differences in the size and vigour of the vegetative material pieces and subsequently shoot weights were not recorded. The roots were removed from the corms and cut into 2 cm pieces and the nematodes extracted from the roots, by placing in a misting chamber for 7 days (Hooper, 1986).

Experiment 5 - Resistance of banana varieties to *Pratylenchus goodeyi*

Tissue culture plants of the banana varieties Bluggoe, Calcutta 4, FHIA-18, Goldfinger (NSW selection), Gros Michel, Improved Ladyfinger, IRFA 909, IRFA 910, IRFA 914, *Musa balbisiana*, Pisang jari buaya, Saba, SH-3142, Yangambi Km 5 and Williams were tested with *Pratylenchus goodeyi.*

Thirteen weeks after deflasking, the banana plants were repotted into 175 mm pot of standard UC mix. A total of 26 weeks after deflasking the pots were inoculated with 250 nematodes per pot. Treatments were replicated five times and pots were kept in a glasshouse at 21° C. One year after inoculation nematodes were extracted from the roots over 7 days in a misting chamber and counted.

Statistics

The data were analysed using Genstat. All nematode counts data were transformed using ln (x+1) to allow data to be normally distributed before being subject to analysis of variance (ANOVA). If a statistical difference ($P \leq 0.05$) was observed, means of treatments were separated using the least significant difference (LSD) method. Means are presented as back transformed (e^x-1) values.

Results and discussion

Yangambi Km5 was able to significantly reduce the recovery of all *Radopholus* spp. isolates relative to Williams in Experiments 2, 3 and 4 (Table 36). This suggested that resistance of Yangambi Km5 is consistent whether the plants are grown using *in vitro* techniques or using vegetative planting material and confirms reports from other trials (De Waele and Elsen, 2002).

The hybrid FHIA-25 was significantly more resistant than Williams, in Experiment 2 and 4 (Table 36). FHIA-25 was the only hybrid that demonstrated consistent resistance to *Radopholus* spp. isolates whether cultivated from *in vitro* or vegetative planting material. *M. balbisiana* and Paka had similar resistance to *R. similis* as Yangambi KM5 in Experiment 5 (Table 36). However, these cultivars were only tested for resistance in one experiment. (De Waele and Elsen, 2002) suggested that *M. balbisiana* was as resistant as Yangambi Km5 to *R. similis*.

The *Musa* cultivar Saba had partial resistance with significantly fewer nematodes on the roots when inoculated with the Tully isolate of *R. similis* in Experiment 2 and 4 (Table 36). However, Saba was not resistant to the Pimpama or Darwin isolates of *Radopholus* spp. (Table 36). Similarly in Experiment 1 the banana cultivar SH-3142 and Ladyfinger significantly reduced the recovery of nematodes from the Pimpama isolate of *R. similis* relative to Williams (Table 36). However, SH-3142 and Ladyfinger were unable to significantly reduce the recovery of nematodes from the Tully isolate relative to Williams (Table 36). These result highlight the need to test *Musa* cultivars against a range of *Radopholus* spp. isolates as the resistance reaction to the plants is not consistent. The differences in reaction appear to be linked to genetic differences in the *Radopholus* spp. isolates and suggested the existence of biotypes within *R. similis*.

Pisang ceylan was significantly more resistant than Williams to *Radopholus* spp. isolates when grown from *in vitro* plants (Table 36). However, when grown from vegetative planting material Pisang ceylan was not significantly more resistant *R. similis* than Williams, even though it had one third of the nematodes recovered from the roots (Table 36). Similarly, Pisang mas was resistant to the Tully and Pimpama isolates of *R. similis* when tested on *in vitro* plants, but was not resistant to the Tully isolate when inoculated on vegetative planting material. The differences in resistance reaction of planting material of the same cultivar highlight the need to test different types of planting material for resistance screening. The results obtained from pot trials should be used to guide field evaluations of the more resistant cultivars.

There was no significant difference in the recovery of *R. similis*. isolates from Pimpama and Tully on both Goldfinger and Williams, which suggested that Goldfinger was as susceptible to *R. similis* isolates as Williams. Goldfinger was also found to be as susceptible to *R. similis* in both *in vitro* and vegetative planting material. This is in contrast with results that have described Goldfinger plants being more resistant to burrowing nematode when at least 28 weeks old (Stanton, 1999).

Similarly, Pisang jari buaya grown from vegetative planting material was not resistant to the Tully isolate of *R. similis* (Table 36). This is in contrast to reports that Pisang jari buaya is a confirmed source of resistance (De Waele and Elsen, 2002) and agrees with observations made in other screening trials of *Radopholus* spp. isolates (Cobon and Pattison, 2003).

Williams was found to be the most susceptible cultivar to *Radopholus* spp. isolates. However, the majority of cultivars were found to have similar susceptibility, which included many hybrid lines developed with partial resistance to *R. similis* (De Waele and Elsen, 2002). This highlights the need for regional screening of *Musa* germplasm that is being developed by worldwide breeding projects and the need test against different isolates of *Radopholus* spp.

There was no significant difference in the number of *P. goodeyi* recovered from the roots of the fifteen banana varieties (Table 37). Yangambi KM5 was reported to have some resistance to *P. goodeyi* (Fogain and Gowen, 1998; Pinochet, *et al.* 1998). However, in this trial Yangambi KM5 was found to be as susceptible as Williams to *P. goodeyi* (Table 37). *M. balbisiana* had the lowest number of *P. goodeyi* recovered from the roots (Table 37). This was consistent with the resistance of *M. balbisiana* to *R. similis* grown from vegetative planting material observed in Experiment 4 (Table 36).

Pisang jari buaya and its progeny, SH-3142 and Goldfinger, were not resistant to *P. goodeyi*. Care would be needed if using Pisang jari buaya as the source of resistance to burrowing nematode, as in mixed populations of endoparasitic nematodes *P. goodeyi* would not be controlled by cultivar selection based on this cultivar.

Table 36. Recovery of isolates of *R. similis* from Tully and Pimpama and one isolate of *R. musicola* from Darwin from the roots of banana cultivars in four separate experiments.

Cultivar	Genotype	*Radopholus* spp. in 100 g of root								
		Experiment 1		Experiment 2			Experiment 3			Experiment 4
		Tully	Pimpama	Pimpama	Tully	Darwin	*Pimpama*	Tully	Darwin	*Tully*
Williams	AAA	730 a	1479 c	763 bc	1295 d	1092 cd	3483 b	646 b	265 a	115 e
SH-3142	AA	243 a	7 a							
Ladyfinger	AAB	931 a	224 b							52 de
Goldfinger	AAAB	1245 a	530 bc							50 de
Yangambi KM5	AAA			104 a	325 abc	282 a	102 a	99 a	63 a	2 a
Pisang Ceylan	AAB			188 ab	300 abc	319 a				36 de
FHIA-25	AAA			311 ab	124 a	371 ab				15 abcd
Pisang Mas	AA			395 ab	256 ab	993 bcd				44 de
FHIA-18	AAAB			712 bc	420 bc	482 abc				31 de
Saba	ABB			801 bc	492 bcd	929 bcd				4 abc
Cachaco enano	ABB			2501 c	791 cd	1624 d				
TMB 5295-1	AAAB						775 b	831 b	34 a	
FHIA-03	AABB						1754 b	783 b	34 a	57 de
M. balbisiana	BB									2 ab
Paka	ABB									3 abc
Ney Poovan	AB									14 abcd
FHIA-23	AAAA									20 bcde
Bluggoe	ABB									27 cde
Calcutta 4	AA									35 de
Grande naine	AAA									38 de
Pisang lemak mas	AAB									47 de
Selangor	AA									52 de
Pisang jari buaya	AA									79 de

Numbers are the means of 5 replicates back transformed from ln(x+1). Means in columns with the same subscript are not significantly different *($P<0.05$).*

Table 37. Recovery of *P. goodeyi* from the roots of 15 banana cultivars 52 weeks after inoculation.

Variety	*P. goodeyi* in 100g of roots
Musa balbisiana	885
Saba	1115
FHIA-18	1177
IRFA 910	1241
Yangambi Km 5	1269
IRFA 909	1328
Calcutta 4	1436
Gros Michel	1592
SH-3142	1614
Improved Ladyfinger	1696
Pisang jari buaya	1777
IRFA 914	2111
Williams	2190
Bluggoe	2298
Goldfinger NSW selection	2853

Numbers are the means of 5 replicates back transformed from ln(x+1). Means in the columns are not significantly different from one another *($P<0.05$)*.

Conclusion

There was very little resistance to endoparsitic nematodes amongst the banana cultivars in collection in Australia. Yangambi Km5 demonstrated the most consistent resistance, with lower number of *Radopholus* spp. compared to Williams.

M. balbisiana was also resistant to the Tully isolate of *R. similis* and had the lowest number of *P. goodeyi* recovered from the roots. However, there were no significant sources of resistance to *P. goodeyi* among the 15 *Musa* cultivars screened.

The different reaction of the isolates of *Radopholus* spp. means that testing for resistance in *Musa*, more than one isolate may be needed to determine the durability of the resistance genes among *Musa* germplasm.

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5. Alternative methods of treating banana planting material

Introduction

Burrowing nematode (*Radopholus similis*) is the most widespread and important nematode of bananas world wide (Gowen and Queneherve, 1990). It damages the root system of bananas, causing destruction of the primary root, disrupting anchorage and severe root damage causes the plant to topple (Gowen and Queneherve, 1990). Vegetative planting material, taken from growing banana plants, is the most common method of dispersal of burrowing nematode (Blake, 1961; Blake, 1963).

Vegetative planting material can be treated to reduce burrowing nematode before planting. The corm of the banana plant is cut into bits (Figure 5), which are usually directly planted into the field. The currently registered method in Australia for disinfesting bits of burrowing nematodes is dipping in a solution containing the equivalent of 1 $ml.L^{-1}$ of Nemacur 400® (400 g fenamiphos L^{-1}). Blake (1961) tested a number of chemical nematicides to reduce the infestation of nematodes in vegetative planting material without success. However, Broadley (1979) using organophoshate and oxime carbamate nematicides was able to achieve good nematode control with light paring and dipping. Broadley (1979) found that 600 ppm of ethoprophos for 20 minutes, 250 ppm of fenamiphos for 10 minutes and 1000 ppm of oxamyl for 20 minutes were able to reduce the number of burrowing nematode in planting material. Nematicidal solutions, while able to reduce nematode numbers in vegetative planting material, are hazardous to operators and require stringent safety precautions (Broadley, 1979)

Blake (1961) demonstrated that by immersing vegetative planting material in hot water at 55°C for 20 minutes and thorough paring could remove the incidence of symptoms of nematodes when plants were three months old. Although hot water treatment are considered superior to nematicidal dips, the technique is quite difficult due to the critical balance required between a temperature that is lethal to nematodes in the corm tissue and one that causes permanent damage to the plant (Gowen and Queneherve, 1990). An alternative method of reducing nematodes in planting material uses 1 % sodium hypochlorite solution for 5 minutes (Lordello, *et al.* 1994). Using low temperatures, (Fallas and Sarah, 1994) found that *R. similis* was unable to reproduce at 15°C and that the initial population disappeared after 135 to 260 days of incubation. (Holdeman, 1986) reported that very low temperatures were able to eliminate burrowing nematode without killing the banana plant.

The aim of the trials reported was to investigate alternative methods of dipping to control burrowing nematode in vegetative banana planting material.

Material and methods

Bleach treatment

Trial 1

Corms from banana plants (*Musa* AAA Cavendish subgroup cv. Williams) infested with burrowing nematode were divided into bits, which were washed free of any adhering soil and trimmed to remove roots and some surface tissue. The trimmed bits weighed between 500 g and 1.0 kg (Figure 1).

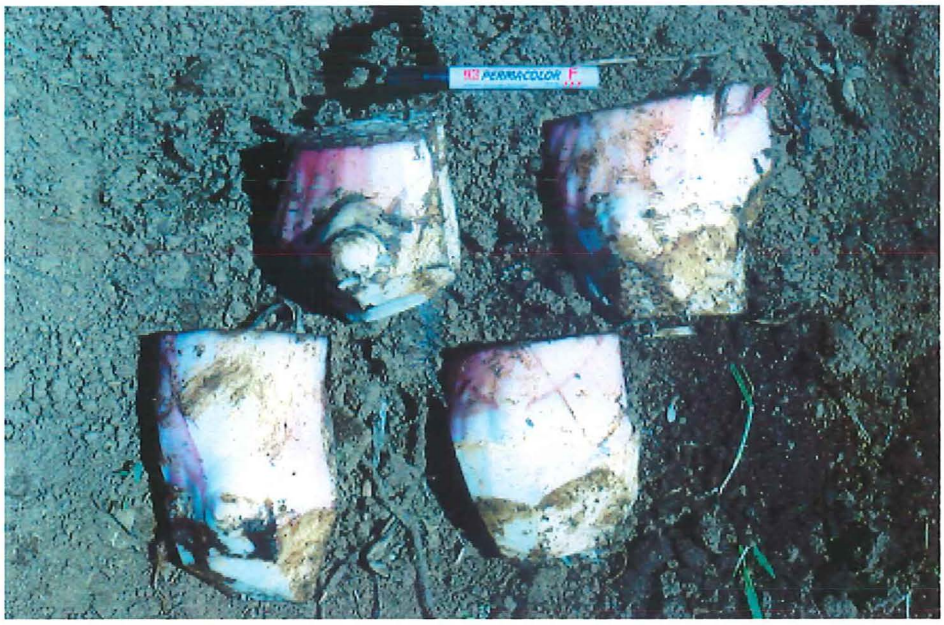

Figure 5. Banana bits washed and trimmed for use as vegetative planting material.

After trimming 10 bits were thoroughly immersed in either a 0.5, 1.0 or 2.0 % sodium hypochlorite solution or a water control for 10 minutes. Each bit was planted into a 10 L polyethylene bag and filled with pasteurised river sand and allowed to grow for 8 weeks. Plants were harvested; the height, area of the last fully emerged leaf and the shoot dry weight were determined. The sand was then washed from the roots and corm and the appearance of the root system was given a rating on the appearance of lesions and necrotic tissue; 0 for healthy tissue and 10 for a dead root system (Netscher and Sikora 1990). The roots were then cut off the surface of the banana corm and divided into 20 mm lengths. Nematodes were extracted from the roots using a misting technique for seven days before being quantified under a compound microscope (Hooper, 1986).

Trial 2

Corms from banana plants (*Musa* AAA Cavendish subgroup cv. Williams) infested with burrowing nematode were divided into bits, washed free of any adhering soil and trimmed to remove roots and some surface tissue. The trimmed bits weighed between 500 g and 1.0 kg.

After trimming, the bits were then thoroughly immersed in a 2 % sodium hypochlorite solution for 10 minutes, in hot water at 55°C for 20 minutes, a solution of 1 mL of Nemacur 400® (400 g fenamiphos.L^{-1}) in a litre of water for 10 minutes or a water control. Each treatment was replicated eight times. The bits were then placed into 10 L polyethylene bag and filled with pasteurised river sand and allowed to grow for 12 weeks. The plants were harvested and nematodes extracted as described in trial 1.

Temperature treatment

Trial 3.

Corms from banana plants (*Musa* AAA Cavendish subgroup cv. Williams) infested with burrowing nematode were divided into bits, which were washed free of any adhering soil and trimmed to remove roots and some surface tissue. The trimmed bits weighed between 500 g and 1.0 kg.

Bits were placed in a multi-temperature incubator with temperature data loggers placed in pseudostem of the planting bits recording the temperature every 10 minutes. The pieces achieved the desired temperatures 5, 8 12 and 22 (+/- 0.5)°C after 12 hours and remained at that temperature for a further 48 hours. Each treatment was replicated 10 times.

Bits were then potted into 10 L polyethylene bag, filled with pasteurised river sand and allowed to grow in the glasshouse at ambient temperature, which ranged from 12 to 32 °C for 50 days before being harvested. At harvest plant height, size of the last fully emerged leaf and shoot dry weight were determined. The soil was washed away from the roots. The roots were scored on the appearance of lesions (Netscher and Sikora, 1990), cut into 20 mm lengths and the nematodes extracted using the misting technique (Hooper, 1986).

Trial 4

Corms from banana plants (*Musa* AAA Cavendish subgroup cv. Williams) infested with burrowing nematode were divided into bits, washed free of any adhering soil and trimmed to remove roots and some surface tissue. The trimmed bits weighed between 500 g and 1.0 kg.

Bits were placed in temperature incubators or left at room temperature with temperature data loggers placed in the pseudostem of the planting bits recording the temperature every 10 minutes. After 2, 5, 7 and 14 days, seven bits were selected at random from the incubator and each bit was planted into a 10 L polyethylene bag and filled with pasteurised river sand. The plants were harvested 12 to 14 weeks after planting. At harvest, plant height, size of the last fully emerged leaf and shoot dry weight were determined. The soil was washed away from the roots. The roots were scored on the appearance of lesions (Netscher and Sikora, 1990), cut into 20 mm lengths and the nematodes extracted using the misting technique (Hooper, 1986).

Oxamyl dipping

Trial 5

Corms from banana plants (*Musa* AAA Cavendish subgroup cv. Williams) infested with burrowing nematode were divided into bits, washed free of any adhering soil and trimmed to remove roots and some surface tissue. The trimmed bits weighed between 500 g and 1.0 kg.

The bits were then immersed in a solution of 1 ml of Nemacur 400® (400 g fenamiphos.L^{-1}) in a litre of water for 10 minutes, 2 mL of Vydate 240L® (240 g oxamyl.L^{-1}) in a litre of water for 10 or 20 minutes or 5 mL of Vydate 240L® (240 g oxamyl.L^{-1}) in a litre of water for 10 or 20 minutes and compared to an untreated control. Following treatment the plants were allowed to air dry for two hours before being planted into 10 L polyethylene bag and filled with pasteurised river sand and allowed to grow for 8 weeks. The plants were harvested and the height, area of the last fully emerged leaf and the shoot dry weight were determined. The sand was washed from the roots and corm and then roots cut from the surface of the banana corm into 20 mm lengths. Nematodes were extracted from the roots using a misting technique for seven days before being quantified under a compound microscope (Hooper, 1986).

Results and discussion

Bleach treatment

Trial 1

There were no significant differences amongst treatments in the reduction of nematodes or the growth of plants (Figure 6). There was a decrease in the number of nematodes with increasing concentration of bleach. However, as suggested by (Broadley 1979) the objective of treatment of planting material is to eradicate burrowing nematodes not merely to reduce its numbers. The bleach treatment failed to significantly reduce the nematode numbers let alone eradicate the nematodes.

There were no significant differences in plant growth between the treatments (data not shown). There was greater than 80 % germination in all treatments, indicating that the bleach treatment was not detrimental to plant growth.

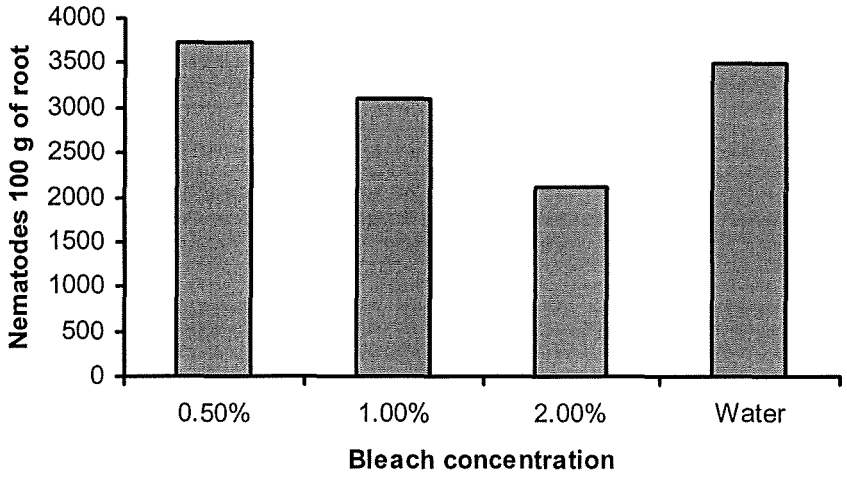

Figure 6. Effect of the dipping vegetative planting material into three different concentrations of bleach solution compared to a water control.
(There were no significant differences (P=0.05) between treatments.)

Trial 2

There was no significant difference in nematode control between the treatments (Table 38). Very low numbers of nematodes were recovered from all treatments including the untreated control. However, the germination of bits treated with hot water for 20 minutes was significantly reduced relative to the other treatments (Table 38). This result suggested that hot water treatment was not as safe for the growth of the plant compared to a bleach or Nemacur 400 dip®. There was no significant difference in the growth of plants after 12 weeks (data not shown).

Table 38. Effect of three bit treatments on the number of burrowing nematodes extracted from the roots and germination of banana bits

Treatment	Nematodes in 100 g of root	Germination (%)
Untreated	0.8 a	100 b
2 % NaOCl	0.4 a	88 b
Nemacur 400®	0.1 a	100 b
Hot Water	1.9 a	50 a

Means in columns with same subscript are not significantly different at the 5% level.

Dipping banana bits into a bleach solution failed to significantly reduce the number of nematodes and would not be considered an efficacious treatment of planting material for the banana industry. This in contrast to the findings of previous work that found that dipping banana bits into a 1 % sodium hypochlorite solution for 5 minutes significantly reduced nematodes in the planting material (Lordello, *et al.* 1994). However, the bits that were used by(Lordello, *et al.* 1994) weighed between 100 and 300 g, whereas the bits planted commercially and used in the trials reported were between 500 g and 1.0 kg. The increased mass of corm tissue may have resulted in a reduced efficacy of the bleach solution.

Temperature treatment

Trial 3

There was no significant difference in the number of nematodes or the growth of plants when the planting material was kept at the four different temperatures for 48 hours (Table 39). There was also no difference in the germination of the planting material or the weight or size of the plants after 50 days growth.

Table 39. Burrowing nematodes recovered from the roots of banana plants 8 weeks after the bits were treated at four different temperatures for 48 hours.

Temperature (°C)	Nematodes in 100 g of root
5.0	1110
8.0	591
12.0	3176
21.0	492

(There were no significant differences (P=0.05) between treatments)

Trial 4

The viability of the planting material was significantly affected by the interaction between the time that planting material was stored and the temperature it was stored at (Table 40). Bits stored at the low temperatures, 4 and 0°C, exhibited no germination after five days storage (Table 40). Germination of bits was unaffected by the storage time when maintained at 24°C (Table 40). Therefore, prolonged exposure of banana planting material to cool temperatures to prevent the reproduction of burrowing nematode is detrimental to the growth of the plant and would not be an efficient method of disinfesting planting material

There were no significant differences amongst the treatments in the reduction of nematodes due to the temperature treatment. An average of 1033, 453 and 1339 burrowing nematode were recovered 100 g of root of vegetative planting material maintained at 0, 4 and 24°C respectively. Due to the poor germination of planting material maintained at 4°C and the poor germination of planting material stored for longer than 5 days at 0° C cold temperature treatment is not a viable option for the treatment of banana planting material for disinfestation to burrowing nematode.

Table 40. Effects of temperature and storage time on the germination of banana bits.

Temperature (°C)	Storage time (days)	Germination (%)
0	2	71 cd
	5	43 bc
	7	0 a
	14	0 a
4	2	14 ab
	5	0 a
	7	0 a
	14	0 a
24	2	71 cd
	5	86 d
	7	100 d
	14	86 d

Means with same subscript are not significantly different at the 5% level.

Oxamyl dipping

Trial 5

Nemacur 400® was the only treatment to significantly reduce the number of nematodes in the roots of banana plants relative to the untreated control (Figure 7). However, though Nemacur 400® dip was able to significantly reduce the number of nematodes recovered from the roots it was unable to eliminate the nematodes from the planting material.

There was no significant reduction in nematode numbers when banana bits were immersed in any of the oxamyl (Vydate 240 L®) solutions for any length of time (Figure 7). Broadley (1979) found that immersion of banana bits for 20 minutes in a 1000 ppm solution of oxamyl was significantly reduced nematode numbers. The concentration of oxamyl in the highest treatment was equivalent to $1.2x10^6$ ppm for 20 minutes but failed to significantly reduce nematodes relative to the untreated control (Figure 7). The lack of reduction of nematodes in the planting material may be due to the bits not being as heavily parred as the planting material reported in (Broadley, 1979) trial.

There was no significant difference in the germination or the growth of banana plants after dipping in the nematicide solutions relative to the untreated control (data not shown).

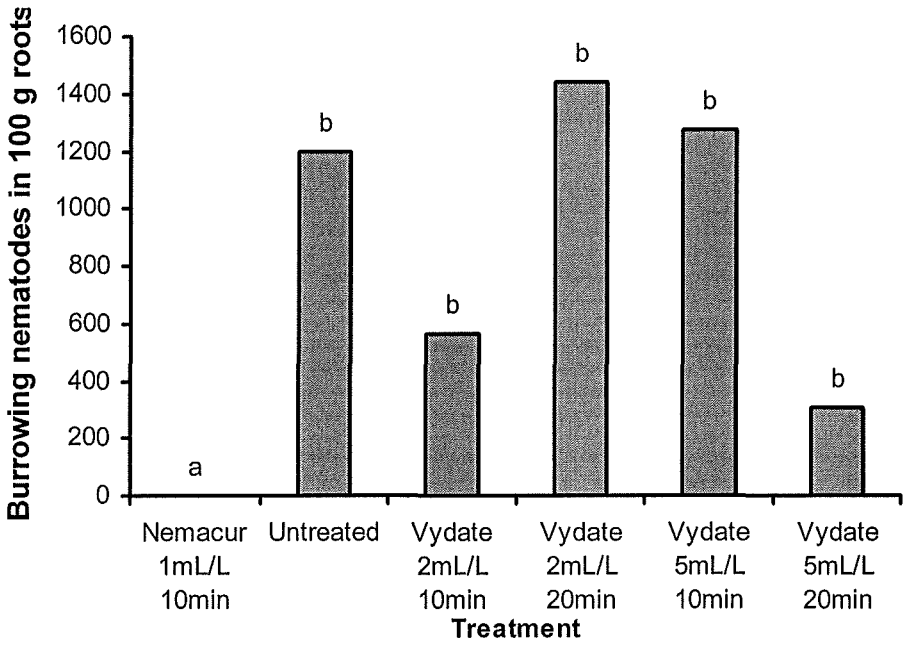

Figure 7. Burrowing nematodes recovered from the roots of banana bits treated with two different concentrations of Vydate 240 L for two time periods relative to Nemacur 400® and an untreated control. (Columns with the same letter above are not significantly different from one another at the 5 % level)

Conclusion

No alternative treatment to the currently recommended use of dipping banana bits in a solution of 1 ml of Nemacur 400® (400 g fenamiphos L^{-1}) in a litre of water for 10 minutes could be recommended from the five trials conducted. No treatment was able to completely eradicate burrowing nematode from the vegetative planting material including the use of Nemacur 400®. The use of a bleach solution, cold temperatures and oxamyl dips all failed to reduce the number of nematodes recovered from treated banana bits below the number of nematodes recovered from untreated bits. Prolonged storage of banana bits at cool temperatures, below 4 °C, was detrimental to the germination of the bits. Similarly, hot water treatment was also found to significantly reduce the germination of banana bits and failed to eradicate nematodes from the planting material.

Since dipping treatments have not been able to eradicate burrowing nematode from planting material selection and preparation of banana planting material is critically important in establishing banana paddocks without nematodes. Tissue culture plants are an alternative to corm derived planting material. Tissue culture plants reduce the risks of reintroduction or contamination of burrowing nematode into banana fields. Tissue culture plants can also be used to create nursery areas in nematodes free areas from which planting material can be taken.

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6. Technology transfer

Industry survey

A mail out survey was sent to over 1000 banana growers in sub-tropical and tropical banana production areas. A copy of the survey questionnaire is included in Appendix A. Banana growers were asked to respond to questions referring to;

1. Producer background,
2. Farm practices for managing nematodes and
3. Sources of information, advice and training.

The responses to the questions were compared to a survey of tropical banana growers conducted in 1994 (Stanton, 1997) and sub-tropical banana growers in 1997 (Stanton and Pattison, 2000), which presented similar questions about farm practices for nematode management. The aim of comparing the surveys was to determine how industry funded projects on nematode management had influenced the knowledge and the current management practices of Australian banana growers.

Producer background

A total of 132 survey sheets were returned. 75% of the respondents were from north Queensland, north of Cardwell. The area of land under cultivation from the respondents in north Queensland was 2,934 Ha and 123 Ha from south of Cardwell. There was a further 142 Ha that were not allocated to a production zone.

43% of the respondents considered that they had a nematode problem on bananas on their property. A further 43% did not consider nematodes as a problem and 14 % were unsure if they had a nematode problem.

In the 1997 sub-tropical survey 53% of growers considered that they had a problem with nematodes, 26% said that nematodes were not a problem and 20% were unaware if nematodes were a problem on their property. In the 1994 survey of the tropical banana industry 54% of respondents considered nematodes as being very important banana pests.

The funding of nematode projects in the banana industry may have increased the awareness of the nematode status of banana crops by growers on their properties.

Farm practices for managing nematodes.

Planting material

95% of respondents considered planting material was critically important or important in preventing the spread of nematode infestations on bananas. 87% of the respondents considered that they used clean planting material, so that 13% of the respondents either were not using clean planting material or did not know.

In previous surveys 55% of respondents in the sub-tropics and 75% in the tropics had adopted the use of clean planting material as a management practice for the reduction in plant-parasitic nematodes. This improvement may be attributed to the increased awareness raised in industry funded projects where planting material was shown to be the prime source of nematode contamination of banana fields.

Detailed information was sought from banana growers on the planting material practices they used to manage nematodes. 24% of the respondents were using tissue culture plants regularly as the preferred source of planting material (Table 41). Whereas 33% had used it once or twice and a further 42% were aware or considered using tissue culture plants (Table 41).

55% of the respondents were aware or considered using a dedicated nursery in clean ground started from tissue cultured plants. However, only 15% were regularly using nurseries started from tissue culture plants as the source of planting material. Whereas, 37% of respondents were using planting material derived from a nursery originating from bits and suckers. This type of nursery is more prone to contamination from nematodes and leaves the potential for reintroduction of plant-parasitic nematodes into previously uninfested or disinfested ground.

60% of the respondents used or were using regularly the dipping of bits and suckers into a Nemcur 400® solution. Although, the use of Nemcur 400® as a dip does not entirely eradicate burrowing nematode from the planting material (Chapter 5). In contrast only 15% of the respondents had used or were regularly using hot water dipping of bits and suckers.

The other method of respondents used to disinfest nematodes from planting material was the use of heavy paring to remove the roots and outer surface. This method had been used by 80% of the respondents and could have been used in conjunction with other disinfestation methods.

While the awareness among banana growers had increased regarding the importance of clean planting material there is a need for further education on the methods which achieve the cleanest planting material. The most recent survey probed the management methods used by banana growers, with most respondents believing that they were disinfesting their planting material. However, recent trial work has shown that some methods used do not ensure nematode free planting material.

Table 41. Planting material practices to reduce nematode problems used by banana growers (%).

Management practice	Not aware	Aware	Considered it	Used it once or twice	Using it regularly
Using tissue-cultured plants	1	28	14	33	24
Using a dedicated nursery in clean ground strated with tissue cultured plants	5	36	19	25	15
Using a dedicated nursery in clean ground using bits and suckers	1	29	15	18	37
Dipping bits and suckers in Nemcur 400®	5	28	7	20	40
Hot water dipping for bits and suckers	14	47	14	16	9
Heavy paring of bits and suckers (removal of roots and outer surface)	6	13	1	22	58

Chemical use

39% of the respondents had used a nematicide in the past 12 months. This in contrast with past surveys where 61% of the respondents had used a nematicide in the 1994 survey of the tropical banana industry and 42% in the sub-tropical banana industry in 1997. The reduction in the use of nematicides could be attributed to increased awareness of the nematode status of the banana crops due to industry funded projects. Furthermore, increased awareness of other methods of managing nematodes such as the use of clean planting material and fallows may have contributed to the reduced need for nematicides.

In the 2003 survey, Vydate® and Nemacur® were the two most popular products, with 33 and 28% of respondents, respectively, having used them in the past 12 months (Table 2). Terbufos products (Counter®, Hunter® and Terbuforce®), Rugby® and Mocap® were also used by 16, 11 and 2 % of respondents respectively. This is in contrast with the previous survey of the tropical banana industry where Nemacur® had been used by 70% of the banana industry (Table 42). There had been a reduction in the use of Rugby® in both the tropics and the sub-tropics. Mocap® was withdrawn from use by the manufacturers in 1999 and subsequently there was very little used in the 2003 survey.

Table 42. Nematicide use by respondents in the past 12 months (%).

Nematicide	Year of survey		
	1994 Tropical	1997 Sub-tropical	2003 Industry
Nemacur®	70	14	28
Rugby®	21	42	11
Counter®/Hunter®/Terbuforce®	n/a	1	16
Mocap®	16	26	2
Vydate®	16	44	33
Other			10

The reasons respondents had not used a nematicide in the past 12 months included concern about the effects on the soil and environment (20%), health risks to spray operators and workers (20%), chemicals were too costly and not cost effective (16%), no nematodes on new ground (15%) and very few nematodes or damage (15%).

There was no change in how banana growers decided to use nematicides. Approximately 40% of respondents applied nematicide routinely in the 1997 and 2003 surveys. Similarly, 20% in both surveys monitored the roots for nematode damage.

65% of respondents were aware of the root disease index method of monitoring for burrowing nematode damage. Of those aware of the root disease index method for checking for nematode damage, 69% had used it in some form, either regularly or occasionally as a plant toppled. This is in contrast with the survey in 1994 where only 31% had used the root disease index. The increased use of the root disease index method corresponds to an increase in the knowledge that banana growers have about the nematode status of their crops. However, only 30% of respondents using the root disease index method were keeping regular records for blocks and checking root disease index over time.

When asked about the practice of chemical rotation for agricultural chemicals, including nematicides 77% of respondent were aware of the practice. However, only 41% of those aware of the practices were currently rotating nematicides on their farm. Of those that were rotating chemicals, 32% were changing chemicals after one application of nematicide as recommended by current research findings. A further 34% were changing chemicals after two or three applications.

54% of respondents were aware of the term 'enhanced biodegradation' and of those that were aware of the term, 77% were able to select the correct definition for the term. This suggested that while banana growers were aware of enhanced biodegradation, most were not practicing proper chemical rotation to avoid the problem. Therefore, further extension on how to avoid enhanced biodegradation of soil applied nematicides is required to ensure the longevity of the chemicals.

When asked which method they used to apply nematicides, 24% of respondents were applying nematicide into the pseudostem of the banana plant by injection. The practice of pseudostem application of nematicides is suited only to liquid formulations and development has concentrated on one product. The lack of alternative products for this method of nematicide application may reduce chemical rotation opportunities for banana growers.

Use of fallows

87% of respondents were aware that a fallow period was able to reduce nematode populations in the soil. 63% of the respondents used a fallow period before replanting bananas in the past three years. This contrasts with 38% of the respondents in the 1997 sub-tropical survey and 81% in the 1994 tropical banana. However, the number of respondents using a fallow period was related to the length of time that respondents had been growing bananas. New growers to the industry would be unlikely to have the need to fallow ground to reduce nematode numbers.

33% of the respondents using a fallow were using a bare fallow before they planted bananas, which is the same proportion using a bare fallow in the 1994 survey of the tropical banana industry. The use of a bare fallow remains the most common fallow in the banana industry.

13 and 12% of respondents were using either a Brassica or Rhodes grass fallow respectively. The use of these two types of fallows is a result of development from nematology projects in conjunction with the extension project FR99009. Lindsay (2003) concluded that current use of a well-managed fallow such as, the use of Brassica and Rhodes grass represented a saving of $3,000,000 annually in reduced nematicide costs. The satisfaction of respondents using a fallow crop was high, with 80% of respondents not using a bare fallow saying they would continue to grow a fallow crop as a standard management practice for the management of burrowing nematode.

87% of respondents considered that freedom from weeds and banana regrowth were critically important or important for nematode control in the fallow. The majority (50%) of respondents were using cultivation to remove banana plants from the previous crop. However, 33% of the respondents were using a herbicide to help remove banana plants from the previous crop either as injection prior to cultivation (10%) or a spray after cultivation (23%). The use of herbicide to remove volunteer banana plants to reduce nematode carry over has been promoted in recent nematology and extension projects.

Soil amendments and organic matter management

Some type of soil amendment had been used by 76% of the respondents (Table 43). The majority had used mill mud (14%) or mill ash (12%) (Table 43).

Table 43. Use of pre-plant amendments prior to planting bananas

Product	Percentage used
No product applied	24
Mill ash	12
Mill mud (filter press)	14
Molasses	8
Rock dust	12
Compost	10
Other	20

When deciding which soil amendment to use, respondents ranked information provided by the DPI as the most important source when making the decision on what and how to use the amendment.

When asked about trash (leaf and stem material) management, 26% of respondents placed the trash in the interrow, 32% placed trash around the base of the plants and a further 31%

allowed leaf material to lay where it fell and placed harvested pseudostem into the interrow. The placement of trash and the use of organic amendments is seen as increasing importance with concerns about soil health management.

Sources of information, advice and training

29% of responding banana growers had attended a field day or field walk in the past three years. However, field days and farm walks were seen as important sources of information (Table 44). Industry newsletters and DPI staff were also seen as important for providing information on nematode management to banana growers. The internet was seen as the least important source of information on nematode management.

There have been 11 industry publications, 8 conference presentations, 4 newsletter articles and two scientific publications produced as an outcome of FR99011. There have also been four field days on nematode management issues for banana growers throughout Queensland.

Table 44. Importance of sources of information used by banana growers for the management of nematode on bananas.

Activity	Ranking (0=not important, 10=very important)
Field days / farm walks	7.4
Training activities or workshops	6.8
Industry seminars or meetings	6.1
The national banana industry conference	5.5
DPI staff	7.7
Consultants	5.9
Industry newsletters or bulletins	7.8
Other growers/managers	7.0
Agricultural retailers	5.6
Chemical/fertiliser company staff	5.6
The internet	4.2
Other	6.5

Conclusion

The survey of banana grower's practices over the past 9 years has revealed some important shifts in the way banana growers manage plant-parasitic nematodes and an increase in the knowledge of the nematode status of their crop. Only 14% of respondents were unsure of the nematode status of their crop in the 2003 survey. This compares with 20% of banana growers who were unsure of the nematode status of their crop in the 1997 survey. Respondents perceived the DPI and their publications as the most important source of information on nematode management in bananas.

The survey revealed a decrease in nematicide use since an initial survey in 1994. In the latest survey 39% of the respondents had used a nematicide in the past 12 months compared with 61% in the 1994 survey of the tropical banana industry and 42% in the sub-tropical industry in 1997. 77% of respondents were aware of the need to rotate chemicals, but only 32% were rotating correctly after each application.

95% of respondents were aware of the importance of clean planting material as a management practice to reduce nematode problems. However, most respondent were using practices that could allow nematodes to infest their crop via planting material, mostly by using nurseries derived from bits and pieces from corm material of older banana plants rather than tissue cultured plants.

87% of respondents were aware that fallows could reduce nematodes in bananas. 30% of those aware of the need for fallows had adopted either Rhodes grass or Brassica as a cover crop as part of their standard farm practice. The importance of these two cover crops in the fallow period had been developed in recent nematology and extension projects.

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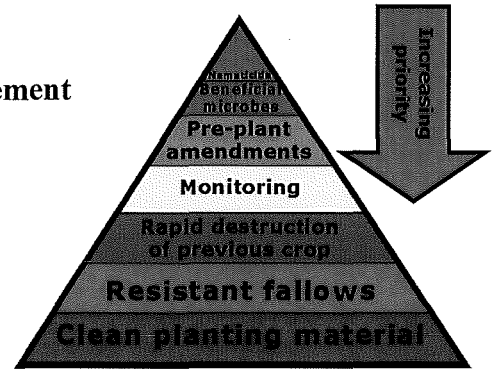
7. Recommendations

A combination of the findings from this project and previous projects on nematode management on banana has led to the development of a nematode management pyramid (Figure 8). The priority for growers should be to develop a sound base for nematode management, which starts with clean planting material. Increased emphasis is required on ensuring that planting material is free of burrowing nematode, as current banana bit disinfestation practices are ineffective for removing all nematodes from within the corm.

Figure 8. Nematode management pyramid for bananas.



The next priority is to ensure that the paddocks where bananas are to be planted are free of burrowing nematodes. Although, it is extremely difficult to entirely eradicate burrowing nematodes from infected paddocks, fallowing and rapid crop destruction methods can be used to reduce nematode numbers to very low numbers. The need for nematicide application can be prolonged or excluded with proper fallow management. Although there is some information on the crops grown in the fallow period, there is a need to screen new cultivars and crops for nematode resistance and suitability for rotation with bananas.

There is already adoption of the use of herbicide sprays to reduce the growth of volunteer bananas in the fallow period. However, emphasis on reducing nematode host plants in the fallow needs to be reinforced.

Economic thresholds and nematode sampling strategies have been developed in previous projects. However, there is poor utilisation of information by growers.

There is little information on the use of pre-plant amendments and the improvement of soil health in the suppression of burrowing nematodes. This is currently being addressed in FR02025. The concept behind the project is to develop nematode suppressive soils as early as possible in the life of the banana plantation.

The use of biological antagonists to nematodes can be successfully introduced into tissue culture banana plants in the nursery phase of production. These organisms would increase the plant's defence system and improve the vigour of the plant. However, if planting material is heavily infested with nematodes these organisms would have little impact on suppressing the population of nematodes.

The need for nematicide application is a signal that something has gone wrong with the nematode management system. Nematicides should be used as a last resort in existing banana crops. Nematicides have little impact on the nematode population compared to other strategies in the nematode management pyramid (Figure 8), such as clean planting material and nematode free fallows. However, rotation of soil applied nematicides is crucial to

maintain the efficacy of the products. The injection of nematicides into the pseudostem of the following sucker is still in the development phase and requires further work to ensure safe efficacious application of nematicides. The practice of pseudostem injection of nematicides increases the likelihood of new, less toxic products being available to the banana industry. By removing the soil phase, chance of decomposition by microorganisms is reduced.

The concept of the nematode management pyramid (Figure 8) can be extended to the Australian banana industry to encourage a systems approach to nematode management. The nematode management pyramid requires that banana growers develop a strong base using nematode free planting material. If nematodes are introduced at the base of the pyramid other management strategies above will be required resulting in premature nematicide use and significant nematode damage.

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9. Appendix A: List of figures

10. Appendix B: List of tables

11. Appendix C: Nematode management survey of the Queensland banana industry

DPI survey of nematode management practices in the Queensland banana industry

1. Producer background.

The following questions give us some background information on you – where you farm, how long you've been farming etc. Please make a short answer where space is provided. There is no way of identifying individual producers in this survey so an individual's information is kept confidential and anonymous.

1.1 How long have you been growing bananas?

..... years

1.2 What age is the key decision-maker on the farm?

.....years

1.3 What district or postcode covers your banana farm/s?

Postcode	District or town name or
eg. *4860*	*Innisfail*

1.4 Do you think nematodes are a problem on your property?

- ❑ **Yes**
- ❑ **No**
- ❑ **Not sure**

1.5 What area do you currently have under bananas?

..... hectares

2. Farm practices for managing nematodes

The following questions relate to different practices that you might use on your banana farm to manage nematodes.

2.1 Planting material

2.1.1 How important do you rate planting material as a source of nematode infestation and spread in bananas? (please tick)

- ❑ **Critically important**
- ❑ **Important**
- ❑ **Not at all important**
- ❑ **Not sure**

2.1.2 Do you currently use 'clean' planting material to establish new plantings of bananas? (please tick)

- ❑ **Yes**
- ❑ **No**
- ❑ **Not sure**

2.1.3 Listed below are different management practices that eliminate or reduce the risk of spreading burrowing nematodes in planting material. For each practice, we would like to know if you are aware of it, and if so whether you have considered using it, have used it once or twice, or use it regularly on your property.

Please tick the appropriate boxes.

Management practice	Not Aware	Aware	Considered it	Used it once or twice	Using it regularly
Using tissue-cultured plants					
Using a dedicated nursery in clean ground started with tissue-cultured plants					
Using a dedicated nursery in clean ground using bits and suckers					
Dipping bits and suckers in Nemacur 400®					
Hot water dipping for bits and suckers					
Heavy paring of bits and suckers (removal of roots and outer surface)					

2.1.4 This question refers to the list of management practices in 2.1.3. For any of the practices where you have answered that you are aware of a practice but do not use it regularly, give a brief reason in the space provided.

Management practice	Reason
Using tissue-cultured plants	
Using a dedicated nursery in clean ground started with tissue-cultured plants	
Using a dedicated nursery in clean ground using bits and suckers	
Dipping bits and suckers in Nemacur 400®	
Hot water dipping for bits and suckers	
Heavy paring of bits and suckers (removal of roots and outer surface)	

2.2 Chemical use in the field

The questions in this section relate to your use of chemicals (called nematicides) to control nematodes in the field.

2.2.1 Have you used a nematicide on your property in the last 12 months?

- ❑ Yes *(go to (a) and (b))*
- ❑ No *(go to (c))*

(a) If YES, please tick in the list below which product/s you have used (including alternative or biological products), and how many applications you made per year.

Product	Number of applications
❑ Nemacur®	
❑ Rugby®	
❑ Counter®/Hunter®/Terbuforce®	
❑ Mocap®	
❑ Vydate®	
❑ Other (please list)	

(b) If YES, how do you decide whether to apply nematicide? (tick those which apply)

- ☐ I use it when I can afford it
- ☐ I use it on a regular schedule or routine
- ☐ When I think the nematodes are getting bad
- ☐ I monitor nematode damage on the roots and apply chemicals when it reaches a certain level
- ☐ When plants fall over
- ☐ Other reasons .....

(c) If NO, indicate your reasons in the list below (tick those which apply)

- ☐ No nematodes/new ground
- ☐ Concerned about the effects on the soil and environment
- ☐ Very few nematodes or damage
- ☐ Chemicals too costly/not cost-effective
- ☐ Health risk to spray operators/workers
- ☐ Chemicals don't work
- ☐ Other reasons .....

2.2.2 Are you aware of the Root Disease Index method of monitoring for burrowing nematodes in bananas? (please tick)

- ☐ Yes *(go to (a))*
- ☐ No
- ☐ Not sure

} *(go to question 2.2.3)*

(a) If YES, pick from the list below to show your level of use.

- ☐ Don't currently use it
- ☐ Use it myself occasionally when plants fall out
- ☐ Get a pest consultant to do it occasionally when plants fall out

} *(go to question 2.2.3)*

- ☐ Use it myself regularly *(go to (b))*
- ☐ Get a pest consultant to do it regularly *(go to (b))*

(b) If you are using it regularly, do you keep records for blocks and check the changes in root disease index over time?

- ☐ Yes
- ☐ No

2.2.3 Are you aware of the recommended practice of chemical rotation for agricultural chemicals, including nematicides? (please tick)

- ☐ Yes *(go to (a))*
- ☐ No
- ☐ Not sure

} *(go to question 2.2.4)*

(a) If YES, are you currently rotating the use of nematicides on your farm? (please tick)

- ☐ Yes *(go to (b))*
- ☐ No

(b) If YES, pick from the list below to show how you are rotating? (please tick)

- ❑ Change chemical after only one application
- ❑ Change chemical after 2-3 applications
- ❑ Change chemicals each year
- ❑ Other .....

2.2.4 Are you aware of the term 'enhanced biodegradation' with regard to nematicides? (please tick)

- ❑ Yes *(go to (a))*
- ❑ No
- ❑ Not sure

} *(go to question 2.2.5)*

(a) Can you select an explanation from the list below that best matches your understanding of the term 'enhanced biodegradation'?

- ❑ Where nematodes become resistant to the chemicals
- ❑ Where soil microbes 'eat' the chemicals before they have the required effect on the nematodes
- ❑ Other .....

2.2.5 How do you currently apply your nematicides or alternative products? (please tick)

- ❑ Don't apply any products
- ❑ Through the irrigation system
- ❑ Hand spray onto the ground around the plant
- ❑ Band spray with tractor
- ❑ Inject into stem of following sucker
- ❑ Apply granules using a mechanised applicator
- ❑ Apply granules by hand
- ❑ Other .....

2.3 Using fallows

The questions in this section relate to the use of fallows to control nematodes.

2.3.1 Are you aware that fallowing for periods of time with particular crops and pastures can reduce the nematode population in the soil? (please tick)

- ❑ Yes *(go to (a))*
- ❑ No
- ❑ Not sure

} *(go to question 2.3.2)*

(a) If YES, have you used a fallow period/crop before replanting bananas in the last 3 years? (please tick)

- ❑ Yes *(go to (b) & (c))*
- ❑ No *(go to (d))*

(b) If YES, please tick in the list below the rotation practice you use, and write in the usual length of fallow.

Rotation practice	Length of fallow
❑ Bare fallow	months/years (please circle)
❑ Legume cover crop	months/years (please circle)
❑ Brassica field crops (BQ® Mulch, canola)	months/years (please circle)
❑ "Callide" Rhodes grass	months/years (please circle)
❑ Jarra grass	months/years (please circle)
❑ Other pasture grasses (please list)	months/years (please circle)
❑ Forage sorghum	months/years (please circle)
❑ Self-sown grasses/weeds	months/years (please circle)
❑ Sugarcane (list varieties)	months/years (please circle)
❑ Others (please list)	months/years (please circle)

(c) If you have used a FALLOW CROP (not bare fallow) in the last 3 years, will you continue to use fallow crops as a standard farm practice to manage burrowing nematodes in the future? (please tick)

- ❑ Yes
- ❑ No
- ❑ Not sure

(d) If you have NOT used a fallow period/crop before replanting bananas in the last 3 years please give a brief reason why?

.....
.....
.....

2.3.2 How important do you consider freedom from weeds and 'regrowth' bananas is for a successful fallow period/crop for nematode control? (please tick)

- ❑ Critically important
- ❑ Important
- ❑ Not at all important
- ❑ Not sure

2.3.3 Eradicating the previous banana crop is important to prepare for fallowing or replanting. Check the list of practices below and tick any that you may have used in the last 3 years.

- ❑ Cultivation used to kill the plants and destroy the crop residue
- ❑ Injection with herbicide to kill the plants, followed later by cultivation to destroy the crop residue
- ❑ Crop knocked down and then sprayed with herbicide to kill the plants, followed later by cultivation to destroy the crop residue
- ❑ Other (please list).....

2.4 Soil amendments and organic matter management

The questions in this section relate to the use of soil amendments, like mill mud, mill ash or molasses, and the management and placement of leaf and stem trash.

2.4.1 Please check the list below and indicate which pre-plant amendments you have used in the last 3 years (please tick) and the rate of application.

Product	Application rate per hectare
❑ No product applied	
❑ Mill ash	
❑ Mill mud (filter press)	
❑ Molasses	
❑ Rock dust (eg. Minplus®)	
❑ Compost	
❑ Other (please list)	

2.4.2 Please check the list below and score on a scale of 1 to 10 the importance of these sources of information about the products listed in 2.4.1. (10 = very important to 1 = not at all important) (please circle)

10 9 8 7 6 5 4 3 2 1 Other growers/managers
10 9 8 7 6 5 4 3 2 1 Consultants
10 9 8 7 6 5 4 3 2 1 DPI staff or DPI bulletins/newsletters
10 9 8 7 6 5 4 3 2 1 Agricultural retailers eg. Primac, Grow Force etc.
10 9 8 7 6 5 4 3 2 1 Chemical and fertiliser company representatives
10 9 8 7 6 5 4 3 2 1 Other (please list) .....

2.4.3 Check the list below and identify the trash (leaf and stem material) management practice that most closely applies to your situation. (please tick)

- ❑ All trash is placed away from the base of the plants (in the interrow)
- ❑ All trash is placed around the base of the plants
- ❑ Leaf trash from deleafing stays where it falls but leaf and stem material from harvest is deliberately placed in the row area.
- ❑ Other practice (please list) .....

.....
.....

2.4.4 Please provide a brief reason for your particular practice identified in the above question.

.....
.....
.....
.....
.....
.....
.....

3. Sources of information, advice and training.

The questions in this section are designed to help us understand which are the most important sources of information and training for you. We also want to know how best to meet your information needs.

3.1 Attendance at grower activities.

3.1.1 Have you attended any of the following activities, with regard to nematode management , in the last 3 years? (please tick)

- ❑ A field day or field walk for growers
- ❑ A seminar or presentation of research or extension results
- ❑ A workshop or training activity
- ❑ The national banana industry conference – presentation or indoor field day
- ❑ A product launch or promotion for any nematicide
- ❑ A grower discussion group

3.1.2 Please check the list below and score on a scale of 1 to 10 the importance of these sources of information on nematode management in bananas. (10 = very important to 1 = not at all important) (please circle)

10 9 8 7 6 5 4 3 2 1	Field days/farm walks
10 9 8 7 6 5 4 3 2 1	Training activities or workshops
10 9 8 7 6 5 4 3 2 1	Industry seminars or meetings
10 9 8 7 6 5 4 3 2 1	The national banana industry conference
10 9 8 7 6 5 4 3 2 1	DPI staff
10 9 8 7 6 5 4 3 2 1	Consultants
10 9 8 7 6 5 4 3 2 1	Industry newsletters or bulletins
10 9 8 7 6 5 4 3 2 1	Other growers/managers
10 9 8 7 6 5 4 3 2 1	Agricultural retailers eg. Primac, Grow Force etc.
10 9 8 7 6 5 4 3 2 1	Chemical/fertiliser company staff
10 9 8 7 6 5 4 3 2 1	The internet
10 9 8 7 6 5 4 3 2 1	Other (please list) .....

3.1.3 How would you most like to receive information about managing burrowing nematodes in bananas?

.....
.....
.....
.....
.....
.....
.....

Thank you for your time and cooperation in completing this survey questionnaire. Please place it in the Reply Paid envelope provided and post it.