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Suppressive soils for biological control of root-knot nematodes on vegetable crops

Dr. Graham Stirling
Biological Crop Protection Pty Ltd

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Final report of Horticulture Australia project VG01087
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Purpose of the report

Root-knot nematode is a serious pest of vegetable crops and many growers routinely apply nematicides and soil fumigants to achieve control. However, these nematode control strategies are now under threat because of concerns about the effects of chemicals on human health and the environment. This report describes research aimed at enhancing the natural biological control mechanisms that exist in all soils. Results of laboratory and field studies are presented which show that amendments of organic matter increase soil microbial activity, stimulate the activity of predators and make the soil more suppressive to root-knot nematode and Pythium root rot. This research therefore demonstrates that conserving soil organic matter is a critical component of soil-borne disease management in vegetable farming systems.

Acknowledgments

This work would not have been possible without Liz Wilson and Marcelle Stirling, who helped with laboratory experiments, nematode counts and other biological analyses. Ms Rosemary Kopittke advised on statistical analyses, Neil Halpin kept a watchful eye on field trials and Lois Eden helped with the *Pythium* work. Nutri Tech Solutions Pty. Ltd supplied products for field and glasshouse studies (Chapter 5) and Heritage Seeds and Pacific Seeds supplied seeds for the experiments described in Chapter 6. The field work was done on Don Halpin's farm at Bundaberg. Don was an excellent co-operator, as he managed the farm professionally and was always willing to accommodate experimental requirements that inevitably made his job more difficult. The Australian capsicum industry provided financial support for the project.

Date: 25 January 2005

Media Summary

Research in sandy, vegetable-growing soils at Bundaberg has shown that amending soil with organic matter reduces losses from root-knot nematode in capsicum by increasing soil microbial activity and enhancing naturally-occurring mechanisms of biological control.

Dr Graham Stirling from Biological Crop Protection in Brisbane, who did the work on behalf of Horticulture Australia and the vegetable industry, found that many Bundaberg vegetable-growing soils were low in biological activity and diversity, and were therefore prone to nematode problems. Large inputs of organic matter were necessary to re-establish the missing fungi and predatory nematodes that normally keep nematode pests under control.

Results from Dr. Stirling's laboratory experiments indicated that materials with a high C:N ratio were most likely to enhance biological control of root-knot nematode. A field trial was therefore established to examine the effect of amending soil with sugarcane trash (12.5 t/ha) and some additional nitrogen (100 kg N/ha as ammonium nitrate). Forage sorghum was planted as a rotation crop into beds of amended and non-amended soil and when the crop reached maturity, it was either incorporated as green manure and plastic was laid, or it was sprayed with glyphosate and the above-ground biomass was used as mulch. Observations on capsicums planted 18 weeks after the amendment was first incorporated showed that sugarcane trash increased microbial activity, increased numbers of free-living (beneficial) nematodes, reduced the severity of Pythium root rot, decreased populations of root-knot nematode and reduced the severity of galling caused by root-knot nematode. In contrast, a treatment consisting of 14 commercial products that are often promoted as soil health improvers and alternatives to nematicides, had no effect. Two components of this program (neem cake and a product purported to contain various fungal predators of nematodes) also failed to show efficacy against root-knot nematode in glasshouse tests.

Although organic amendments improve the balance between beneficial organisms and pests, Dr. Stirling warned growers that adding large amounts of organic matter to soil just before planting could have unforeseen side effects. He therefore suggested that growers experiment with amendments before using them on a large scale. The optimum time for adding organic matter to soil will depend on soil type, soil moisture and temperature, but is probably 3-6 months prior to planting. Amendments are beneficial in modern vegetable production systems that deplete soil organic matter (e.g. cultivated beds covered with plastic), but Dr. Stirling indicated that they are likely to be even more useful in farming systems that include organic mulches, appropriate crop rotations and minimum tillage, as these practices will reduce the amount of organic matter required to achieve a more balanced soil biology.

Technical Summary

Root-knot nematode (*Meloidogyne* spp.) is a serious pest of vegetable crops and many growers routinely apply nematicides and soil fumigants to achieve control. However, nematode control programs based on chemicals are now at risk because of concerns about their effect on human health and the environment. This research aimed to achieve control by enhancing naturally-occurring mechanisms of biological control. The approach taken was based on results of previous work in the sugar industry which had shown that suppressiveness to root-knot nematodes is enhanced for at least 4 months by amendments with a high carbon to nitrogen (C:N) ratio.

All experiments were done in light-textured soils from Bundaberg, as root-knot nematode invariably causes problems when such soils are used for vegetable production. The first step was to determine whether these soils naturally contain soil biota capable of suppressing root-knot nematode. The second step was to compare different types of organic matter for their capacity to enhance suppression of the pest. Finally, the effect of soil management practices that enhance levels of soil organic matter (e.g. organic amendments, minimum tillage, crop rotation and organic mulching) was examined in the field.

When soil from an undisturbed grass pasture that had never previously been used for vegetable production was autoclaved, or fumigated with methyl iodide, and then inoculated with root-knot nematode, the nematode multiplied readily and caused severe galling on tomato seedlings. In contrast, galling was negligible in untreated soil and nematode multiplication was about 80% less than in sterilised soil. This demonstrates that biological factors capable of suppressing the pest occur naturally in the test soil. Nematode community analysis clearly indicated that the suppressive pasture soil had a more active and diverse soil biology than typical vegetable-growing soils. Total numbers of free-living nematodes were 5-10 times higher in the pasture than in soils used for vegetable production, and numbers of omnivorous nematodes were 25 times higher.

The results of laboratory experiments with organic amendments showed that materials with a high C:N ratio are most likely to enhance suppression. In one experiment, soil amended with sugarcane trash was suppressive to root-knot nematode, while in a second experiment, sawdust, sugarcane trash and grass hay were more effective than nitrogen-rich amendments in inducing suppression. In both cases, amended soils remained suppressive long after the organic matter was added. Suppressive soils were fungal dominant and had high populations of fungal-feeding, omnivorous and predatory nematodes.

The effect of amending soil with organic matter (with and without later disturbance due to tillage) was investigated in a field at Bundaberg that had recently grown capsicum followed by zucchini and was heavily infested with root-knot nematode. Forage sorghum was planted as a rotation crop into non-

amended soil and into beds to which sugarcane trash (12.5 t/ha) plus ammonium nitrate (100 kg N/ha) had been added. When the forage sorghum crop reached maturity, it was incorporated as green manure and plastic was laid on beds, or it was sprayed with glyphosate and the above-ground biomass was used as mulch. Observations on capsicums that were planted 18 weeks after the amendment was first incorporated showed the biological benefits of adding sugarcane trash, as microbial activity and numbers of free-living nematodes were higher in amended plots. The amendment also reduced the severity of Pythium root rot, decreased pre-plant populations of root-knot nematode and reduced the severity of galling caused by the nematode on capsicum.

Tillage did not affect responses to the organic amendment, but there were higher numbers of root-knot nematode in non-tilled plots than cultivated plots, probably because nematicidal cyanogens are not released when forage sorghum tissue is not disrupted by cultivation. Non-tilled plots mulched with forage sorghum yielded poorly because nutrients (particularly N, K, Ca and Mg) were leached from the profile following heavy rainfall.

A treatment consisting of 14 commercial products that are promoted as soil health improvers and alternatives to nematicides was included in the above experiment, but it had no effect on root-knot nematode. Two components of this treatment (neem cake and a product purported to contain various nematophagous fungi) also failed to show efficacy against root-knot nematode in glasshouse tests.

In conclusion, these results show that amending soil with sugarcane trash or other materials with a high C:N ratio enhances the soil biology and reduces the severity of soil-borne diseases caused by root-knot nematode and *Pythium*. The optimum time for adding organic matter to soil depends on soil type, soil moisture and temperature, but is probably 3-6 months prior to planting. Benefits from amendments were obtained under the soil management practices currently used in the vegetable industry (e.g. cultivated beds covered with plastic), but in the long-term, the emphasis must shift towards using amendments within a farming system that is less destructive of organic matter. Future research should therefore concentrate on redesigning the vegetable farming system to accommodate organic amendments, organic mulches, appropriate crop rotations and minimum tillage.

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CHAPTER 1. AN ECOLOGICAL APPROACH TO ENHANCING NATURAL BIOLOGICAL CONTROL OF PLANT-PARASITIC NEMATODES

Introduction

Plant-parasitic nematodes attack most agricultural and horticultural crops, but are particularly important pests of vegetables in warm-temperate and tropical climates, and in light-textured soils. In these situations, nematodes are a constant problem and control is usually achieved by routinely applying a nematicide. However, the withdrawal of soil fumigants such as ethylene dibromide and methyl bromide, the high mammalian toxicity of organophosphate and carbamate nematicides, consumer preferences for food that is free of pesticides and successes in biological control against plant and insect pests have ensured that there is a continuing search for non-chemical methods of nematode control.

Efforts to develop biological controls for plant-parasitic nematodes commenced more than sixty years ago, and in most cases, the approach used was to try and replace chemical nematicides with a biological alternative. Nematode-trapping and/or egg-parasitic fungi were mass-produced in the laboratory and applied to soil prior to planting a crop, in the hope that they would proliferate in soil and control nematodes. It is now clear that this approach was largely unsuccessful. Promising results were sometimes obtained in glasshouse experiments and small-scale field trials, but the high level of nematode control required in modern farming systems was never consistently achieved in the field (Stirling 1991). Recent attempts to overcome the problems involved in mass-producing and formulating nematophagous fungi did not result in the development of commercial products (Stirling *et al.* 1998a,b), while most fungal preparations that have been marketed commercially have been plagued by quality control problems and inconsistency of performance (Stirling 1991).

Another approach which reached its zenith in the 1980's was the use of chitin to stimulate populations of chitinolytic microorganisms in soil. Chitin is the main structural component of the nematode egg shell, and so the aim was to develop a chitinolytic microflora capable of controlling nematodes by degrading or breaching the egg's protective chitin layer. Clear evidence that chitin reduces nematode populations was obtained (Mian *et al*. 1982; Culbreath *et al*. 1985), but evidence that chitinolytic organisms were involved is somewhat tenuous (Stirling 1991; Fegan 1993). It is more likely that most of the nematode control is due to ammonia, as it is produced at concentrations which are toxic to nematodes when materials with a high carbon to nitrogen (C:N) ratio are added to soil (Mian *et al*. 1982). Many other organic wastes with high nitrogen contents (e.g. animal manures, oil-cakes and legume hays) have also been used over the years to enhance natural biological control mechanisms, but it is now accepted that these materials also act by producing ammonia and/or nitrous acid (Rodriguez-Kabana 1987; Lazarovits *et al*. 2001). Since nitrogen is potentially damaging to the environment and is also detrimental to predatory nematodes (Stirling *et al.*

2003; Tenuta and Ferris 2004), organic amendments with high nitrogen contents do not have a place in ecologically-based nematode management programs unless they are used at low application rates.

Given the problems with other biological control strategies against plant-parasitic nematodes, an approach that deserves more attention is the use organic amendments to enhance natural mechanisms of biological control. This approach was first used by Linford *et al*. (1938), who showed that damage caused by root-knot nematode on pineapple was reduced by amending soil with pineapple trash. Although the reasons for this effect were never conclusively determined, it was suggested that natural enemies (specifically predatory mites and nematode-trapping fungi) were responsible. Since that time, it has been assumed that the addition of organic matter to soil stimulates the activity of natural enemies of nematodes, but it is only recently (Jaffee 2002; 2003; 2004) that quantitative evidence has been obtained to show that the populations and/or activity of some species of nematode-trapping fungi are enhanced by organic amendments.

Considerations when using organic matter to enhance biological control

Methodology. One of the problems with much of the work on organic amendments for nematode control is that the effect of an amendment on nematode populations or the symptoms caused by plant-parasitic nematodes is measured within a few weeks of the amendment being applied. Experiments on ammonia production by nitrogenous amendments are often done in this way, and the fact that immediate effects are observed suggests that the nematicidal activity of these amendments has little to do with biological control. Biological approaches to nematode control start with the addition of organic matter to soil, and this sets off a succession of biological changes that result in the development of a new biological community. The critical issue is whether this community is more suppressive to nematode pests than the one that it replaced.

Although some organic amendments act by producing ammonia, there are many other situations where the mode of action of amendments involves more subtle chemical mechanisms. Phenols, tannins, various organic acids, and a range of pre-formed chemicals in amendments such as neem cake (e.g. azadarachtin) are some examples (Stirling 1991). It is therefore difficult to determine whether an amendment is acting through chemical or biological mechanisms, or both. Difficulties in interpreting experimental results are compounded by the problems involved in measuring population densities of fungal predators, and quantifying their predatory activity (Jaffee 2004). The complexity of the soil food web is also an issue, because predation on nematodes in organically-amended soil is probably due to a wide range of organisms, including fungi, nematodes and arthropods. Many of those organisms are likely to be taxonomically obscure, poorly understood or unknown to science.

Ecological issues. Any attempt to use organic matter to enhance biological control must recognize that ecological constraints govern what is achievable. Our knowledge of the components and functions of the detritus food web and the interactions that occur between organisms in the food web has improved markedly in the last 25 years, and this knowledge must be used to improve our chances of success. The organisms that prey on nematodes form the third or highest trophic level in the detritus food web, and therefore only thrive when the lower trophic levels of the food web are functioning properly. A source of organic matter must be available at all times, as it forms the resource base for fungi and bacteria in trophic level 1. These microorganisms support the fungal and bacterial-feeding nematodes in trophic level 2, and they in turn, are an important food source for predators and parasites of nematodes in trophic level 3 (Wardle 1995). Although the interactions which occur between various levels of the soil food web are poorly understood, it is known that predatory nematodes at the end of the food chain, for example, are regulated by resource availability via the microbial biomass (Wardle and Yeates 1993). Continual inputs of organic matter are therefore the primary key to maintaining a diverse range of predators capable of keeping plant-parasitic nematodes under control. Lack of soil disturbance may also be important because microarthropods that prey on nematodes are particularly vulnerable to damage during cultivation (Wardle 1995).

Issues related to the farming system. One of the main reasons that pests such as nematodes are a problem in modern farming systems is that many soil management practices are detrimental to soil biological activity and diversity. The practices used in vegetable production provide perhaps the best example, as fumigation, cultivation, herbicides, plastic mulches, long periods of bare fallow, lack of carbon inputs, and regular inputs of inorganic fertilizer create soils that are low in organic matter and almost devoid of biological activity. Given the level of investment in such farming systems, current methods of managing soil will not be easily modified, but it should be possible to eliminate some of the most destructive practices.

If the full biological potential of vegetable-growing soils is to be restored, greater inputs of organic matter will be required. However, this is not as simple as just growing more green manure crops or using more organic amendments. Any change in soil management will have effects on other components of the farming system (e.g. economics, crop nutrition, other soil-borne pathogens), and so the consequences of such changes on the whole system must be considered.

Approach taken in this work

The objective of the work described in this report was to enhance the natural suppressiveness of vegetable-growing soils to root-knot nematode. The approach taken was based on previous work in the sugar industry (Stirling *et al.* 2003; Pankhurst *et al.* 2004), which showed that organic amendments with a high

C:N ratio (e.g. sawdust, grass hay and sugarcane trash) induced suppressiveness to root-knot and lesion nematodes four and seven months after they were added to the soil. In contrast, nitrogen-rich amendments (e.g. lucerne hay, feedlot manure, poultry manure, chitin and mill mud) did not suppress plant-parasitic nematodes. Follow-up work in the field at Bundaberg showed that there were 95% fewer lesion nematodes in roots taken from soil amended with sugarcane trash (with or without additional nitrogen) than in roots from the non-amended control. The mechanisms by which sugarcane trash induced suppressiveness were not determined, but low levels of nitrate-nitrogen in soil, a fungal-dominant soil biology and high numbers of omnivorous nematodes were associated with suppression (Stirling *et al.* 2003).

The experiments described in this report were done in light-textured soils from Bundaberg, as root-knot nematode invariably causes problems when these soils are used for vegetable production. The first step was to examine undisturbed sandy soils in this region and determine whether they contained soil biota capable of suppressing root-knot nematode. The second step was to compare different types of organic matter for their capacity to suppress populations of root-knot nematode. The third component of the work was limited by lack of resources (particularly with regard to agronomic input), but an attempt was made to evaluate soil management practices that could form part of a more sustainable system of vegetable production. The practices investigated included organic amendments, minimum tillage, crop rotation and organic mulching.

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CHAPTER 2. NATURAL SUPPRESSION OF ROOT-KNOT NEMATODE IN A SOIL NEVER PREVIOUSLY USED FOR VEGETABLE PRODUCTION

Introduction

During a study of the physics, chemistry and biology of soils used for capsicum production in Australia (Pung *et al* 2003), a preliminary laboratory test showed that soil from a reference site at Bundaberg was suppressive to root-knot nematode (*Meloidogyne javanica*). This site was a small area of unused grass pasture on a vegetable farm and its sandy loam soil was similar in texture to adjacent fields where high populations of root-knot nematode invariably occurred on vegetable crops such as zucchini and capsicum. Since soils suppressive to root-knot nematode are rarely reported, the objective of this work was to confirm the suppressive nature of this soil.

Methods

The study site was predominantly a pasture of couch grass and other grasses, with an occasional *Eucalyptus* tree. The pasture had not been cultivated for many years, but was occasionally grazed by horses. Previous sampling had established that root-knot nematode was not present at the site. In March 2002, soil collected from depths of 0-15 cm was gently mixed and sub-divided into three aliquots, which were either autoclaved for 30 minutes, fumigated with methyl iodide or left untreated. Fumigation was done by adding 160 µL of methyl iodide to each 1 L pot and then incubating the pots in a sealed plastic bag for four days. The level of suppressiveness in the untreated soil was assessed by adding root-knot nematode to treated and untreated soils and using one of four assays to compare the number of nematodes recovered or the numbers of galls produced.

Suppression experiments

Ten replicate 200 mL polystyrene drinking cups were filled with autoclaved soil, methyl iodide-treated soil, untreated soil or pasteurized washed river sand. Each cup was then inoculated with 4,000 eggs of *M. javanica* obtained by removing eggs from tomato root systems with NaOCl containing 0.5% available chlorine. For the soil assay, cups were incubated at ambient temperatures (about 23 °C) for 10 days and then nematodes were extracted from five replicate cups by placing the soil on a standard nematode extraction tray for 2 days (Whitehead and Hemming 1965). The remaining five replicate cups were used for a root assay in which a tomato seedling (cv. Tiny Tim) was planted in each cup and 4 days later, nematodes in roots were stained with acid fuchsin/lactoglycerol and counted.

When suppression was assessed using root gall and egg production assays, only three treatments (methyl iodide-treated soil, untreated soil and pasteurized sand) were included in the experiments. For the root gall assay, 400 mL pots of each soil were inoculated with 4,000 root-knot nematode eggs and a tomato seedling was planted 10 days later. After plants had grown in the glasshouse for 30 days, roots were rated for

galling on a 0-10 scale (Zeck 1971). Galled roots were then softened by soaking them in water containing 20g/L Pectinase II (Amano Pharmaceutical Company, Nagoyo, Japan), and nematodes in roots were recovered by macerating them in a blender. In the egg production assay, 1 L pots were used, but otherwise, pots were inoculated, incubated and planted in the same way as for the root gall assay. Differences in nematode multiplication were assessed 8 weeks after planting by placing roots in NaOCl (1% available chlorine) for 5 minutes and pouring the suspension through a 38 µm sieve to retrieve the eggs.

Free-living nematodes in pasture soil

To obtain an indication of the biology of the soil used in the above experiments, free-living nematodes were extracted from 200 mL samples using the extraction method described above. Nematodes were then separated into three trophic groups (fungivores, bacterivores and omnivores) and counted under a microscope.

Results

Suppression experiments

The number of second-stage juveniles extracted from soil 10 days after they were inoculated, and the number of second-stage juveniles invading roots 10-14 days after inoculation, was much lower in untreated pasture soil than in heated or fumigated soil or sand (Table 2.1). The lower number of nematodes in roots growing in pasture soil was reflected in the lower level of galling at 30 days, as plants in untreated soil had very few galls compared to plants growing in fumigated soil or pasteurized sand (Table 2.2, Figure 2.1). Also, there were 98% fewer root-knot nematodes in roots from untreated soil than in the heavily-galled roots from methyl iodide-treated soil (Table 2.2). Egg production was also lower in roots from untreated soil, although the effect was only significant in comparison to roots from pasteurized sand (Table 2.2).

Table 2.1. The number* of root-knot nematodes recovered from soil or roots 10 and 14 days, respectively after the nematode was inoculated into untreated pasture soil from Bundaberg and into soil or sand sterilized by fumigation or heat.

Treatment	Soil assay J2 in soil	Root assay J2 in roots
Untreated	1.51 (31)	0.62 (3)
Methyl iodide	2.65 (446)	1.79 (61)
Autoclaved	3.05 (1121)	2.58 (379)
Pasteurised sand	2.94 (870)	2.36 (228)
LSD (P=0.05)	0.139	0.56

* Values are log transformed means [log (x+1)], with equivalent means in parentheses

Table 2.2. The level of galling caused by root-knot nematode on tomato and the number of root-knot nematodes in roots at 30 days, and egg production on tomatoes at 56 days, when the nematode was inoculated into untreated pasture soil from Bundaberg and into fumigated soil or heat-pasteurised sand.

Treatment	Gall assay	Egg production assay	
	Root gall index	Nematodes in roots	No. eggs/plant
Untreated	2.2	0.99 (9)	4.32 (20,900)
Methyl iodide	6.4	2.74 (549)	4.64 (43,650)
Pasteurised sand	7.4	-	5.34 (218,750)
LSD (P=0.05)	1.45	0.636	0.33

* Nematode numbers are log transformed means [log (x+1)], with equivalent means in parentheses

Figure 2.1. Galling caused by root-knot nematode 30 days after tomatoes were planted into untreated pasture soil (left) or methyl iodide-treated soil (right) that had been inoculated with the nematode.

Free-living nematodes in pasture soil

The soil used for the above experiments contained an average of 5,970 free-living nematodes/200 mL of soil. About 64% of the nematodes were bacterivores, 32% were fungivores and 4% were omnivores (Dorylaimida).

Discussion

The much higher recovery of root-knot nematode and the higher level of nematode multiplication and damage in fumigated or pasteurised soil compared with the untreated soil is clear evidence that biological factors capable of suppressing the pest occurred naturally in the test soil. Since root-knot nematode was not present in this soil, the suppressive factors were not-specific to the pest and are presumed to consist of general suppressive forces that probably occur in all soils with complex food webs.

The level of suppression observed in the test soil was unexpectedly high. Galling was negligible in untreated soil and regardless of the assay used to measure nematode populations, numbers of root-knot nematodes were reduced by 54-95% in untreated soil compared with fumigated or heated soil. From a practical perspective, this result is encouraging, because it shows that natural biological suppression can provide a level of nematode control that is similar to that obtained with other possible physical, chemical and cultural control measures.

When this work commenced, several non-agricultural soils from the Bundaberg region were examined for suppressiveness and all except the above soil were found to be conducive to root-knot nematode. The reason that only one soil was suppressive is not known, but it may be significant that suppression developed under an undisturbed pasture. Perhaps continual inputs of organic matter and lack of disturbance allow the food web in a pasture soil to develop the complexity that is needed for nematode predation and other multitrophic interactions to occur.

Nematode community analysis clearly indicated that the suppressive pasture soil was biologically different to soils of the same texture that are used for vegetable production. The main difference from vegetable-growing soils was the high number of free-living nematodes and the relatively high number of omnivorous nematodes. Total numbers of free-living nematodes were 5-10 times higher in the pasture soil than in soils from adjacent fields that had previously grown vegetable crops, and they also contained an average of 230 omnivorous nematodes/200 mL of soil. The latter figure is particularly high, as previous studies (Pung *et al*. 2003) have shown that population densities of omnivorous nematodes in vegetable-growing soils are usually <5 nematodes/200 mL soil. These observations suggest that the suppressive pasture soil has a much more active and diverse biology than soils used for vegetable production. They therefore provide an insight into the type of biology that may be required to restore suppressiveness to root-knot nematode in vegetable-growing soils.

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CHAPTER 3. THE POTENTIAL OF USING ORGANIC MATTER TO ENHANCE THE SUPPRESSIVENESS OF SOIL TO ROOT-KNOT NEMATODE

Introduction

As indicated in the literature review (see Chapter 1), most studies on organic amendments for nematode control have concentrated on effects that are measurable within a few weeks of adding the amendment to soil. These effects are often due to chemical factors and biological changes that could lead to long-term suppression of plant-parasitic nematodes have not necessarily occurred. The objective of the work described in this chapter was to enhance the antagonistic potential of the soil biota with organic matter and then sustain biological control activity for long enough to provide durable suppression of root-knot nematode. The comparative effects of various amendments were therefore assessed by measuring the suppressiveness of soil to root-knot nematode several months after organic materials were added to soil.

Experiment 1

Methods

The soil used for the experiment was a sandy loam from Bundaberg that had been cropped to zucchini, capsicum and cucumber over the previous three years. The soil was thoroughly mixed in a cement mixer and amended with sugarcane trash, lucerne hay, poultry manure (deep litter) or Organic Xtra^{TM} (a pelletised organic product produced by Queensland Organics) at 10, 20 and 40g organic matter/kg dry soil. The amended soils and a non-amended control were placed in 3L pots, kept moist by regular watering and re-mixed after 3 and 6 weeks to aid the decomposition process.

Two months after the amendments were added, amended soils contained large amounts of non-decomposed organic matter and the nitrogenous amendments (poultry manure and Organic Xtra) were phytotoxic at 40 g/L of soil. Consequently, only the 10 and 20 g/L application rates were assessed at this time. To obtain an indication of the biological changes induced by the amendments, the nematode community was characterized and microbial activity was measured. Suppressiveness to root-knot nematodes was assessed using soil and root gall assays.

All treatments were assessed at 4 months using the above techniques, except that numbers of culturable microorganisms were measured and an additional suppression assay was included. Total C, total N, labile C, NO_3-N, NH_4-N, pH and EC were also measured for two replicates of the 20 g/L treatment by the Queensland Department of Natural Resources and Mines using standard methods.

Characterisation of the nematode community. Nematodes were extracted by spreading 200 mL soil on a Baermann tray and leaving it for four days at a temperature of 24-28°C (Whitehead and Hemming 1965). Plant parasitic nematodes were identified to genus level and free-living nematodes were separated into trophic groups as follows: fungal-feeding nematodes (Tylenchida, Aphelenchida and some Dorylaimida), bacterial-feeding nematodes (Rhabditida) and omnivore-predators (Dorylaimida and Mononchina). If nematodes were required for further characterization and identification, they were killed in hot formalin-acetic acid and stored in the fixative until required.

Total microbial activity. The method used to assess microbial activity is based on the hydrolysis of water-insoluble fluorescein diacetate (3',6' -diacetylfluorescein; FDA) by a number of soil enzymes to produce a water-soluble end-product (fluorescein) that is quantified with a spectrophotometer (Schnurer and Roswall 1982). Briefly, soil was gently but thoroughly mixed and 5g dry weight equivalent of soil was incubated with FDA and phosphate buffer at 26º-27ºC on an orbital shaker for 30 minutes. The reaction was then terminated with acetone, the mixture was centrifuged and the absorbance of the supernatant was measured at a wavelength of 490 nm. Readings were corrected for background absorbance and the appropriate standard curves were used to calculate microbial activity (Chen *et al*. 1988). Results for total microbial activity are expressed as µg FDA hydrolysed / g dry soil/min.

Populations of microorganisms. Populations of culturable bacteria and fungi were measured using a combination of procedures including serial dilution, the plate dilution frequency technique (Harris and Sommers 1968) and the most probable number technique (MPN) (Cochran 1950). Soil was gently but thoroughly mixed and a sample (10g dry weight equivalent) was shaken in 90 mL of phosphate buffered saline on an end-over-end shaker for 20 minutes. A 10-fold dilution series was then prepared in phosphate buffered saline. Five replicate 20µL aliquots per dilution were pipetted as spots onto tryptic soy agar + cycloheximide (1/5 commercial preparation; Difco) for bacteria and Martin's medium (Martin 1950) for fungi. After plates were incubated at 26ºC for two days, all spots that showed evidence of growth were recorded and the MPN was obtained from a table in Meynell and Meynell (1970). The total number of microorganisms was expressed as $\log_{10}$ colony forming units (cfu) per g dry weight of soil.

Assays for suppression of root-knot nematodes. Several assays were used to assess suppression, but in every case the number of root-knot nematodes or the level of galling in amended soil was compared to that in non-amended, pasteurized or fumigated soil. In the soil assay, soil was added to 200 mL styrofoam cups and inoculated with 4000 root-knot nematode eggs extracted from tomato roots using sodium hypochlorite (0.5 g/L available chlorine). The cups were then placed in polystyrene boxes and covered with moist paper to prevent soil from drying out. After 10 days at ambient temperatures (22-26°C), second-stage juveniles (J2) were extracted on a Baermann tray and counted.

Two assays that involved plants commenced in the same way as the soil assay, but instead of extracting nematodes, tomato seedlings (cv. Tiny Tim), were planted in the cups. In the stained root assay, roots were washed free of soil after 4 days, nematodes in roots were stained with acid fuchsin in lactoglycerol and counted. In the root gall assay, the level of galling was assessed after 25-30 days in one of two ways. In cases where all treatments were lightly infested, the number of galls was counted. Heavier infestations were rated on a 0-6 scale where 0 = no galls, 1 = < 20 individual small galls, 2 = > 20 individual small galls, 3 = occasional multiple galls, 4-6 = light, moderate and heavy multiple galling respectively. In some cases where the root gall assay was used, numbers of female nematodes in galled roots were estimated by submerging 2-4 cm segments of roots in an enzyme solution (20 g/L Pectinase II, Amano Pharmaceutical Company, Nagoyo Japan) for 2 days at 22-26°C. The roots were then macerated in a blender for 10 seconds and the macerate passed through 850 and 53 µm sieves. Female nematodes in the material retained on the 53 µm sieve were counted.

A pot assay measured the number of nematodes remaining after populations multiplied on tomato and then declined in the absence of the host plant. A tomato seedling (cv. Tiny Tim) was planted in 400 mL of soil in a plastic pot and 10 days later, soil was inoculated with 4000 eggs of root-knot nematode. After the plants had grown for two months, the tops were cut off and the roots were left to decompose in soil for a further eight weeks. Root-knot nematodes were then extracted from the soil in a Baermann tray and counted.

Results

After two months, total microbial activity and populations of free-living nematodes were higher in all amended soils than in the control (Table 3.1). Results of the soil assay suggested that soil amended with sugarcane trash was suppressive to root-knot nematodes, as significantly fewer nematodes were recovered from both application rates of this treatment than from the non-amended control (Table 3.1). Soil amended with lucerne hay, poultry manure or Organic Xtra was not suppressive. The root gall assay produced different results, as a significant reduction in galling was obtained only in the soil amended with lucerne hay (Table 3.2). Numbers of galls per plant were increased significantly by Organic Xtra at 20g/L soil. All amendments increased root dry weight and all except sugarcane trash increased shoot dry weight (Table 3.2).

After four months, results of the soil assay showed that soil amended with sugarcane trash, lucerne hay, poultry manure and the lower rates of Organic Xtra were suppressive to root-knot nematodes (Table 3.3). This suppression was also reflected in the low gall ratings in the root gall assay for some of the treatments

(Table 3.4). Overall, sugarcane trash was the most suppressive across all rates of application. It was also the only amendment that reduced nematode populations in the pot assay (Table 3.3).

When compared to the non-amended soil, total microbial activity was significantly higher in all organically-amended soils, as were populations of bacterial-feeding nematodes (Table 3.3). However, populations of fungal-feeding nematodes and those belonging to the omnivore/predator group were the highest in the soil amended with sugarcane trash (Table 3.3). Populations of bacteria and fungi did not differ appreciably between treatments, but fungal populations tended to be highest in some of the treatments containing sugarcane trash (Table 3.3).

Chemical analyses (Table 3.5) showed that sugarcane trash had little impact on any of the parameters measured, whereas the nitrogenous amendments decreased pH and increased EC, NO_3 –N and leco N.

Results of plant assays were confounded by nutritional and toxicity problems. Plants in treatments with high C:N ratios (e.g. sugarcane trash) sometimes suffered from N deficiency and therefore had small root systems (data not shown). On the other hand, plants growing in treatments with high levels of N had poor root systems, possibly because of NH_3 toxicity.

Table 3.1. Effect of organic amendments on suppression of root-knot nematode (measured using a soil assay), microbial activity and populations of free-living nematodes, two months after amendments were added to soil.

Treatment	Rate (g/kg)	Number of root-knot nematodes in soil	Total microbial activity	Total free-living nematodes
		$\log_{10}$ (J2 +1)/ 200 mL	µg/FDA hydrolysed/ g /min	$\log_{10}$ (no. +1)/ 200 mL
Sugarcane trash	10	1.84	0.27	3.89
	20	1.82	0.36	3.96
Lucerne hay	10	1.92	0.49	4.22
	20	1.99	0.64	4.83
Poultry manure	10	2.47	0.33	4.22
	20	2.43	0.54	4.13
Organic Xtra	10	2.14	0.41	4.07
	20	2.18	0.47	4.24
Non-amended soil		2.37	0.19	3.36
LSD (P=0.05)		0.48	0.13	0.26

Table 3.2. Effect of organic amendments on plant growth and suppression of root-knot nematode (measured using a root gall assay) two months after amendments were added to soil.

Treatment	Rate (g/kg)	Log no. galls/plant	Shoot dry weight (g)	Root dry weight (g)
Sugarcane trash	10	1.97	1.64	0.46
	20	1.97	1.31	0.31
Lucerne hay	10	1.60	3.00	0.54
	20	1.76	3.46	0.64
Poultry manure	10	2.15	2.80	0.51
	20	2.12	3.87	0.70
Organic Xtra	10	2.10	2.60	0.52
	20	2.58	2.66	0.65
Non-amended soil		2.14	1.60	0.28
LSD (P=0.05)		0.33	0.91	0.18

Table 3.3: Numbers of root-knot nematodes recovered in two suppression assays, total microbial activity, and populations of free-living nematodes and microorganisms, four months after amendments were added to soil.

Treatment	Rate g/kg	No. nematodes (log_{10} (J2+1)/ 200 mL)		Microbial activity	Free-living nematodes/200 mL soil (log_{10} (no. +1))[A]				Microorganisms (log_{10} cfu/ g)	
		Soil assay	Pot assay	µg FDA hydrolysed /g /min	BF	FF	O/P	FL	Bacteria	Fungi
Sugarcane trash	10	2.01	2.83	0.55	3.69	3.31	1.45	3.85	7.52	5.87
	20	1.83	2.42	0.69	3.89	3.83	1.66	4.17	7.36	6.65
	40	1.69	2.43	0.62	3.66	3.28	1.70	3.82	7.61	6.06
Lucerne hay	10	1.64	3.25	0.51	3.53	2.31	1.03	3.56	7.91	5.16
	20	1.67	3.23	0.80	3.71	2.62	0.79	3.75	7.17	5.34
	40	1.98	3.46	0.87	3.90	1.84	0.49	3.69	7.99	5.42
Organic Xtra	10	2.11	3.52	0.50	3.69	2.18	0.68	3.69	7.96	5.60
	20	2.01	3.74	0.60	3.87	2.80	0.33	3.87	7.96	5.76
	40	2.32	3.19	0.69	3.95	1.45	0.0	3.95	7.72	5.60
Poultry manure	10	1.96	nd[B]	0.45	3.63	2.72	0.30	3.67	7.22	5.30
	20	1.86	3.32	0.58	3.54	2.55	0.56	3.65	8.07	5.55
	40	1.95	3.38	0.73	3.94	2.18	0.42	3.96	7.02	5.76
Non-amended		2.65	3.14	0.25	2.88	2.60	0.24	3.09	7.56	4.69
LSD (P=0.05)		0.35	0.63	0.12	0.26	0.47	0.43	0.25	ns[C]	ns

[a]BF = total bacterial-feeding nematodes, FF = total fungal-feeding nematodes, O/P = omnivore /predators, FL = total free-living nematodes. [B] ns = no significant differences. [C] nd = not done

Table 3.4. Gall ratings and root dry weights of tomato (cv. Tiny Tim) seedlings in a root gall assay four months after soil was amended with organic matter.

Amendment	Rate g/kg	Gall rating	Root dry weight (g)
Sugarcane trash	10	2.8	0.06
	20	2.5	0.08
	40	2.5	0.08
Lucerne hay	10	2.0	0.09
	20	3.0	0.15
	40	3.5	0.09
Organic Xtra	10	2.3	0.08
	20	5.8	0.08
	40	6.0	0.08
Poultry manure	10	2.5	0.09
	20	3.9	0.12
	40	5.3	0.05
Non-amended soil		4.3	0.11
LSD (P=0.05)		1.5	n.s.

Table 3.5. Chemical analyses 4 months after various organic materials were incorporated into soil at an application rate of 20 g/kg soil.

	pH_w	EC (mS/m)	NH_4 –N (mg/kg)	NO_3 –N (mg/kg)	Leco C (%)	Leco N (%)
Nil	7.8	10.3	0.7	18.6	1.6	0.10
Cane trash	8.1	10.9	1.8	9.2	1.8	0.11
Lucerne hay	7.2	30.7	4.8	88.9	2.0	0.15
Poultry manure	6.9	44.7	1.1	125.3	1.7	0.14
Organic Xtra	7.1	56.9	0.7	149.1	1.5	0.17

Experiment 2

Methods

This field experiment was established in a sandy loam soil at Bundaberg and was part of the sugarcane soil biology program within the Sugar Yield Decline Joint Venture. The site had a history of vegetable production, but was planted with sugarcane prior to the commencement of the experiment. The sugarcane was harvested in 2000, the land was cultivated and various amendments (Table 3.6) were spread on the

surface of 3 × 3m plots in March 2001. The amendments were incorporated with a rotary hoe and plots were then kept free of weeds with herbicides.

On 10 January 2002, 10 months after amendments were applied, the opportunity was taken to measure any biological differences due to organic matter that may have developed at this site. Approximately 4 L of soil was collected from each plot and three replicate sub-samples were used to characterize the nematode community and estimate microbial activity (see experiment 1 for details). A further sub-sample was sent to the Analytical Services Laboratory, Queensland Department of Natural Resources and Mines for estimation of KCL extractable NH4-N and NO3-N, leco-C and leco-N. Three replicate samples were also assayed for suppression to root-knot nematodes using assays described previously. For the soil assay, pasteurised sand and soils from treatments 1, 5, 8, 13, 18, 20 and 22 that had been fumigated with methyl iodide (160 µL/L soil) were included as standards. The standard for the root gall assay was fumigated non-amended soil (i.e. treatment 1). Methyl iodide was applied to the soil in plastic bags, the bags were sealed for 3 days and then the soil was aerated for 2 days before it was used.

Table 3.6: Application rates of amendments applied to soil at Bundaberg in March 2001.

Treatment no.	Treatment, with application rate (t C/ha) in parentheses	Application rate (t dry matter /ha)	Additional N (kg/ha) applied as ammonium nitrate
1, 24	Nil	0	0
2	Sawdust (20)	50	0
3	Sawdust (20) + N	50	200
4	Sugarcane trash (20)	50	0
5	Sugarcane trash (20) + N	50	200
6	Sugarcane trash (20) + feedlot manure (4)	50 + 10	0
7	Grass hay (20)	50	0
8	Grass hay (20) + N	50	200
9	Grass hay (20) + poultry manure (4)	50 + 10	0
10	Grass hay (10) + N	25	100
11	Grass hay (4) + N	10	50
12	Lucerne hay (20)	50	0
13	Lucerne hay (20) + N	50	200
14	Lucerne hay (10) +N	25	100
15	Lucerne hay (4) + N	10	50
16	Feedlot manure (10)	40	0
17	Feedlot manure (4)	16	0
18	Poultry manure (10)	40	0
19	Poultry manure (4)	16	0
20	Chitin (10)	21	0
21	Chitin (4)	8.5	0
22	Mill mud (11.3)	38	0
23	Molasses (4)	14	0

Results

All amendments increased the carbon content of soil (Table 3.7). However, effects on nitrogen were variable and depended on the nitrogen content of the amendment. Poultry manure, chitin and mill mud had the greatest impact, increasing NO_3 –N above levels in the nil treatment (Table 3.7). The C/N ratio was relatively high in most treatments, with chitin the only treatment where the C/N ratio was less than 16.

Most of the organic amendments had little effect on microbial activity (Table 3.8). The exceptions were some of the lucerne hay treatments which increased microbial activity, and some of the grass hay and sawdust treatments, where microbial activity decreased.

Observations on the nematode community (Table 3.8) showed that there were high numbers of fungal-feeding nematodes following sawdust, sugarcane trash, grass hay and some lucerne hay treatments. Numbers of omnivorous and predatory nematodes were highest in the sawdust treatment and relatively high following all the amendments with a high C/N ratio. Large quantities of poultry manure, chitin, lucerne hay and mill mud had a negative impact on this group of nematodes.

Suppression assays clearly showed that soils amended with sawdust, sugarcane trash or grass hay were suppressive to root-knot nematode (Table 3.8).

Table 3.7. Carbon and nitrogen analyses of organically-amended soils

Treatment no.	Treatment, with application rate (t C/ha) in parentheses	NH_4-N (mg/kg)	NO_3-N (mg /kg)	Leco-C (%)	Leco-N (%)	C/N
1	Nil	0.9	22.2	1.54	0.088	17.5
24	Nil	1.6	43.1	1.57	0.089	17.6
2	Sawdust (20)	1.2	10.7	1.75	0.091	19.2
3	Sawdust (20) + N	1.8	9.0	1.69	0.095	17.8
4	Sugarcane trash (20)	1.2	14.1	1.80	0.096	18.8
5	Sugarcane trash (20) + N	1.1	9.0	1.83	0.107	17.1
6	Sugarcane trash (20) + feedlot manure (4)	0.5	14.3	1.74	0.099	17.6
7	Grass hay (20)	0.4	4.6	1.63	0.084	19.4
8	Grass hay (20) + N	1.0	14.4	1.65	0.102	16.2
9	Grass hay (20) + poultry manure (4)	0.9	14.4	1.67	0.099	16.9
10	Grass hay (10) + N	0.5	8.1	1.57	0.084	18.7
11	Grass hay (4) + N	1.0	23.9	1.61	0.090	17.9
12	Lucerne hay (20)	2.1	7.8	1.74	0.105	16.6
13	Lucerne hay (20) + N	1.6	29.2	1.80	0.111	16.2
14	Lucerne hay (10) +N	1.8	39.2	1.67	0.106	15.8
15	Lucerne hay (4) + N	1.3	23.2	1.70	0.098	17.3
16	Feedlot manure (10)	1.6	25.8	1.84	0.108	17.0
17	Feedlot manure (4)	1.6	16.3	1.70	0.103	16.5
18	Poultry manure (10)	2.0	60.0	1.76	0.092	19.1
19	Poultry manure (4)	1.8	59.2	1.69	0.090	18.8
20	Chitin (10)	2.8	134.0	1.69	0.106	15.9
21	Chitin (4)	1.5	47.4	1.64	0.106	15.5
22	Mill mud (11.3)	1.7	50.3	1.89	0.095	19.9
23	Molasses (4)	1.4	8.6	1.72	0.082	21.0

Table 3.8. Nematode populations by trophic group and microbial activity of organically-amended soils and their suppressiveness to root-knot nematode as measured by two different assays.

Treatment no.	Treatment, with application rate (t C/ha) in parentheses	Root-knot nematode suppression assays		Populations of free-living nematodes [(log_{10} (no. +1)]			Microbial activity
		Soil assay log_{10} (no. J2 +1) /200 mL soil	Root assay log_{10} (no. J2+1) /root system)	Fungal-feeding	Bacterial-feeding	Omnivore/ predators	(µg/ FDA hydrolysed/g /min)
Sand	Pasteurised	2.84	2.29	-	-	-	-
1	Nil fumigated with methyl iodide	2.79	2.38	-	-	-	-
5F	Trt. 5 fumigated with methyl iodide	2.83	nd	-	-	-	-
8F	Trt. 8 fumigated with methyl iodide	2.67	nd	-	-	-	-
13F	Trt. 13 fumigated with methyl iodide	2.79	nd	-	-	-	-
18F	Trt. fumigated with methyl iodide	2.80	nd	-	-	-	-
20F	Trt. 20 fumigated with methyl iodide	2.70	nd	-	-	-	-
22F	Trt. 22 fumigated with methyl iodide	2.84	nd	-	-	-	-
1	Nil	2.82	2.18	2.39	2.41	1.45	1.48
24	Nil	2.36	2.46	2.16	2.32	1.29	1.25
2	Sawdust (20)	***2.14***	***1.25***	**3.34**	2.67	**1.79**	1.34
3	Sawdust (20) + N	***1.94***	***1.27***	**3.21**	2.38	**1.84**	***0.81***
4	Sugarcane trash (20)	***2.14***	***1.49***	**3.14**	**3.01**	1.68	1.68
5	Sugarcane trash (20) + N	***2.22***	***1.54***	**2.62**	2.50	1.39	1.27
6	Sugarcane trash (20) + feedlot manure (4)	***2.41***	***1.32***	**3.09**	**2.73**	1.44	1.27
7	Grass hay (20)	***2.28***	***0.97***	**2.74**	2.49	1.27	1.51
8	Grass hay (20) + N	2.58	2.07	**2.69**	2.28	1.06	1.47
9	Grass hay (20) + poultry manure (4)	***2.35***	1.89	**2.83**	***1.97***	1.48	***0.95***
10	Grass hay (10) + N	2.72	***1.44***	**2.71**	2.16	1.25	***0.54***
11	Grass hay (4) + N	2.58	1.94	2.44	2.11	1.63	1.17
12	Lucerne hay (20)	2.69	2.36	**2.65**	2.23	***0.74***	1.73
13	Lucerne hay (20) + N	2.65	2.36	2.04	2.39	***0.65***	**2.11**
14	Lucerne hay (10) +N	2.63	2.08	**2.80**	2.12	1.15	**1.87**
15	Lucerne hay (4) + N	2.61	2.13	2.37	2.50	1.52	1.48
16	Feedlot manure (10)	2.47	2.11	2.05	2.52	1.40	1.69
17	Feedlot manure (4)	2.60	2.19	2.06	2.18	1.39	1.52
18	Poultry manure (10)	2.49	2.81	2.30	2.32	***0.0***	1.54
19	Poultry manure (4)	2.84	2.73	2.37	2.09	1.21	1.20
20	Chitin (10)	2.76	2.56	2.53	**2.86**	***0.10***	1.43
21	Chitin (4)	2.78	2.22	2.48	2.51	1.61	1.71
22	Mill mud (11.3)	2.59	2.07	2.12	2.69	***0.94***	1.45
23	Molasses (4)	2.53	2.29	2.07	2.13	1.21	1.42
	LSD (P=0.05)	0.31	0.46	0.29	0.33	0.33	0.35

For nematode numbers and microbial activity, numbers in bold are significantly higher and numbers in bold italics significantly lower than the average of the two controls. For suppression assays, numbers in bold italics are significantly lower than the average of the fumigated soils and pasteurized sand.

Relationships between soil biological parameters and suppressiveness

Methods

Correlations between the biological parameters measured in experiments 1 and 2 and the suppressiveness of soil to root-knot nematode were used to determine the factors most closely associated with suppression. The biological data used (Tables 3.3 and 3.8) included microbial activity, components of the free-living nematode community, and numbers of bacteria and fungi, while suppression was assessed by the number of root-knot nematodes recovered in soil and pot assays.

Results

Regression analysis showed that numbers of fungi, numbers of fungal-feeding nematodes and numbers of omnivorous and predatory nematodes were associated with suppression, as the number of root-knot nematodes recovered from soil and plant assays decreased as these parameters increased (Table 3.9).

Table 3.9. Relationships* between various soil biological parameters and suppression of root-knot nematode (measured as the number of root-knot nematodes in soil and plant assays) in experiments 1 and 2.

Experiment no.	Parameter	Soil assay		Plant assay	
		P	R^2	P	R^2
1	Microbial activity	ns		ns	
	Bacteria	ns		ns	
	Fungi	ns		0.049	0.31
	Bacterial-feeding nematodes	ns		ns	
	Fungal-feeding nematodes	ns		0.011	0.46
	Omnivore-predators	0.037	0.34	<0.001	0.64
2	Microbial activity	ns		ns	
	Bacterial-feeding nematodes	ns		ns	
	Fungal-feeding nematodes	0.004	0.32	<0.001	0.50
	Omnivore-predators	ns		ns	

* P = probability, R = regression coefficient.

Validation of suppression assays

Introduction

The gelatinous material that normally surrounds the eggs of root-knot nematode is thought to have a protective function, but it was removed with NaOCl when inoculum was prepared for experiments 1 and 2. These eggs may therefore have been more vulnerable to attack from soil organisms than eggs in their natural state. Since the level of suppression may therefore have been overestimated in the above

experiments, suppression assays were done with hypochlorite-treated eggs and eggs in egg masses to check that they responded in a similar manner.

Methods and results

Validation experiment 1. Soil from an undisturbed pasture at Bundaberg that did not contain root-knot nematode was either sterilized by autoclaving or left untreated and then 28 styrofoam drinking cups were filled with either autoclaved or untreated soil. A sample of eight egg masses from a tomato root system was found to contain 3520 ± 68 eggs, and so half the cups of each soil were inoculated with eight egg masses and the remaining half with 3500 separated eggs of root-knot nematode extracted from the same root system with NaOCl (0.5 g/L available chlorine). The cups were then placed in polystyrene boxes, covered with moist paper to prevent soil from drying out, and after 10 days at ambient temperatures (22-26°C), J2 were extracted on a Baermann tray and counted. Thus this experiment consisted of autoclaved or untreated soil × separated eggs or egg masses as inoculum × 7 replicates. The second component was the same, except that tomato seedlings (cv. Tiny Tim) were planted in the cups following the 10 day incubation period and plants were rated for galling 25-30 days later using the first nine categories (0-8) of a gall rating scale developed previously by Zeck (1971).

The results of this experiment (Table 3.10) showed that when separated eggs were used as inoculum, fewer root-knot nematode juveniles were recovered and root galling was less severe in untreated soil than in autoclaved soil. However, the opposite tended to occur when egg masses were used as inoculum.

Table 3.10. Number of second-stage juveniles (J2) of root-knot nematode, and the level of galling caused by the nematode on tomato seedlings after untreated or autoclaved soil from a grass pasture was inoculated with the same number of separated eggs or eggs in egg masses.

	Soil assay [log (no. J2/200 mL soil)]		Plant assay [Gall rating on a scale of 0-8]	
	Separated eggs	Egg masses	Separated eggs	Egg masses
Untreated	1.995	2.501	4.29	5.71
Autoclaved	2.618	2.251	6.71	5.00
LSD (P=0.05)	0.204		0.768	

Validation experiment 2. Fifteen 200 mL styrofoam drinking cups were filled with four sandy loam soils from Bundaberg (designated soil A, B, C and D), and another 15 cups were filled with soil D that had previously been autoclaved. Inoculum of *M. javanica* was obtained by collecting mature egg masses from a culture maintained on tomato plants, by preparing a suspension of eggs from the same root system using NaOCl, or by hatching J2 from an egg suspension in a hatching chamber. Five replicate cups of each

treatment were then inoculated with 2000 newly-hatched juveniles, 4000 separated eggs or six egg masses (containing a total of about 4300 eggs). Cups were then covered with moist newspaper to prevent the soil from drying excessively and incubated at about 23°C for 10 days. Nematodes were then extracted from soil on a standard nematode extraction tray for 2 days, and the number of J2 in each sample was counted.

The results (Table 3.11) suggested that soils B, C and D were suppressive to root-knot nematode, as fewer nematodes were recovered from these treatments than from autoclaved soil or from soil A. There was also an effect of the type of inoculum (Table 2.11). However, there was no interaction between soil source and inoculum, indicating that the three types of inoculum all responded in the same way to each soil.

Table 3.11. Main effects of soil source and type of inoculum on recovery of second-stage juveniles of *Meloidogyne javanica* in a suppression assay

Soil or inoculum treatment	Log (number of second-stage juveniles)
Soil A	2.701
Soil B	2.481
Soil C	2.409
Soil D	2.473
Soil D (autoclaved)	2.646
LSD (P=0.05)	0.149
Egg masses	2.613
Separated eggs	2.570
Second-stage juveniles	2.443
LSD (P=0.05)	0.115

Discussion

The equivalent of 20, 40 and 80 t of dry matter/ha incorporated to a depth of 15 cm was added to soil in experiment 1, and 10-50 t dry matter/ha was applied in experiment 2. These application rates are much greater than would be possible in practice, but were used because the aim was to identify organic materials that enhanced the biological suppressiveness of soil to root-knot nematodes.

The results suggest that materials with a high C/N ratio are most likely to enhance suppression. In experiment 1, soil that had been amended with sugarcane trash four months previously was the only amendment that was suppressive in all three assays. Also, it was the only amendment to suppress the nematode in the pot assay. In experiment 2, sawdust, sugarcane trash and grass hay stood out from the

nitrogenous amendments as the only materials to induce suppression 10 months after they had been incorporated into soil.

Although relatively few biological parameters were measured in these experiments, there were indications that fungal-dominant soils with high populations of fungal-feeding, omnivorous and predatory nematodes were most suppressive to root-knot nematode. In contrast, both experiments showed that addition of large quantities of nitrogenous amendments to soil made it conducive to root-knot nematodes. The effects of poultry manure and Organic Xtra on galling at both 2 and 4 months in experiment 1 (Tables 3.2 and 3.4) provides evidence of the detrimental effects of nitrogen. Interestingly, these amendments were also detrimental to omnivorous and predatory nematodes.

In the suppression assays used in these experiments, separated eggs were added to soil because this was the easiest way to quantify the inoculum. However, data published after the experiments were completed (Orion *et al.* 2001) showed that separated eggs are more vulnerable to attack by soil microorganism than eggs in egg masses. Additional experiments were therefore done to validate the suppression assays, and they produced conflicting results. In the first experiment, fewer second-stage juveniles were recovered from field soil than from autoclaved soil when separated eggs were used as inoculum, whereas this did not occur with egg masses. In the second experiment, however, the type of inoculum did not affect the result of the suppression assay. These results, together with those of Orion *et al.* (2001), suggest that at least in some situations, separated eggs are more likely to be attacked by soil microorganism than eggs in egg masses.

Although it is possible that the level of suppressiveness measured in these experiments is higher than would have been obtained if egg masses rather than separated eggs had been used as inoculum, treatment effects are unlikely to have been influenced by the type of inoculum. My results are therefore useful for identifying suppressiveness. Interestingly, they showed that materials with a high C:N ratio enhance biological suppression of root-knot nematode whereas nitrogenous materials had little effect. Such a conclusion is contrary to conventional thinking on organic amendments for nematode control, as nitrogenous materials producing toxic decomposition products such as ammonia and nitrous acid are generally considered the most effective amendments (Rodriguez-Kabana 1986; Lazarovits *et. al.* 1999; Oka and Pivonia 2002). The problem with these materials is that their effects are relatively short-lived, and their high nitrogen content means that they are detrimental to the environment at the application rates needed to achieve control. Organic materials with relatively low nitrogen contents will be better from an environmental point of view, and my results suggest that they are also likely to provide better long-term control. The nematode counts in the pot assay in experiment 1 showed that sugarcane trash was suppressive 8 months after it was added to soil, while some amendments in experiment 2 were suppressive 10 months after they were applied.

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CHAPTER 4. THE IMPACT OF SOIL MANAGEMENT PRACTICES ON POPULATIONS OF ROOT-KNOT NEMATODE IN A VEGETABLE FARMING SYSTEM

Introduction

Many of the soil management practices used for vegetable production in Australia are detrimental to the soil biota. Land preparation usually involves a combination of ripping, disc or tyne cultivation and rotary hoeing, and these practices kill some of the macrofauna and deplete reserves of organic matter that fuel the detritus food web. Non-selective soil fumigants such as methyl bromide and metham sodium kill beneficial organisms as well as pathogens, while herbicides, plastic mulches and lack of green manuring further minimize the amount of organic matter available to soil organisms. The end result is a soil with little biological buffering capacity and a predominance of soil-borne pests and pathogens (e.g. plant-parasitic nematodes and root rotting fungi).

Root-knot nematode is one of the most important soil-borne pests of vegetables, particularly if crops are grown on sandy soils. Results presented in chapter 2 of this report show that some undisturbed pasture soils are naturally suppressive to this nematode, while the work in chapter 3 showed that suppressiveness to root-knot nematode can be enhanced by amending soil with high C/N materials. These observations suggest that organic inputs and tillage both affect the natural suppressive forces that normally keep this pest under control. The following field experiment was therefore established to determine whether suppressiveness to root-knot nematode could be enhanced by minimizing tillage and amending soil with organic matter.

Methods

In July 2003, a sandy loam soil on a property at Bundaberg was selected for the experiment. At that time, roots of zucchinis growing at the site were moderately to heavily galled by root-knot nematode (*Meloidogyne incognita*), and the root-knot nematode population density was about 1000 nematodes/200 mL soil.

During the following two months, the zucchinis were ploughed out and the site was rotary hoed in preparation for planting a green manure crop of forage sorghum. On 12 September 2003, 32 plots each 12 m wide × 1.4 m long were marked out to accommodate four replicates of the eight treatments listed in Table 4.1. A soil sample (20 cores with a 2 cm –diameter sampling tube) was then collected from each replicate to check the nematode population density before treatments were applied.

Establishment of treatments commenced on 17 September 2003. Sugarcane trash was spread on the surface of 16.8 m^2 plots at 21 kg/plot (i.e. 1.25 kg/m^2 or 12.5 t/ha); compost was applied at 4.2 kg/plot (i.e. 0.25

kg/m^2 or 2.5 t/ha) while nitrogen was applied as ammonium nitrate (478 g/plot or 28 g/m^2), which is equivalent to 100 kg N/ha.

Amendments were incorporated by rotary hoeing and bed-forming equipment was then used to form 0.7 m-wide beds in the centre of each plot. Since the amendments applied to each plot were dragged into the bed as it was formed, the application rates within the bed were approximately double the rates listed above. Two lines of trickle tubing were laid in beds that were not to be cultivated again, and then seeds of forage sorghum (cv. Zulu) were drilled into three rows on each bed.

Table 4.1. The sequence of events involved in establishing eight soil management treatments* prior to planting capsicums on 30 January 2004

Sequence of events	Amendments were incorporated into soil on 17 Sept. 2003	FS was planted on 17 Sept. 2003	On 25 Nov. 2003, FS was either incorporated with a rotary hoe (+) or left standing (-)	On 8 Jan. 2004, previously-cultivated plots were rotary hoed, fertilizer and other additives were applied and beds were formed		On 8 Jan. 2004, plastic was laid on beds in six treatments. The remaining treatments were mulched with FS on 19 Jan. 2004
Treatment no.	**Amendment**	**Forage sorghum**	**Tillage**	**Tillage at bed formation**	**Other additives**	**Mulch**
1	Nil	FS	-	-		FS
2	Nil	FS	-	-		P
3	Nil	FS	+	+		P
4	ST +N	FS	-	-		FS
5	ST +N	FS	-	-		P
6	ST + N	FS	+	+		P
7	Compost	FS	+	+	Compost	P
8	Nil	FS	+	+	Nematicide	P

* ST=sugarcane trash at 12.5 t/ha, N=ammonium nitrate at 100 kg N/ha, FS=forage sorghum, P=plastic mulch.

The forage sorghum crop produced about 13 t of dry matter/ha which was incorporated into 16 plots (treatments 3, 6, 7 and 8) with a rotary hoe on 25 November 2003. The forage sorghum in the remaining plots was slashed and allowed to ratoon. The ratooning foliage in treatments 2 and 5 was sprayed with glyphosate on 22 December 2003, and in treatments 1 and 4 on 6 January 2004.

On 8 January 2004, the previously-sprayed forage sorghum on beds to be used for treatments 2 and 5 was cut at ground level and removed. Gypsum, pelletised poultry manure (3.0% N, 1.6% P, 1.0% K and 2.5% Ca) and inorganic fertilizer (5.6% P, 19.9% K, 9.0% S) was then spread on the surface of all plots at 4 t/ha, 2.5 t/ha and 550 kg/ha, respectively. Compost (5 t/ha) was applied to treatment 7 and granules of Nemacur 100G (100 g fenamiphos/kg) were sprinkled on the soil surface of treatment 8 at 100 kg/ha. Plots were then rotary hoed, beds formed and plastic mulch laid in treatments 3, 6, 7 and 8. The plastic mulch for treatments 2 and 5 was laid by hand onto undisturbed beds. The treatment program listed in Table 4.1 was

completed on 19 January 2004, when the dead forage sorghum that was still standing in treatments 1 and 4 was laid on the surface of the bed to form mulch. Probes were inserted at a depth of 5 cm into plots mulched with plastic and forage sorghum, and soil temperatures were recorded with a data logger.

After all treatments were completed, each plot was split into sub-plots 5 m long, leaving a 50 cm gap between them and 75 cm barriers at either end. Inoculum of *M. incognita* (prepared by cutting galled roots of glasshouse-grown capsicum plants into small pieces and mixing the roots with sand) was then was added to 10 holes in one of the two sub-plots, so that each inoculated sub-plot received about 44,000 nematode eggs. Thus the final experiment consisted of 8 treatments × +/- root-knot nematode × 4 replicates, with the main treatments laid out in a randomized block design and nematode inoculum in split plots.

Capsicum cv. Raptor was planted on 30 January 2004 and the grower then managed the planting according to standard district practice. Application of nutrients through the trickle irrigation system commenced about 3 weeks after planting and the crop was also sprayed with pesticides for disease and insect control as required. Fruit was harvested three times (1, 15 and 29 May) and the number and weight of fruit in each plot was recorded.

Pest and disease damage to seedlings

The stems of some seedlings were severed by cutworms within a few days of planting. All plants were therefore sprayed twice with Lorsban to prevent further damage, and on 17 February 2004 (18 days after planting), the number of plants affected by cutworms was recorded. Symptoms of Pythium root rot caused by *P. apanidermatum* were also apparent within a week of planting and disease severity was assessed on 17 February by giving each seedling a plant health rating of 1-3, where 3= healthy plants showing no symptoms, 2= smaller plants with no other symptoms, 1= small, wilting, plants with some chlorosis, and 0= plants with almost no leaves, or dead.

Bacterial spot (caused by *Xanthomonas campestris* pv. *vesicatoria*) was observed after prolonged hot, wet weather in February and March, and its severity was assessed on 27 March 2004 by rating five randomly-selected plants on a 0-4 scale. Healthy plants showing no symptoms were given a rating of 0, plants with a severity rating of 2 had limited leaf lesions 1-2 mm in diameter and 5-10% of the leaves affected, while a rating of 4 indicated that lesions had merged, leaves were yellow and had started to drop, and more than 20% of leaves were affected. Ratings of 1 and 3 were used for situations of intermediate disease severity.

Effects on initial populations of root-knot nematode

Pre-plant soil samples (20 cores/plot) were collected at a depth of 0-18 cm on 19 January 2004, before plots were split and inoculated with root-knot nematode. A second set of samples (10 cores/plot) were collected from non-inoculated sub-plots on 18 February 2004. The severity of galling caused by root-knot nematode

was assessed on 9 March 2004 (38 days after planting) by removing one plant from each plot and rating the level of galling on the 0-10 scale of Zeck (1971). In cases where Pythium root rot had destroyed most of the roots, a missing value was recorded.

Effects on yield and final populations of root-knot nematode

The weight and number of fruit in each plot was recorded on 1 May, 15 May and 29 May 2004, and data from each harvest were summed to give total yields and numbers of fruit per plot. The number of plants harvested in each plot was also recorded so that yield could be calculated on a per plot or per plant basis. Soil samples for nematode analysis (10 cores per plot to a depth of 18 cm) were collected following the final harvest, and the level of galling on two plants in each plot was assessed as above.

Effects on soil and plant nutrients

Soil samples collected for nematode analysis on 19 January and 18 February 2004 were also used for nutrient analysis. Two composite samples of each treatment (one consisting of replicates 1 and 2, the other of replicates 3 and 4) were forwarded to Crop Tech Laboratories, Bundaberg, for analysis of major nutrients. The same procedure was used for the second sampling time, except that only N and K were analysed.

On 15 April 2004, plants in plots mulched with forage sorghum were yellow-green in colour, whereas plants in plots mulched with plastic were dark green. A leaf sample consisting of 12 of the oldest fully expanded leaves was therefore collected from each plot. Leaves from the four replicate plots of each treatment were combined into one sample and forwarded to Crop Tech Laboratories for nutrient analysis.

Effects on soil biology

The impact of treatments on soil biology was assessed by measuring culturable fungi, microbial activity and numbers of free-living nematodes. All parameters were measured on soil collected at the first two sampling times (19 January and 18 February 2004), while microbial activity was also assessed at the final sampling time (29 May 2004).

Fungi were quantified by adding 10g dry weight equivalent of soil to 90 mL of phosphate buffered saline and shaking the suspension on an end-over-end shaker for 20 minutes. A 10-fold dilution series was then prepared in phosphate buffered saline and 0.1 mL of appropriate dilutions were spread onto one-fifth-strength PDA. Colonies were counted after 1, 2 and 3 days, and counts were used to determine the number of colony-forming units (cfu) per g dry weight of soil.

Microbial activity was assessed by measuring the rate of hydrolysis of fluorescein diacetate (FDA) to fluorescein (Schnürer and Roswall 1982). A soil sample (5g dry weight equivalent) was incubated with

FDA and phosphate buffer at 26º-27ºC on an orbital shaker for 30 minutes. The reaction was then terminated with acetone, the mixture was centrifuged and the absorbance of the supernatant was measured on a spectrophotometer at 490 nm. Readings were corrected for background absorbance and the appropriate standard curves were used to calculate microbial activity (Chen *et al*. 1988). Results were expressed as µg FDA hydrolysed /g dry soil/min.

Nematodes were extracted from 200 mL soil samples using the Whitehead tray technique (Whitehead and Hemming 1965). Free-living nematodes were separated into four groups on the basis of their feeding habits (bacterivores, fungivores, omnivores and predators) and counted.

Results

Pest and disease damage to seedlings

Losses of seedlings due to cutworm damage were mainly confined to plots mulched with forage sorghum (Table 4.2). All deaths occurred within about 10 days of planting, and once seedlings were sprayed with Lorsban, further losses were not observed.

Pythium root rot (caused by *Pythium aphanidermatum*) was observed within a few days of planting, and the disease was almost certainly exacerbated by extreme weather conditions. Heavy rain (44 mm) fell on the day seedlings were planted and a further 242 mm of rain fell in the next four days. Soil temperatures at a depth of 5 cm ranged from 26-29 °C in plots mulched with forage sorghum, but were much higher in plots covered with black plastic (diurnal fluctuations from 28 to 41°C). When root rot data were analysed by analysis of variance, there was a significant treatment effect, but no effect of nematode inoculation and no interactions between treatment and inoculation for both of the parameters measured. When black plastic was laid onto non-amended soil without cultivation (treatment 2), Pythium root rot was particularly severe (Table 4.2). However, damage was much less severe when the same cultivation/mulch treatment was amended with sugarcane trash (treatment 5), suggesting that the amendment had enhanced suppressiveness to Pythium root rot. The other major treatment effect was the relatively low number of plants with severe Pythium root rot in plots mulched with forage sorghum (Table 4.2), almost certainly because of lower soil temperatures.

Two months after planting, bacterial spot was more severe on plants mulched with forage sorghum than on plants mulched with plastic. Average disease severity ratings for plants on plastic generally ranged from 1.25 to 2.0, whereas treatments 1 and 4 had a significantly higher rating of 3.25.

Table 4.2. Effect of soil management practices on losses due to cutworms and the severity of Pythium root rot in capsicum seedlings 18 days after planting.

No.	Treatment	% plants damaged by cutworms	Plant health rating	% plants poor or dead
1	No amendment/no tillage/forage sorghum mulch	11.8	2.15	7
2	No amendment/no tillage/plastic mulch	0	1.15	81
3	No amendment/cultivation/plastic mulch	0	2.15	27
4	Sugarcane trash +N/no tillage/forage sorghum mulch	10.8	2.45	1
5	Sugarcane trash +N/no tillage/plastic mulch	0.6	2.07	16
6	Sugarcane trash + N/cultivation/plastic mulch	0	2.47	14
7	No amendment/ cultivation/compost/plastic mulch	0	1.95	43
8	No amendment/cultivation/fenamiphos/plastic mulch	0	2.42	17
	LSD (P=0.05)	4.6	0.62	31.5

Effects on initial populations of root-knot nematode

Data obtained from soil samples collected prior to planting and 18 days after planting (Table 4.3) showed clear effects of tillage on numbers of root-knot nematode. In non-amended soil, nematode populations were relatively high in non-tilled plots (treatments 1 and 2) and much lower in tilled plots (treatment 3). Similar effects of tillage were observed in amended soil (treatments 4 and 5 v. 6).

The other major effect was the impact of the sugarcane trash + N amendment on numbers of root-knot nematodes. When non-amended and amended soil was compared for the same tillage and mulch treatment (e.g. treatments 1v. 4, 2 v. 5 and 3 v. 6), populations of root-knot nematode were usually about 10 times lower in amended than non-amended soil (Table 4.3).

When galling on roots was assessed 38 days after planting, one plant was sampled from plots inoculated with root-knot nematode and another was taken from non-inoculated plots. However, analysis of the data showed no effects of nematode inoculation and no interactions between treatment and inoculation, and so only treatment effects are shown (Table 4.3). Gall ratings were generally related to initial nematode densities in soil. Tillage reduced the severity of galling in non-amended soil, and gall ratings were much lower in amended than non-amended soil.

Table 4.3. Effect of soil management practices on populations of root-knot nematode in soil prior to planting and 18 days after planting, and the severity of galling caused by root-knot nematode on capsicum seedlings 38 days after planting.

No.	Treatment	No. root-knot nematodes /200 mL soil*		Gall rating
		Pre-plant	18 days	38 days
1	No amendment/no tillage/forage sorghum mulch	2.40 (247)	2.20 (156)	6.12
2	No amendment/no tillage/plastic mulch	2.18 (149)	1.69 (48)	6.62
3	No amendment/cultivation/plastic mulch	1.08 (11)	0.76 (5)	4.42
4	Sugarcane trash +N/no tillage/forage sorghum mulch	1.47 (29)	1.65 (44)	2.62
5	Sugarcane trash +N/no tillage/plastic mulch	1.06 (10)	0.50 (2)	2.92
6	Sugarcane trash + N/cultivation/plastic mulch	0.21 (1)	0.67 (4)	3.92
7	No amendment/ cultivation/compost/plastic mulch	0.75 (5)	0.76 (5)	3.75
8	No amendment/cultivation/fenamiphos/plastic mulch	0.59 (3)	0.41 (2)	1.58
	LSD (P=0.05)	0.552	0.553	1.56

* Values are transformed means [log (no. nematodes + 1)], with equivalent means in parentheses

Effects on yield and final populations of root-knot nematode

Analysis of yield data (kg fruit/plant and kg fruit/plot) showed similar trends, with a significant treatment effect, no effect of nematode inoculation and no treatment × inoculation interaction. The main treatment effect was a reduction in yield in the two treatments mulched with forage sorghum (Table 4.4). The severity of galling was lower in soil amended with sugarcane trash + N than in non-amended soil, with final gall ratings in amended soil comparable with the level of galling in fenamiphos-treated plots (Table 4.3). Final root-knot nematode population densities were lowest in amended soil that was not tilled prior to planting (Table 4.4).

Table 4.4. Effect of soil management practices on yield of capsicum, the severity of galling caused by root-knot nematode and final nematode population densities.

No.	Treatment	Fruit yield (kg/plant)	Gall rating	No. root-knot nematodes/200 mL soil
1	No amendment/no tillage/forage sorghum mulch	0.54	6.9	3.72 (5200)
2	No amendment/no tillage/plastic mulch	0.86	7.3	3.75 (5559)
3	No amendment/cultivation/plastic mulch	0.90	6.1	3.97 (9270)
4	Sugarcane trash +N/no tillage/forage sorghum mulch	0.68	4.9	3.57 (3715)
5	Sugarcane trash +N/no tillage/plastic mulch	0.98	5.3	3.54 (3428)
6	Sugarcane trash + N/cultivation/plastic mulch	0.89	5.1	3.93 (8452)
7	No amendment/ cultivation/compost/plastic mulch	0.87	5.7	4.09 (12218)
8	No amendment/cultivation/fenamiphos/plastic mulch	0.86	4.9	3.80 (6382)
	LSD (P=0.05)	0.212	0.82	0.285

* Values are transformed means [log (no. nematodes + 1)], with equivalent means in parentheses

Effects on soil and plant nutrients

The results of soil nutrient analyses (Table 4.5) showed that prior to planting, concentrations of N, K, Ca and Mg were much lower in plots mulched with forage sorghum than in all other plots. Ca and Mg were not measured in samples taken one month later, but differences in N and K were still apparent.

When nutrients were analysed in leaf samples taken on 15 April 2004, plants growing in plots mulched with forage sorghum had very high concentrations of P, very low concentrations of N and sub-optimal concentrations of Mg (Table 4.6). Concentrations of Ca and Fe were sub-optimal in some treatments, but concentrations of all other elements (e.g. K, Zn, S, Cu, Mn and B) were optimal and did not differ between treatments (data no presented).

Table 4.5. Effect of soil management practices on concentrations of major nutrients (mg/kg) in soil prior to planting capsicums and 19 days after planting.

	Pre-plant (19 January 2004)					Post-plant (18 February 2004)	
Trt. No.	N	P	K	Ca	Mg	N	K
1	15	163	143	899	137	11	71
2	40	194	277	1058	194	23	212
3	59	209	369	1490	208	56	285
4	14	150	135	935	153	17	87
5	51	155	276	1131	202	35	216
6	73	183	320	1445	214	48	247
7	63	168	335	1477	260	56	278
8	57	177	313	1310	230	71	350
LSD (P=0.05)	23	n.s.	106	251	41	20	68

Table 4.6. Effect of soil management practices on concentrations of some nutrients (mg/kg) in capsicum leaves collected 75 days after planting

Treatment no.	N	P	Ca	Mg	Fe
1	88	998	559	488	515
2	492	565	304	580	525
3	492	533	496	698	522
4	84	1200	456	551	80
5	554	629	344	712	504
6	686	593	192	701	522

Effects on soil biology

Numbers of culturable fungi (log cfu/g soil) ranged from 4.88 to 5.46, and did not differ significantly between treatments at either sampling time (data not presented).

Analysis of data for microbial activity using repeated measures procedures showed significant effects of treatment and time, but no interaction between treatment and time. The Greenhouse-Geisser epsilon was 0.6008. The main effects (Table 4.7) show that microbial activity was higher before planting than at the later sampling times, and was higher in soil amended with sugarcane trash + N than in non-amended soil.

When free-living nematode data at two sampling times were analysed by repeated measures, there were significant effects of treatment, but no effects of time or treatment × time (Greenhouse-Geisser epsilon = 1.00). Total numbers of free-living nematodes in amended soil that was not tilled prior to planting were higher than all other treatments (Table 4.8). When plots were covered with plastic mulch, cultivation prior to planting and the nematicide reduced the proportion of fungal-feeding nematodes in the nematode community.

Table 4.7. Main effects of soil management treatments and sampling time on soil microbial activity in a field trial on capsicums at Bundaberg

Main effects	Microbial activity (µg FDA/g/min)
Treatment	
1. No amendment/no tillage/forage sorghum mulch	0.59
2. No amendment/no tillage/plastic mulch	0.62
3. No amendment/cultivation/plastic mulch	0.60
4. Sugarcane trash +N/no tillage/forage sorghum mulch	0.88
5. Sugarcane trash +N/no tillage/plastic mulch	1.00
6. Sugarcane trash + N/cultivation/plastic mulch	0.92
7. No amendment/ cultivation/compost/plastic mulch	0.61
8. No amendment/cultivation/Nemacur/plastic mulch	0.49
LSD (P=0.05)	0.073
Sampling time	
Pre-plant (19 January 2004)	0.96
Post-plant (18 February 2004)	0.54
Final harvest (30 May 2004)	0.64
LSD (P=0.05)	0.155

Table 4.8. Effect of soil management practices on numbers of free-living nematodes* prior to planting capsicums and 18 days after planting.

No.	Treatment	Log total no. free-living nematodes /200 mL soil	Fungivores /Bacterivores + Fungivores
1	No amendment/no tillage/forage sorghum mulch	3.20	0.30
2	No amendment/no tillage/plastic mulch	3.27	0.32
3	No amendment/cultivation/plastic mulch	3.13	0.22
4	Sugarcane trash +N/no tillage/forage sorghum mulch	3.51	0.33
5	Sugarcane trash +N/no tillage/plastic mulch	3.48	0.29
6	Sugarcane trash + N/cultivation/plastic mulch	3.06	0.27
7	No amendment/ cultivation/compost/plastic mulch	3.01	0.24
8	No amendment/cultivation/Nemacur/plastic mulch	3.08	0.12
	LSD (P=0.05)	0.185	0.094

* Values are means for the two sampling times

Discussion

This work clearly demonstrated the biological benefits of adding organic matter to soil. There were higher numbers of free-living nematodes in amended than non-amended soil, and an increase in microbial activity due to the amendments that was sustained for at least eight months. Addition of organic matter also reduced the severity of Pythium root rot, increased the rate of decline in populations of root-knot nematode during a fallow and reduced the severity of galling on a capsicum crop planted more than four months after amendments were applied. The work also demonstrated the benefits of replacing plastic with organic mulch, particularly in summer when high soil temperatures under plastic exacerbate Pythium root rot (Stirling *et al.* 2004).

The results also highlighted the complexity of interactions between rotation crops, green manures, tillage, mulches, organic amendments and the soil biota, and reinforced the need for a farming systems approach to enhancing the activity of naturally-occurring predators of nematodes. One unexpected result was the relatively high numbers of root-knot nematode following forage sorghum. Forage sorghum is an excellent rotation crop for minimizing populations of root-knot nematode in sub-tropical climates (McSorley and Gallaher 1991; 1993; Gallaher *et al*. 1992) and experience in Queensland has shown that when forage sorghum cv. Jumbo is grown as a green manure crop, nematode populations are usually reduced to levels that do not damage vegetable crops (Stirling 1999). However, cultivar Zulu was used in this work and a later experiment (see Chapter 6 of this report) showed that it is more susceptible to root-knot nematode than cultivar Jumbo. This is possibly one reason why numbers of root-knot nematode were generally higher than expected at the time capsicum was planted. However, nematode numbers were also much lower in cultivated than non-tilled plots, suggesting that the cyanogenic compounds released from sorghum and

sudan grass hybrids when they are incorporated as a green manure (Widmer and Abawi 2000; 2002) are also important in achieving good root-knot nematode control. Since these compounds are only produced when tissue is disrupted during the green manuring process, this mechanism could not act when forage sorghum was killed with an herbicide rather than by cultivation.

Since root-knot nematode is a key pest of vegetables in sandy soils, the loss of one of the mechanisms by which forage sorghum controls the nematode has implications for the development of no-till farming systems for vegetable production. Crops to be used as mulch producers must be very poor hosts of the nematode, and so resistance to root-knot nematode will be an important criterion when selecting suitable mulch crops. Another way of tackling this issue would be to reconsider the way forage sorghum is mulched. Instead of rolling the crop and pressing the above-ground biomass onto the soil surface, it is possible that cyanogenic compounds could be released from this biomass by maceration, and then moved into soil by drenching or rainfall. This approach warrants further testing.

Because of the unexpected effect of non-tilled forage sorghum on populations of root-knot nematode, it was difficult to determine whether the soil's biological control potential was improved by minimizing tillage. However, when tillage treatments were compared under the same mulch treatment (e.g. 2 v. 3 and 5 v. 6), final population densities of root-knot nematode were lower in non-tilled plots, suggesting an effect of reduced tillage on suppressiveness.

Although non-tilled, organically amended and organically mulched plots (treatment 4) had high microbial activity and suffered little damage from either Pythium root rot and root-knot nematode, this treatment yielded poorly because of loss of nutrients from the soil profile. Pre-plant fertilizer was applied on 8 January and when soil nutrients were measured on 19 January, concentrations of N, K, Ca and Mg were much lower in organically-mulched plots than in plots covered with plastic. The most likely reason for this difference is that these nutrients leached from the profile in organically-mulched plots, as there was 179 mm of rain during that 11 day period. Since a further 560 mm of rain fell in the first 6 weeks after capsicums were planted, additional nitrogen from the pre-plant fertilizer or added via the trickle irrigation system was also lost, and so concentrations in soil and plant tissue remained at sub-optimal levels throughout the life of the crop. This result raises questions about how crop nutrition should be handled in organically-mulched, minimum till farming systems, particularly during periods of extreme rainfall. One concern is that leaching was as severe in organically-amended plots (treatment 4) as it was in non-amended plots (treatment 1), which suggests that increasing the cation-exchange capacity of soil by adding organic matter had little effect on nutrient losses.

In conclusion, this work showed that a vegetable farming system based on organic amendments, minimum tillage and organic mulching markedly improves the soil biology and reduces Pythium root rot by reducing

soil temperatures and increasing microbial activity. The organic amendment component also reduces populations of root-knot nematode and the severity of nematode damage, while minimum tillage tends to preserve the beneficial organisms responsible for suppressing the nematode. However, nutrient management, losses due to cutworms, increases in the severity of bacterial spot, and carryover of root-knot nematode on the rotation crop used for organic mulch are deficiencies in the alternative farming system that require further attention. Cutworms can be controlled by pest monitoring and appropriate chemical sprays, while resistant varieties and chemicals are available for bacterial spot, but the other two problems will require further research.

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CHAPTER 5. EFFICACY OF COMMERCIAL ORGANIC PRODUCTS AGAINST ROOT-KNOT NEMATODE

Introduction

Interest in soil health and organic production methods has increased in recent years, and this has resulted in increased demand for pest control products that are less toxic to humans and less damaging to the environment than the chemical pesticides widely used in modern agriculture. Since there is little experimental evidence that non-chemical products promoted for nematode control are efficacious, this project provided an opportunity to check their efficacy.

A field experiment investigating the impact of organic amendments and minimum tillage on root-knot nematode was reported in chapter 4. This experiment originally contained 10 treatments, but results from only eight treatments were presented in the previous chapter. The other two treatments were commercial products purported to control nematodes and they were incorporated into the experimental design from the inception of the experiment. However, results were not reported earlier because data collection ceased once the grower's fertigation program started to have an impact on plant nutrition. Details and results from these treatments, together with results from appropriate control treatments and a standard nematicide treatment are reported here. Results from additional experiments in the glasshouse and laboratory are also presented.

Methods

Field experiment

Specific details of the experiment are given in chapter 4, but briefly, this work was done in a sandy loam soil at Bundaberg that was infested with root-knot nematode (*Meloidogyne incognita*). Beds were formed in September 2004 and then forage sorghum was grown in all plots. In some cases, the forage sorghum was ploughed out in November, fertilizer and other additives were applied in January and then beds were re-formed and covered with plastic. In other cases, the forage sorghum was sprayed out, fertilizer and additives were added to undisturbed beds and sorghum biomass was used as mulch. Capsicum cv. Raptor was planted on 30 January 2004.

The two commercial treatments (A and B) consisted of a program based on products supplied by Nutri-Tech Solutions Pty. Ltd., Yandina QLD. The same products were included in both treatments, and they differed only in whether the soil was cultivated and plastic was used as mulch (treatment A) or whether beds were undisturbed and mulched with forage sorghum (treatment B). The treatment program was

designed by an agronomist from Nutri-Tech Solutions Pty. Ltd., and was based on soil analyses processed by the company prior to the commencement of the experiment. Details of the program are given below.

17 September 2003. Before beds were prepared and forage sorghum was planted, Nutritech Life Force™ was sprinkled onto the soil surface at 2.35 kg/plot (i.e. 0.14 kg/m^2 or 1.4 t/ha). This product consisted of a blend of un-specified nutrients, including K, B, Mo and Co.

8 and 19 January 2004. In treatment A, Nutriphos Guano™ granules (150 kg/ha), Huma Tech™ Soluble humate granules (5 kg/ha), Neem cake (500 kg/ha) and Scoria (500 kg/ha) were applied to the soil surface, plots were rotary hoed, beds were formed and plastic mulch was laid. In treatment B, the same products were applied, but they were sprinkled at the base of forage sorghum plants growing on undisturbed beds. On 19 January 2004, the forage sorghum was laid on the surface of the bed to form mulch.

30 January 2004. Prior to planting, seedlings were drenched with a solution containing kelp powder (1 g/L), fulvic acid (10 mL/L of a 1:100 dilution of the trade product), Nutri-Life Bio N™ (2 mL/L) and Nutri-Life VAM™ (12.5 g/L).

30 January 2004. Immediately after planting, a solution containing the following ingredients was drenched around the base of all seedlings at the application rates indicated in parentheses: Microverm™ (10 L/ha), Nutri-Life Bio P™ (1 L/ha), Root Guard™ (2 kg/ha), Molasses (10 L/ha), Sea Change™ (5 L/ha) and Aloe Tech™ (5 L/ha).

7 and 14 February 2004. Microverm™ (10 L/ha) was drenched around the base of all seedlings.

Treatments A and B did not receive any of the standard inorganic nutrient inputs used by the grower until he commenced applying nutrients through the trickle irrigation system about three weeks after planting. Assessments of these treatments were therefore confined to observations on the severity of Pythium root rot on 17 February 2004, to measurements of root-knot nematode populations on 19 January and 18 February 2004, and to observations of root galling on 9 March 2004.

Nematicidal activity of Neem

Neem cake obtained from Nutri-Tech Solutions Pty. Ltd. was mixed with a sandy, vegetable-growing soil from Bundaberg at application rates of 0, 0.33, 0.67, 1.33 and 2.67 g/L of soil. These application rates are equivalent to 0, 400, 1000, 2000 and 4000 kg of product/ha, assuming incorporation to a depth of 15 cm. Ten replicate 200 mL polystyrene drinking cups were then filled with each batch of soil. One set of five replicate cups were inoculated with 4000 eggs of root-knot nematode (*Meloidogyne javanica*) obtained by

immersing roots of galled tomato plants in NaOCl (0.5% available chlorine). Cups were covered to prevent drying, incubated at ambient temperatures for 10 days, and then nematodes were extracted from the soil using standard nematode extraction trays. Root-knot and free-living nematodes in the soil were counted.

The second set of five replicate cups were stored moist for one month, and then the above process was repeated (i.e. cups were inoculated with root-knot nematode, incubated for 10 days and then nematodes were extracted and counted).

Constituents of Root Guard™ and its nematicidal activity

Nutri-Life Root Guard™ is a talc-based formulation purported to contain four nematophagous fungi (*Arthrobotrys oligospora*, *A. conoides*, *Paecilomyces lilacinus* and *Verticillium chlamydosporium*). To confirm these fungi were present, the product was serially diluted in phosphate-buffered saline and five 20µL drops were plated onto one fifth-strength potato dextrose agar. Plates were inspected for fungal growth over a period of 7 days and fungal population densities were determined by most probable number and dilution plate frequency methods.

The same dilutions of the product were also added to one-quarter strength corn meal agar plates and then bacterial-feeding nematodes (*Caenorrhabditis elegans*) were added 24 hours later. Plates were incubated for 1, 2 and 3 weeks and then inspected for nematode-trapping fungi.

The effects of Root Guard™ on second-stage juveniles of root-knot nematode were determined by mixing the product with sandy loam soil at 0, 0.00134, 0.00268 and 0.00536 g/L of soil (the equivalent of 0, 2, 4 and 8 kg of product/ha, assuming incorporation to 15 cm). Five replicate polystyrene drinking cups were filled with 200 mL of each batch of soil, and efficacy was assessed using the same procedures as in the above neem experiment (i.e. soil was inoculated with 4000 eggs of *M. javanica*, cups were incubated for 10 days and then nematodes were extracted and counted).

The capacity of any egg-parasitic fungi in Root Guard™ to parasitise root-knot nematode eggs was determined by filling five replicate 1L pots with each of the four batches of soil (above). A tomato plant cv. Tiny Tim was planted in each pot and one week later, pots were inoculated with 2000 eggs of *M. javanica*. Three weeks after inoculation, Root Guard™ was drenched onto pots at the application rates used previously. Four weeks later (i.e. 7 weeks after plants were inoculated), plants were rated for galling on the 0-10 scale of Zeck (1971), and then five egg masses were removed from each plant and checked for fungal parasitism under a microscope.

Results

Field experiment

Data from the ten treatments included in the experiment were analysed by analysis of variance. Results from the two commercial treatments, two controls and the standard nematicide treatment are presented here.

Eighteen days after planting, many of the seedlings treated with the commercial products were severely affected by Pythium root rot. In each of the mulch regimes, disease severity was greater in the commercial products treatment than in the control, but this difference was not significant (Table 5.1).

Nematode counts taken prior to planting and 18 days after planting (Table 5.2) showed that populations of root-knot nematode in the two commercial product treatments were similar to the control with the same mulch treatment. Also, root galling at 38 days was not affected by the commercial products, whereas fenamiphos reduced galling to relatively low levels (Table 5.2).

Table 5.1. Effect of soil management practices on plant health and losses due to Pythium root rot in capsicum seedlings 18 days after planting.

No.	Treatment	Plant health rating	% plants poor or dead
A	Commercial products/cultivation/plastic mulch	1.56	52
3	No amendment/cultivation/plastic mulch	2.15	27
B	Commercial products/cultivation/forage sorghum mulch	1.80	21
1	No amendment/no tillage/forage sorghum mulch	2.15	7
	LSD (P=0.05)	0.67	35.9

Table 5.2. Effect of soil management practices on populations of root-knot nematode prior to planting and 18 days after planting, and the severity of galling caused by root-knot nematode on capsicum seedlings 38 days after planting.

No.	Treatment	No. root-knot nematodes /200 mL soil*				Gall rating
		Pre-plant		18 days		38 days
A	Commercial products/cultivation/plastic mulch	0.83	(6)	0.73	(4)	5.61
3	No amendment/cultivation/plastic mulch	1.08	(11)	0.76	(5)	4.42
8	No amendment/cultivation/fenamiphos/plastic mulch	0.59	(3)	0.41	(2)	1.58
B	Commercial products/cultivation/forage sorghum mulch	2.36	(228)	2.05	(111)	5.12
1	No amendment/no tillage/forage sorghum mulch	2.40	(247)	2.20	(156)	6.12
	LSD (P=0.05)	0.522		0.562		1.64

* Values are transformed means [log (no. nematodes + 1)], with equivalent means in parentheses

Nematicidal activity of Neem

Analysis of the root-knot nematode data at both sampling times showed significant treatment and time effects but no interaction between treatment and time. At application rates of 500 and 1000 kg/ha, neem cake did not reduce the number of root-knot nematodes recovered from soil (Table 5.3). However, numbers of root-knot nematodes were reduced at higher application rates.

Neem cake behaved like most organic amendments, increasing numbers of free-living nematodes in soil (Table 5.3).

Table 5.3. Effect of Neem cake on numbers* of root-knot nematode and free-living nematodes in soil.

Application rate (kg/ha)	No. root-knot nematodes/200 mL soil		No. free-living nematodes/200 mL soil	
0	2.818	(657)	3.013	(1030)
500	2.670	(467)	3.220	(1660)
1000	2.695	(495)	3.417	(2612)
2000	2.538	(345)	3.703	(5047)
4000	2.620	(417)	3.878	(7551)
LSD (P=0.05)	0.166		0.139	

* Values are log transformed means, with equivalent means in parentheses.

Constituents of Root Guard™ and its nematicidal activity

Aspergillus was the most common fungus recovered from the formulation of Root Guard™ that was tested, as it was present at about 1.1 x 10^8 colony-forming units (cfu)/g of product. Some *Paecilomyces* was detected but its concentration was relatively low (about 1.5 x 10^5 cfu/g).

When bacterial-feeding nematodes were added to dilutions of the Root Guard™ on agar, nematode multiplication occurred but nematodes were not captured by *Arthrobotrys* or any other genus of nematode-trapping fungus.

When Root Guard™ was mixed with soil at 2, 4 and 8 kg/ha, the number of root-knot nematodes recovered (596, 608 and 650 nematodes/200 mL of soil), did not differ significantly from the 646 root-knot nematodes/200 mL of soil recovered from the control.

Plants grown in soil that had received two applications of Root Guard™ three weeks apart were heavily galled (gall ratings of 7 and 8). Galling did not decrease as the application rate increased, and did not differ

significantly from the untreated control. All the egg masses examined were healthy, and there was no evidence of fungal parasitism.

Discussion

Most of the 14 products comprising the commercial program used in the field experiment were not expected to affect root-knot nematode and were recommended by the supplier's agronomist because of their plant nutrient or soil health benefits. However, two of the products (Neem and Nutri-Life Root Guard™) were included because of their purported effects on nematodes.

The technical basis for using neem is that Indian farmers traditionally use it for pest control and products made from neem (e.g. leaf material, seed kernels, seed powders, seed extracts, oil, sawdust and particularly oilcake), provide some control of plant-parasitic nematodes [see Akhtar and Malik (2000) for some of the relevant literature]. Results of the pot experiment with the neem cake supplied by Nutri-Tech Solutions Pty. Ltd. indicated that this product was nematicidal at 4 and 8 times the application rate used in the field experiment. However, even at those application rates, numbers of root-knot nematodes were reduced by only 37 and 47% relative to the control. It was therefore not surprising that neem cake was ineffective in the field at an application rate of 500 kg/ha. Products based on neem cake undoubtedly have potential for nematode control, but these products will never be widely accepted by growers until efficacy and consistency can be assured. There are two ways that suppliers can provide such an assurance: by checking the potency of a product with bioassays similar to those used in this study, or by providing analytical results which indicate the concentration of various active constituents (e.g. nimbin, salanin, thionemone and azadirachtin).

One observation made in the pot experiment was that the addition of neem cake to soil increased numbers of free-living nematodes, and that nematode numbers were related to application rate. Neem therefore acts like any other organic amendment in that it enhances soil microbial activity. It is therefore possible that in some situations, neem-based products stimulate the activity of naturally-occurring predators of nematodes.

Nutrilife Root Guard™ was purported to contain four fungal biological control agents of nematodes, namely two species of nematode-trapping fungi (*Arthrobotrys conoides* and *A. oligospora*), and two species of egg-parasitic fungi (*Verticillium chlamydosporium* and *Paecilomyces lilacinus*). Such products are sold on the assumption that when nematophagous fungi are added to soil, they establish themselves in the rhizosphere and then parasitise or prey on nematodes. Unfortunately, there is little experimental evidence to show that this occurs in field soil. The fungistatic nature of the soil microflora is a major impediment to establishment, usually preventing introduced fungal biological control agents from surviving for long periods in soil (Cooke and Satchuthananthavale 1968). Network–forming nematode trappers such as *A*

conoides and *A. oligospora* tend to be saprophytic rather than predacious and often do not trap nematodes in field soil (Jaffee 2003; 2004), while formulations containing egg-parasitic and nematode-trapping fungi are rarely effective in controlling root-knot nematode (Stirling *et al.* 1998a, b, c). Given these problems, together with the fact that *P. lilacinus* was the only nematophagous fungus detected in Nutrilife Root Guard™, it is not surprising that the product was not effective in either the field or glasshouse.

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CHAPTER 6. EXPERIMENTS WITH ORGANIC AMENDMENTS AND FORAGE SORGHUM

Introduction

Results presented in Chapter 4 indicated that populations of root-knot nematode declined more rapidly in the field when soil was amended with sugarcane trash (approximately 12 t/ha) and nitrogen (100 kg/ha). The experiment described in this chapter aimed to confirm these results and determine whether the impact of sugarcane trash was affected by the amount of nitrogen applied.

Two other experiments are described (one in the glasshouse and the other in the field), and they follow up observations in the above field trial which showed that root-knot nematode populations on non-tilled forage sorghum cv. Zulu were unexpectedly high (see Chapter 4). The aim of these experiments was to compare the multiplication rates of root-knot nematode on several cultivars of forage sorghum and various grasses.

Effect of sugarcane trash on the rate of decline in populations of root-knot nematode

Methods

Inoculum of root-knot nematode (*Meloidogyne incognita*) was prepared by chopping nematode-infested capsicum roots into pieces 1-2 mm long and mixing the chopped roots with 2 L of sandy loam soil from a vegetable farm at Bundaberg. Egg counts on samples of chopped roots before the inoculum was mixed with soil indicated that 20 mL samples contained about 8,800 eggs.

Sixty 1 L pots were filled with the above field soil and all pots were inoculated with root-knot nematode by adding 20 mL of inoculum to each pot. Three organic amendment treatments were then imposed on 20 replicate pots. In treatment A, 8 g of sugarcane trash (chopped into pieces less than 10 mm long) and 0.143 g of urea were mixed into each pot, which was equivalent to 12 t dry matter/ha and 100 kg N/ha. Treatment B received the same amount of sugarcane trash but 0.715 g urea/pot (i.e. 500 kg N/ha), while treatment C was a non-amended control. Soil samples from the control processed at the time the experiment was established indicated that the initial nematode population density in pots was about 9, 670 root-knot nematodes/200 ml soil.

Pots were placed in polystyrene boxes, covered with paper to minimize drying, incubated at ambient temperatures ranging from 12-30°C and watered when the soil surface was dry (i.e. every 1-2 weeks). At four times after the commencement of the experiment (4, 8, 12 and 16 weeks), soil from five replicate pots of each treatment was mixed and a 200 mL sample of the soil was placed on a nematode extraction tray for two days. Numbers of second-stage juveniles of root-knot nematode and numbers fungal-feeding,

bacterial-feeding and omnivorous (Dorylaimida) nematodes were counted. A 5 g sample of soil from each pot was also taken to estimate microbial activity using methods described in previous chapters.

The fate of the nitrogen added with the sugarcane trash was studied during the first 19 days of the decomposition process. Additional pots of treatments A, B and C, together with soil amended with sugarcane trash without additional N were sampled 2, 8 and 19 days after amendments were added, and the concentration of nitrate-N and ammonium-N in soil was determined.

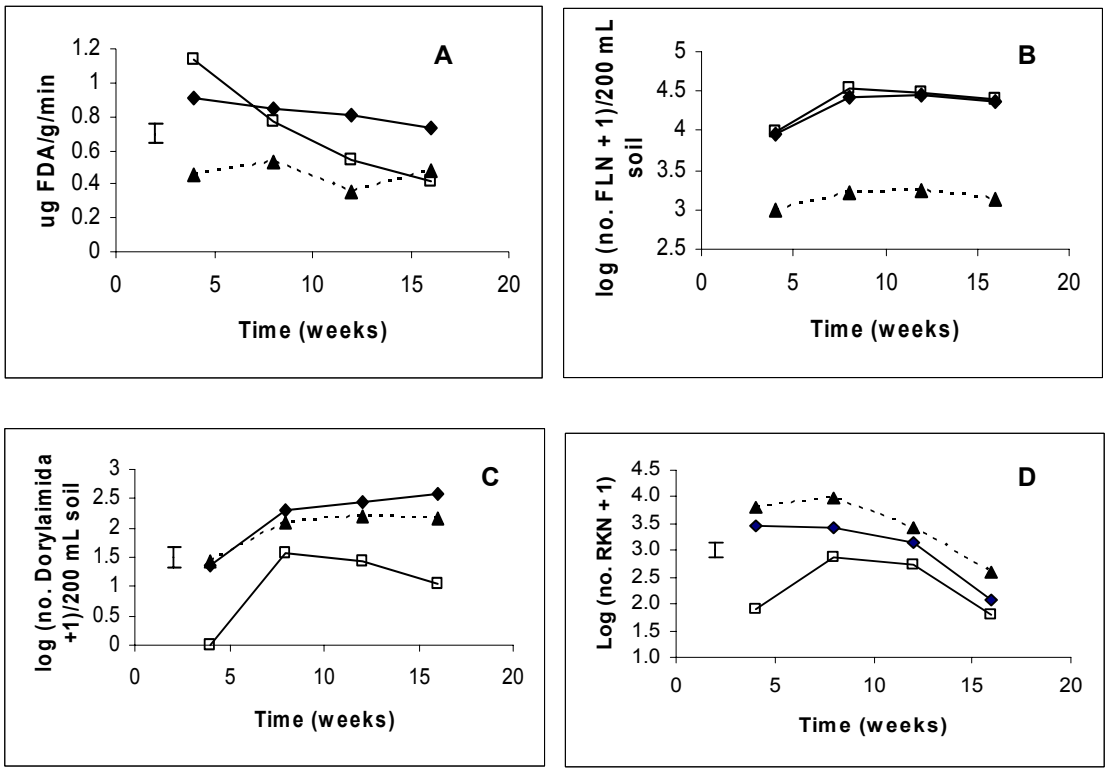
Microcosms were buried in an additional four replicate pots of each treatment to obtain information on biological control agents that were active in soil during the course of the experiment. Microcosms were prepared by gluing synthetic mesh (100 μm mesh size) to the base of plastic cylinders 25 mm in diameter and 10 mm high. At the time the experiment commenced, five microcosms filled with about 5 mL of soil from each pot were buried in the pots from which the soil was obtained. These pots were then incubated under the same conditions as the pots used for the previous experiment. Microcosms were retrieved from one replicate pot of each treatment at each sampling time and placed (mesh base down) on the surface of water agar in a Petri dish. After 6 or 7 days, microcosms were removed and a drop of water was added to the agar previously in contact with the microcosm. This area was then covered with a coverslip and the agar surface was scanned for evidence of predatory activity.

Results

Nematode data and estimates of microbial activity obtained from the pot experiment are presented in Figures 6.1 A-D. Addition of sugarcane trash + 100 kg N/ha markedly increased microbial activity compared with the non-amended control, and this difference was maintained throughout the experiment (Figure 6.1 A). The sugarcane trash amendment behaved differently when 500 kg N/ha was added, as microbial activity was highest at week 4 and then declined with time, so that at week 16 it did not differ significantly from the non-amended control. Both amendments increased populations of free-living nematodes to much the same extent (Figure 6.1 B), except that numbers of Dorylaimida were affected differently. These nematodes were not recovered at 4 weeks from soil treated with the high rate of nitrogen, and numbers of Dorylaimida remained lower than in the non-amended control for the remainder of the experiment (Figure 6.1 C).

Amending soil with sugarcane trash + nitrogen increased the rate of decline in populations of root-knot nematode compared with the non-amended control (Figure 6.1 D). However, the addition of 500 kg N/ha had a greater impact than 100 kg N/ha, particularly at the first and second sampling times.

Figure 6.1. Microbial activity and nematode populations in non-amended soil (--▲--) and in soil amended with sugarcane trash + 100 kg N/ha (♦) or sugarcane trash + 500 kg N/ha (□), 4, 8, 12 and 16 weeks after soils were potted and amendments were added.



Results of chemical analyses (Table 6.1) showed that there was very little NO_3-N or NH_4-N in non-amended soil or soil amended with sugarcane trash. Sugarcane trash + 100 kg N/ha had some impact on both forms of nitrogen, but the addition of 500 kg N/ha had a major impact. In this treatment, the concentration of NH_4-N was 259 µg/g soil after 8 days, while NO_3-N levels reached 112 µg/g soil after 19 days.

Fungal predators of nematodes were not observed when microcosms containing amended and non-amended soil were placed on an agar surface at the first three sampling times. However, at 16 weeks, a fungal-feeding nematode captured by a fungus was observed in soil amended with sugarcane trash + 100 kg N/ha. This fungus, which was not identified because it did not sporulate, is not a nematode-trapping hyphomycete. It has been observed previously in organically-amended soil from Bundaberg, and is capable of capturing large numbers of nematodes on agar plates (Stirling *et al.* 2005).

Table 6.1. Concentrations of nitrate and ammonium nitrogen in soil 2, 8 and 19 days after soil was amended with sugarcane trash with and without nitrogen (as urea).

Amendment	NO_3-N (µg/g soil)			NH_4-N (µg/g soil)		
	2 days	8 days	19 days	2 days	8 days	19 days
No amendment	10	-	-	0.3	-	-
Sugarcane trash (12 t/ha)	2	-	-	0.8	-	-
Sugarcane trash (12 t/ha) + 100 kg N/ha	5	16	15	31	14	1
Sugarcane trash (12 t/ha) + 500 kg N/ha	10	32	112	171	259	132

Discussion

Populations of root-knot nematode in this experiment (when averaged over the four sampling times) were 60% lower in soil amended with sugarcane trash + 100 kg N/ha than in non-amended soil. Root-knot nematode populations therefore declined at a grater rate in amended soil, thus confirming observations made previously in the field. Increasing the amount of urea added with the sugarcane trash increased the level of root-knot nematode control, but was detrimental to the soil biology. For example, omnivorous nematodes (Dorylaimida) were killed by nitrogen at 500 kg/ha and populations had not recovered after 16 weeks. The high levels of ammonium and nitrate nitrogen in this treatment soon after urea was applied probably explain the effect on both root-knot and dorylaimid nematodes, as both groups (but particularly the dorylimids) are sensitive to nitrogen (Tenuta and Ferris 2004).

Observations made by transferring microcosms containing amended or non-amended soil to agar plates provided no indication of whether fungi were involved in reducing root-knot nematode populations in amended soil. An unidentified predatory fungus was seen in soil amended with sugarcane trash + N, but was not recovered consistently. Further work is therefore required to determine whether the microcosm method is appropriate for monitoring predatory activity, and to identify the predators that are active in amended soil.

Multiplication of root-knot nematode on various grasses

Methods

Pot experiment. Seedlings of Rhodes grass, oats and forage sorghum (5, 3 and 1 seeding/pot, respectively) were established in1.5 L pots of pasteurised washed river sand. One month later, four pots were inoculated with 4000 eggs of *M. javanica* and another four pots with *M. incognita*. After nine weeks in a glasshouse, nematodes were extracted from 200 mL soil samples and nematode eggs were retrieved from roots with NaOCl. The number of nematodes in each pot was determined by multiplying the soil count by 7.5 and adding the egg count. Nematode counts were transformed [log (no. nematodes + 1)], and data were analysed by two-way analysis of variance.

Field experiment. Thirty 2 × 2 m plots were marked out in sandy loam soil at Bundaberg and five pre-plant soil samples (each consisting of 12 cores taken at a depth of 0-15 cm) were collected on 23 September 2004. Six cultivars of forage sorghum were then sown into each of five replicate plots at a seeding rate of 2.5 g seed/m^2, and all plots were sampled just before the crop was incorporated as a green manure (i.e. two months after planting). Initial and final root-knot nematode population densities were determined by placing 400 mL samples on a standard nematode extraction tray for 4 days.

Results

Pot experiment. More root-knot nematodes were recovered from pots inoculated with *M. javanica* than *M. incognita*. There was also a significant effect of plant species but no interaction between nematode species and plant species. Nematode counts averaged across both nematode species are presented in Table 6.2.

Only two grass cultivars (forage sorghum cultivars Jumbo and Pac F8350) were highly resistant to root-knot nematode. There was limited multiplication on most grasses, but forage sorghum cultivars Sugargraze and Nectar, oat cultivar Saia, and tomato cultivar Tiny Tim supported relatively high nematode populations.

Field experiment. The initial population density of root-knot nematodes in the field was very low (1.2 nematodes/400 mL soil), largely because the field had been fallowed for 9 months following capsicums the previous summer. Only three replicate plots were sampled when the forage sorghum matured because the crop grew poorly in the other replicates due to soil compaction from a previous roadway. Nevertheless, the data showed that root-knot nematode multiplied on all cultivars (Table 6.2). Final population densities did not differ significantly between cultivars but trends were similar to the pot experiment, with cultivars Jumbo and Pac F8350 having the lowest nematode densities.

Table 6.2. Final populations of root-knot nematode (averaged for *Meloidogyne javanica* and *M. incognita*) on various cultivars of forage sorghum, Rhodes grass, oats and tomato, 9 weeks after pots were inoculated with 4000 eggs of the nematode (pot experiment) or 8 weeks after forage sorghum was planted into a field infested with *M. incognita* (initial density 1.2 nematodes/400 mL soil)

	Final population of root-knot nematodes*			
	Pot experiment		Field experiment	
Sugargraze forage sorghum	4.708	(51050)	2.36	(229)
Nectar forage sorghum	4.532	(34039)	1.92	(82)
Hunnigreen forage sorghum	3.963	(9183)	-	-
Everlush forage sorghum	3.639	(4355)	1.83	(67)
Zulu forage sorghum	3.032	(1076)	2.37	(234)
Jumbo forage sorghum	1.823	(65)	1.14	(13)
Pac F8350 forage sorghum	1.408	(24)	1.69	(48)
Katambora Rhodes grass	2.830	(695)	-	
Callide Rhodes grass	3.694	(4942)	-	
Saia oats	4.337	(21727)	-	
Nugene oats	3.906	(8053)	-	
AS-5 oats	3.379	(2393)	-	
Enterprise oats	3.557	(3605)	-	
Tomato (Tiny Tim)	4.624	(42072)	-	
LSD (P=0.05)	0.8146		n.s.	

* Data are nematode numbers/pot (pot experiment) and nematodes/400 mL soil (field experiment). Transformed means [log (no. nematodes + 1)] are presented, with equivalent means in parentheses

Discussion

This work showed that resistance to root-knot nematode is an important consideration when selecting a rotation/mulch crop for a no-till vegetable cropping system. The economic threshold for root-knot nematode on capsicum is in the order of 10-30 root-knot nematodes/400 mL of soil, and this threshold was exceeded following several forage sorghum cultivars in the field trial, and by forage sorghum cv. Zulu in the field experiment described in Chapter 4. Forage sorghum cv. Jumbo is relatively resistant to both *M. incognita* and *M. javanica*, and appears to be the best option at present, but the result with Pac F8350 in the glasshouse suggests that it may be worth testing further in the field.

Of the other grasses tested, Rhodes grass cv. Katambora warrants consideration for no-till vegetable cropping systems, as it was a relatively poor host of root-knot nematode in the glasshouse. It grows well in a sub-tropical environment and limited tests in small plots have shown that it produces satisfactory mulch when planted on beds to be used for vegetable production.

References

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