THE INCIDENCE OF PLANT-PARASITIC NEMATODES ON SUGARCANE IN QUEENSLAND, AND STUDIES ON PATHOGENICITY AND ASSOCIATED CROP LOSSES, WITH PARTICULAR EMPHASIS ON LESION NEMATODE (*PRATYLENCHUS ZEAE*)

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ABSTRACT

In Queensland, sugarcane has been cropped as a monoculture for 80 years or more in most districts. In the last 30 years, plough-out and replant (no fallow) has increased, as has reliance upon inorganic fertilisers, and intensive tillage to remove soil compaction. An associated decline in the productive capacity of the soil to grow sugarcane has been identified, and has been termed 'yield decline' (YD). Root health and sugarcane yields are increased after fallowing, crop rotation, and soil fumigants (Magarey and Croft 1995; Garside *et al*. 2001; Meyer and Van Antwerpen 2001), implicating root pathogens in YD. However, in the past, nematode studies have been confined to testing the economics of using nematicides.

It was the objective of this work to explore the association between plant-parasitic nematodes and sugarcane in Queensland. Firstly, this thesis examines the incidence of nematodes on field crops. The regional distribution of nematodes is reported, together with nematode populations and dynamics relating to (a) root habit, (b) root distribution across the row to inter-row profile, and (c) temporal changes during the crop cycle.

Secondly, this thesis explores the parasitism of Queensland sugarcane by nematodes, and role in YD. The importance of sett roots, nematodes, and general YD biota on early plant establishment from 0-100 days after planting is examined in field miniplots. Crop losses due to nematodes are assessed at 16 field sites using non-volatile nematicides, and the pathogenicity of *Pratylenchus zeae* is examined in glasshouse pots and field miniplots.

The lesion nematode (*P. zeae*) was found to be ubiquitous in sugarcane fields, and usually at higher densities than other species. The density of root-knot nematode (*Meloidogyne* spp.) was also high in sandy soils (<20% clay), but a high proportion of other soils also contained this nematode, albeit at lower densities. The ectoparasites, spiral nematode (*Helicotylenchus dihystera*), stubby-root nematode (*Paratrichodorus minor*) and stunt nematode (*Tylenchorhynchus annulatus*) were also detected in a high number of fields (>66%). Historically, the sugar industry has perceived nematode problems to be confined to very sandy soils in south

Queensland. However, plant-parasitic nematodes occur in all soils, suggesting a more widespread role in YD.

Within sugarcane fields, nematodes were distributed in aggregated patterns. Thus, densities of lesion nematode varied up to five-fold across short distances (1.4 m) even at a constant distance (20 cm) from the sugarcane stool. Ring and spiral nematode were more aggregated than lesion nematode, perhaps due to more sedentary feeding habits and greater sensitivity to edaphic gradients (eg. soil texture and moisture) across the field at the macro-distributional level. The 'negative binomial model' was used to predict the sampling effort required to estimate mean nematode densities with degrees of precision.

Mean nematode densities across the row, near row (20-30 cm from the stool), and inter-row were very similar during the crop cycle. Because high densities of nematodes were regularly recovered from 'near the row' this zone was recommended for standard sampling. During the crop cycle, nematode densities were related to the volume of the root system and its growth rate, as influenced by season. Because sugarcane develops a new root system annually, nematode densities increased and then declined each year. At planting, up to 400 lesion nematodes and up to 100 spiral nematodes/200 mL of soil were present, which was usually more than other pest species (<50 nematodes/200 mL of soil). Lesion nematode generally persisted at higher densities than other pest species during the crop cycle.

Lesion nematode was pathogenic to sugarcane in 1.5 L pots, reducing root weight and sometimes reducing shoot biomass. In 50 L pots, this nematode caused a general blackening of roots and reduced fine root length by over 50%. Shoot biomass was generally not reduced, suggesting that YD is induced by a combination of root pathogens.

At planting, prior studies have related poor primary tiller emergence to poor sett root growth in field soil (Cadet and Spaull 1985; Garside *et al.* 2002 a; Pankhurst *et al.* 2002). However, this study showed that buds can rely entirely upon the stem cutting to shoot and become established primary tillers. It was concluded that damaged buds, dormant buds, a poorly nurtured seed source, and poor sett root growth, all contribute

to poor primary tiller establishment. Deleterious soil biota and nematodes also reduced the health and volume of shoot roots, which reduced the number of secondary tillers emerging at early establishment. While the experimental sites had a history of consistent fumigation responses (>80%), nematicide responses were quite variable (0-50%). Experiments in glasshouse pots confirmed that nematodes contribute in part to fumigation responses in YD soils.

To assess crop losses, nematodes were controlled for the entire crop cycle using non-volatile nematicides at 16 field sites. Fertile sandy loam to clay soils were chosen where losses from nematodes have only been speculated on previously. While poor tillering due to serious nematodes problems is well documented in sandy soils (<10% clay) in Queensland and around the world (Bull 1981; Spaull and Cadet 1990), stalk numbers were increased with nematicides only at some of the sites reported in this thesis. This contrast was attributed to the relatively low populations of root-knot nematodes (*Meloidogyne* spp.) at planting, and higher soil fertility. However, stalk length was significantly increased in nematicide-treated plots at most sites. Thus, responses in harvest yield of 0-20 T/ha were usually observed in both plant and ratoon crops. Untreated crop yields were average for the surrounding districts, as were nematode densities, suggesting the responses were robust across regions. Upon extrapolation, lost productivity from nematodes is estimated at over A$ 100 million annually. These results indicate that nematodes are a subtle but important pest, and contribute to YD on the sandy loam to clay soils on which 95% of Australia's sugarcane is grown.

The environment and/or level of crop management influenced yield losses from nematodes, and nematicides responses were related to the control of a number of species, especially in ratoons. However, lesion nematode was correlated most consistently with reduced sugarcane yield. It was concluded that lesion nematode is the most important nematode pest of sugarcane in Queensland, and contributes to YD by reducing the health of primary and secondary roots, and by decreasing the length and number of fine roots.

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LIST OF ABBREVIATIONS

A	Australian
BSES	Bureau of Sugar Experiment Stations
CCS	commercial cane sucrose
DAP	days after planting
DAR	days of ratoon
DOF	days of fallow
EM	environmental factors and/or level of management
P_i	nematode density in the soil at planting
PVC	poly vinyl chloride
QDPI	Queensland Department of Primary Industries
®	registered trading name
SL	stalk length
SN	shoot or stalk numbers
YD	yield decline
UC	University of California
CEC	cation exchange capacity
EDB	ethylene dibromide
Ca	calcium
K	potassium
Mg	magnesium
P	phosphorus
a.i.	active ingredient
cv.	cultivar
dry wt.	oven dry weight
wt.	weight
eg.	for example
i.e.	specifically
n	number of sub-samples
no.	number of
pers comm.	unpublished personal communication
unpub.	unpublished observation by Blair

spp.	species
°C	degrees celcius
ha	hectares
T/ha	tonnes per hectare
ML	megalitres
mL	millilitres
m	metres
cm	centimetres
mm	millimetres
µm	micrometres
kg	kilograms
g	grams

ANOVA	analysis of variance
CV	coefficient of variation
E	standard error/mean ratio
F test	A test of data variance, estimating the probability that observations are random events (eg. $P<0.05$ = the probability that data sets are random is less than 5%).
LSD	least significant difference
ns	not significant at $P=0.05$
P	probability
R^2	coefficient of determination
s^2	sample variance
0	sample mean
x	sample mean in an equation
%	percent of
<	is less than
≤	equal to or lower than
>	is greater than
≅	is approximately equal to
≈	approximately
×	multiplied by

CHAPTER 1

A REVIEW OF THE PARASITISM OF SUGARCANE ROOTS BY NEMATODES: A QUEENSLAND PERSPECTIVE

1.1 Preamble

This review discusses the parasitism of sugarcane roots by plant-parasitic nematodes, and its relation to the commercial production of sugarcane in Queensland.

Material under review includes:

(a) the nematode genera deemed to be important pests in parasitising the roots of sugarcane in Queensland,

(b) the symptoms of nematode attack upon the root system and the density of nematodes required to cause those symptoms,

(c) environmental factors that affect nematode damage levels on sugarcane roots,

(d) prospects for control that are applicable to the commercial production of sugarcane in Queensland.

1.2 Introduction

The sugarcane cultivars grown today are crosses between species of the genus *Saccharum.* The primary ancestors are *S. spontaneum* Brandes and Jesweit and *S. officinarum* Linn, the latter being characterised by its thick stems and a high sucrose content (Julien *et al.* 1989). In Australia, sugarcane is grown over an area of 540,000 ha, on coastal plains from Grafton in northern New South Wales to Mossman in far-north Queensland (Canegrowers 2000). Approximately 3.6 million T of sugar are produced annually from the crop in Australia, which is worth over A$1 billion to the export economy (Canegrowers 2000). The tropical to subtropical climate, and the extensive fibrous root system of sugarcane, provides an ideal environment for root-parasitising nematodes. Thus, a diversity of nematode species

are abundant in sugarcane fields (Spaull and Cadet 1990). Plant-parasitic nematodes are small, elongated (0.3-2 mm), pseudocoelomate eelworms of the Phylum *Nematoda.* They possess a cuticular exoskeleton, simple digestive system and longitudinal muscles for locomotion (Siddiqi 1985).

1.3 Endoparasitic nematodes

Endoparasitic nematodes such as *Meloidogyne, Pratylenchus* and *Achlysiella* spp. enter root tissue and spend the majority of their life cycle there. They are considered to be the most damaging nematode group associated with sugarcane roots.

1.3.1 *Pratylenchus* spp.

Lesion nematode (*Pratylenchus* spp.) is the most frequently encountered nematode pathogen of sugarcane (Spaull and Cadet 1990), and *P. zeae* Graham is the most widespread nematode species of this genus. *Pratylenchus* spp. is migratory, moving and feeding intra- or inter-cellularly in the root cortex (Trudgill 1991) (Plate 1.3.1.1 and 1.3.1.2). Death of the cortical cells leads to the formation of cavities and secondary infection by fungi and bacteria (Luc *et al*. 1990 a). Symptoms of *Pratylenchus* spp. damage to sugarcane grown in pots are:

(a) slightly shortened, coarse and thickened primary roots,

(b) fewer feeder roots,

(c) fewer root hairs,

(d) root lesions at the point of nematode entry (Plate 1.3.1.3),

(e) necrotic areas that encircle the primary roots where lesions are extensive (Harris 1974).

The pathogenicity of *P. zeae* on sugarcane has been demonstrated in glasshouse pot experiments in Louisiana (Khan 1963), India (Nath *et al*. 1975) and Puerto Rico (Valle-Lamboy and Ayala 1980). A significant reduction in plant growth occurred when the initial *P. zeae* population exceeded 1000/kg of soil according to Nath *et al*.

(1975). Valle-Lamboy and Ayala (1980) recorded a similar result using an initial *P.zeae* inoculum of 1200 nematodes/kg of soil.

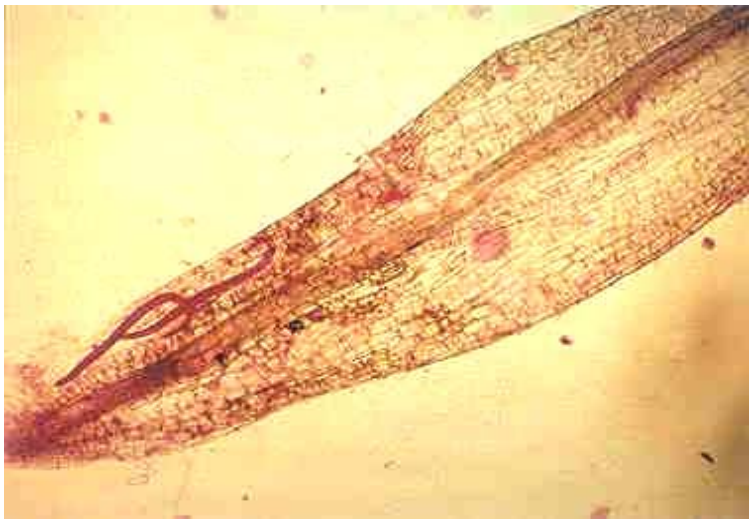


Plate 1.3.1.1: *Pratylenchus zeae* parasitising a secondary root-tip of sugarcane (magnification × 50).

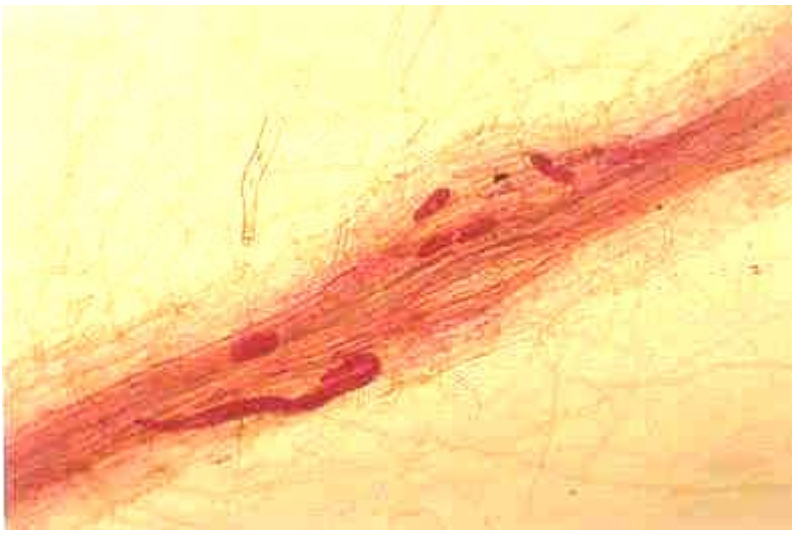


Plate 1.3.1.2: *Pratylenchus zeae* and eggs inside a tertiary root of sugarcane (magnification × 100).

Plate 1.3.1.3: Lesions on new primary roots from the entry of *Pratylenchus zeae* (magnification × 2).

Plate 1.3.2: Terminal galls on the primary roots of sugarcane cultivar Q141 caused by *Meloidogyne javanica* Treub (magnification × 1/2).

1.3.2 *Meloidogyne* spp.

Root-knot nematode (*Meloidogyne* spp.) is a sedentary endoparasite. Vermiform juveniles disperse through the soil and locate and penetrate host roots. Root tissue is stimulated into producing giant nurse cells that act as a nutrient source for the enlarging adult nematode and its associated egg sac. As a result, the tips of primary and fine roots develop terminal galls or clubs (blind rot) (Plate 1.3.2). This process effectively halts the progress of roots through the soil. The uptake and translocation of water and nutrients by the roots is also disrupted (Stirling 1992). The reduced growth of sugarcane roots and shoots has been demonstrated in the glasshouse in short-term pot experiments when *Meloidogyne* spp. are present at sufficient densities (Jensen *et al*. 1959; Harris 1974; Valle-Lamboy and Ayala 1980).

1.3.3 Effect on planted crops

In order to understand the effect of endoparasitic nematodes on root health, an introduction to root establishment is required. In the field, the crop is initiated vegetatively by planting stem lengths (setts) that have 2-3 nodal buds. Sett roots grow from the node region on the stem and stimulate the adjoining bud to initiate growth, which emerges from the soil as a primary shoot. Secondary shoots then bud out from the base of the primary shoot by a process known as tillering. As the shoots tiller, shoot roots are initiated from their base and the sett root system makes no further contribution to growth.

The period that the tillers rely upon the sett roots is uncertain, with periods of 4-6 weeks (Martin 1961) and 2-3 months (Julien *et al.* 1989) suggested in the literature. The growth of tillers is impaired if the sett roots are damaged (Bonazzi 1928), so it could be reasoned that any factor that restricts sett root growth (eg. nematode attack), could cause a reduction in tillering.

Cadet and Spaull (1985) made a distinction between nematode attack on sett and shoot root systems, in nematicide field studies in Africa. A brief summary of these findings is represented below.

In West Africa, high levels of *Meloidogyne* spp. (3000/g of root, dry weight) and *Pratylenchus* spp. (750/g of root, dry weight) built up in the sett roots of untreated sugarcane by 30 days after planting (DAP). The control of these nematodes in nematicide-treated plots improved tillering by 46%. In South Africa, while comparably high numbers of *Meloidogyne* spp. (1200/10 g of wet roots) and *Pratylenchus* spp. (5000/10 g of wet roots) were recorded in the sett roots as in West Africa, it took 150 DAP for nematode populations to reach a maximum inside the roots. Sett roots could still be found up to 210 DAP. In nematicide-treated plots, the improvement in tillering was estimated to be only 20%. Cadet and Spaull (1985) therefore concluded that the attack by nematodes on sett roots within 30 DAP, reduced tillering, thereby causing a major reduction in sugarcane growth. In another African study, reduced tillering was attributed to attack by *Pratylenchus* and *Meloidogyne* spp. (8000/g of dry roots) on sett roots within a month of planting (Cadet *et al*. 1982).

No other field studies have specifically studied sett root parasitism by nematodes. The growth response to nematicides, in the form of increased tillering as found in Africa, was also found in the Australian studies examined below (Chandler 1978, 1980; Bull 1979, 1981). In north Queensland, varying numbers of *P. zeae*, *Meloidogyne* spp. and *Achlysiella williamsi* Siddiqi (500-3000/10g of wet roots) were found in the roots of sugarcane in untreated plots where tillering was poor. It was concluded that the best sugarcane yields occurred when nematicide applications induced a numerous and even growth of primary and secondary tillers (Chandler 1978), presumably because nematodes were being controlled.

In Bundaberg, the greatest improvement in yield occurred when nematicides were applied during the emergence of primary and secondary tillers. If the application of nematicides was delayed by 60 DAP the yield improvement was less (Bull 1981). The endoparasite nematodes, *Meloidogyne* and *Pratylenchus* spp., were the genera found in untreated shoot roots. In another Bundaberg field experiment, improved tillering was emphasised as the major benefit of nematode control (Bull 1979). *Pratylenchus* spp. appeared to be the dominant parasite present. No benefit was observed when nematicide was applied following active tillering (Chandler 1978).

In summary, the poor tillering observed in some sugarcane fields in Australia appears to be caused by endoparasitic nematodes attacking sett roots and/or young shoot roots. The Australian studies made no distinction regarding the type of root being parasitised. In view of the West African data, the importance of endoparasitic nematode attack on sett roots cannot be discounted. The yield benefit in controlling nematodes at plant crop establishment (0-80 DAP) is well documented in sandy soils. However, 95% of Australia's sugarcane is cultivated on sandy loam to clay soils, and nematode damage in these soils has only been speculated on around the world (Sasser and Freckman 1987). It is surprising that nematicide responses are disappointing when applied after tillering, because the bulk of biomass accumulation occurs after 125 DAP (Muchow *et al*. 1993).

1.3.4 Effect on ratooned crops

A large corm-like structure, the stool, develops in the soil beneath the initial plant crop. Below ground, nodes on the stem bases carry axillary buds that shoot when the stem is harvested (ratooning). The new tillers immediately initiate shoot roots from their base in the same manner that the plant crop produces shoot roots.

The crop may be ratooned for up to six years before loss of vigour warrants the ploughing up of the stool. The loss in vigour is not due to poor tillering. In fact, ratoon crops often tiller better than the planted crop (Muchow *et al.* 1993). In ratoon crops, *Meloidogyne* and *Pratylenchus* spp. did not interfere with crop growth until temperatures were high enough to stimulate active root growth. Thus, delaying nematode control over winter for 60 and 140 days after harvest, when roots were inactive, had no detrimental effect on yield (Rostron 1976; Spaull and Donaldson 1983). While plant crops can be very vulnerable to nematode attack at tillering, ratoon crops appear to be less susceptible (Spaull and Cadet 1990). Despite old roots being in a state of decay at harvest, the stele is generally intact and capable of conducting nutrients and water from the less deteriorated root tips (Van Dillewijn 1952). Ratoon tillers may rely on this source for water and nutrients, as well as stool reserves, until their own roots become established. Planted crops do not have that reserve and so are more susceptible to nematode attack.

Nematode numbers decreased in the soil and remained at low levels in the roots over winter, despite ratooning of the stool (Spaull and Donaldson 1983). This phenomenon was also observed in Queensland (unpub.). Conditions of low temperature, dry soil and slow root growth are factors that could contribute to low nematode activity.

1.4 Ectoparasitic nematodes

The ectoparasitic nematodes differ from the endoparasites in that they do not enter the root tissue, or only partially penetrate the outer layers of the root cortex (Jensen *et al*. 1959). A feeding tube (stylet) is used to puncture the cell walls of outer root cells. The ectoparasitic nematodes are generally regarded as mildly pathogenic on sugarcane roots in comparison to the aforementioned endoparasitic nematodes and certain fungal pathogens. Ectoparasitic nematodes may be less important from a parasitic viewpoint because:

(a) feeding is concentrated only on the surface cortex of the root,

(b) feeding tends to be periodic (Stirling *et al*. 1992 a),

(c) particular species, while being found at high densities in some fields, tend to be localised in their distribution.

Paratrichodorus, *Helicotylenchus*, *Tylenchorhynchus* and *Xiphinema* spp. appear to be the most common ectoparasites that parasitise sugarcane roots (Spaull and Cadet 1990). When present in sufficiently high numbers (Table 1.3-4), these genera produce similar symptoms of root damage on sugarcane in glasshouse pots.

Those symptoms of damage include (Jensen *et al*. 1959; Harris 1974):

(a) thickened, malformed and coarse primary roots that are typically stubby,

(b) greatly reduced fine root growth,

(c) reduced number of root hairs,

(d) generally darker appearance of the root system.

1.4.1 *Helicotylenchus* spp.

A significant reduction in root and shoot weight was found when sugarcane seedlings were inoculated with more than 250 *H. dihystera* Cobb/kg of soil in pots (Apt and Koike 1962 a). In Hawaii, *H. dihystera* was found to be a common soil inhabitant and specimens were found feeding on roots (Jensen *et al*. 1959). As a result of these observations, the nematode was considered to be a pest. From a West African study it was concluded that *H. dihystera* did not impair root function enough to suppress yield, despite being the dominant ectoparasite present in planted sugarcane (Cadet *et al*. 1982).

1.4.2 *Tylenchorhynchus* spp.

A 50% reduction in shoot weight was found when sugarcane seedlings were inoculated with *Tylenchorhynchus* spp. in pots (Harris 1974). Variable reductions in root and shoot weight were recorded in the presence of *T. martini* Fielding, despite high inoculum levels and active nematode multiplication (Birchfield and Martin 1956). *Tylenchorhynchus* spp. are not considered to be serious pathogens of sugarcane.

1.4.3 *Trichodorus* and *Paratrichodorus* spp.

Paratrichodorus minor Allen was found to be parasitic on and pathogenic to sugarcane seedlings in pots and caused a significant reduction in root and shoot weight when the initial nematode inoculum exceeded 500 nematodes/kg of soil (Apt and Koike 1962 b). In West Africa, coarse brittle and stunted root growth was attributed to *Trichodorus, Paratrichodorus* and *Xiphinema* spp. parasitism on untreated sugarcane. When these nematodes were controlled, sugarcane had significantly longer stalks at harvest (Cadet *et al*. 1982). It is difficult to single out the effect of specific ectoparasitic nematodes in the field. It is usual to find combinations of ectoparasites and endoparasites on sugarcane roots, each potentially exerting a subtle effect on the root systems.

A summary of the types of nematode genera and density believed to cause a reduction in sugarcane yield is given in Table 1.3-4.

Table 1.3-4: Nematode densities considered responsible for reduced sugarcane growth.

Nematode(s)	Nematodes/g root (dry wt.)	Nematodes/kg soil (dry wt.)	Experimental source and reference
Pratylenchus zeae		>1000	glasshouse (Nath et al. 1975)
Paratrichodorus minor		>1000	glasshouse (Apt and Koike 1962 b)
Helicotylenchus dihystera		>6500	glasshouse (Apt and Koike 1962 a)
Pratylenchus zeae		600	field shoot roots (Bull 1979)
Pratylenchus zeae + *Meloidogyne* spp.	3000 750		field sett roots (Cadet and Spaull 1985)
Pratylenchus zeae + *Meloidogyne* spp. + *Achlysiella williamsi*	>500		field shoot roots (Chandler 1978)
Pratylenchus spp. + *Meloidogyne* spp.	1000		field ratoon roots (Spaull and Donaldson 1983)
Trichodorus spp. + *Xiphinema* spp.		100-200 400-800	field shoot roots (Cadet and Spaull 1985)

1.5 Co-pathogenic relationships

The infection of roots by fungi and bacteria can be enhanced by the activity of phytoparasitic nematodes on the root. Nematodes can facilitate infection by providing points of entry through the root cell wall or by altering the plant's resistance and/or tolerance physiology (Powell 1971). This relationship has been demonstrated on many crops. Infection of banana roots by *Fusarium* spp. was facilitated by the burrowing nematode *Radopholus similis* Cobb (Blake 1961). *Meloidogyne* spp. facilitate the attack of *Fusarium* spp. on alfalfa, banana, beans, cotton, cowpeas and tomato (Mai and Abawi 1987). *Pratylenchus penetrans* Cobb and *Trichoderma viride* Persoon cause a greater reduction in the root growth and shoot biomass of alfalfa and celery than either organism alone (Jones 1978). Of importance to note is that *Trichoderma* spp. are normally regarded as weakly pathogenic.

The characteristic lesions caused by *Pratylenchus* spp. on sugarcane have been

attributed to secondary infection by fungi and bacteria (Jensen *et al*. 1959). A darkening around the feeding sites of *Helicotylenchus* spp. was attributed to secondary infection (Jensen *et al*. 1959), but these conclusions were drawn from observing the roots, only. A facilitated pathogenicity between nematodes and fungi on sugarcane roots in pot experiments has not been well established. One experiment on the pathogenicity of *Meloidogyne incognita* Kofoid and White, *Pratylenchus zeae* and *Pythium graminicola* Subramaniam indicated that the two nematodes and the fungus were antagonistic to each other (Valle-Lamboy and Ayala 1980). When *P. zeae* and the seed-piece rotting fungus (*Phytophthora megasperma* Drechsler) were inoculated in combination, the results indicated that they acted independently (Khan 1963). When *Helicotylenchus dihystera* and *Pythium graminicola* were inoculated in combination, roots grown in the presence of both parasitic agents did not exhibit damage significantly more severe than those caused by either agent alone (Apt and Koike 1962 a). In this experiment, a generally darker appearance of roots was observed in the presence of *Pythium graminicola*. This discolouration is characteristic of the northern Queensland disease complex known as 'northern poor root syndrome' (NPRS). *Pythium* spp. have been regularly isolated from NPRS soils and shown to be pathogenic, and *Pachymetra chaunorhiza* Croft and Dick, an indigenous fungus to Queensland sugarcane soils, has been shown to rot primary roots (Croft and Magarey 1984; Croft 1988). Recent research indicates that as yet unknown factors, but possibly other fungi, are important contributors to NPRS (Magarey *et al.* 1997 a). The interaction between nematodes, *Pythium* spp., *Pachymetra chaunorhiza* and other fungi in Queensland sugarcane soils is largely unknown. However, following the use of nematicides in the field, Chandler (1984) concluded that poor root development occurred in the absence of nematodes. A closer examination of the synergism between nematodes and root rotting fungi is required under controlled conditions.

A number of nematode species usually co-habit in sugarcane fields (Blair *et al*. 1999 a, b). Their relative abundance depends on the suitability of the soil environment for each species, the vigour of the root system to sustain multiple populations, and the ability of nematode species to compete where the root resource is limiting. For example, *Meloidogyne* spp. are more abundant in sandy soils. In a sugarcane field in

Africa, *Hoplolaimus pararobustus* Schuurmans, Stekhoven and Teunissen represented only 10% of the endoparasites in the roots of a plant crop, but composed 80% of the population by 4th ratoon to the detriment of *P. zeae* (Spaull and Cadet 1990). Similarly in surveys of sugarcane fields in India, *P. zeae* abundance was negatively associated with *Hoplolaimus indicus* Sher because both endoparasites are expected to compete for the same niche within the root system. Because *Helicotylenchus dihystera* is an ectoparasite largely confined to the rhizosphere, there was a positive association with *P. zeae* (Sundararaj and Mehta 1993 a). The abundance of *H. dihystera* has been negatively associated with other ectoparasites that are likely to share the same feeding niche, such as *Tylenchorhynchus annulatus* Cassidy (Sundararaj and Mehta 1995). In a sandy field in South Africa, Cadet *et al*. (2002) examined the mix of *Meloidogyne, Pratylenchus*, *Helicotylenchus*, *Xiphinema* and *Paratrichodorus* spp. relating to patchy sugarcane growth, and found *Meloidogyne* spp. were dominant where sugarcane growth was poorest. Sugarcane growth was the best where *H. dihystera* tended to dominate the community. However, differences in this community balance could not be correlated with differences in abiotic factors such as soil texture, pH, organic matter, nutrient levels or CEC, that could significantly affect sugarcane growth. Thus it was inferred that when *H. dihystera* dominated the rhizosphere, serious damage to the crop from *Meloidogyne* spp. was minimised.

1.6 Effect of soil physical factors

Soil texture, degree of water saturation and organic matter content of the soil affect the movement and activity of phytoparasitic nematodes. These factors also affect crop growth, which in turn influences parasitism by nematodes. It is well known that nematodes are potentially more damaging to crops on light sandy soils than on clay-loam soils, as documented by Donaldson (1985) in an African study (Table 1.6).

Table 1.6: Effect of clay content on sugarcane yields and related nematicide responses (Donaldson 1985).

Clay (%)	2	3	5	6	7	8	9
Untreated sugarcane yield (T/ha)	44	49	45	63	66	82	93
Treated sugarcane yield (T/ha)	71	75	68	95	78	97	110
Response to nematicide (% of untreated)	61	53	51	51	18	18	15

As the percent clay increases the water holding capacity and cation exchange capacity of the soil increases. These factors provide an improved soil environment for plant growth, hence the increasing yields observed as percent clay increased. As the conditions for plant growth are improved, the effect of nematodes upon plant growth is lessened (Stirling 1991). By this reasoning, nematode control becomes less important as the potential sugarcane yield increases, as observed with Donaldson's nematicide treatments (Table 1.6).

The direct effect of soil particle size on nematode movement is also important. Cadet and Spaull (1985) found that parasitism of sett roots by *Meloidogyne* and *Pratylenchus* spp. was more rapid in sandy soil than clay soil. As a result, there was poor tillering of the sugarcane grown in the sandy soil, whilst the sugarcane grown in clay soil was able to establish tillers. Nematode survival and movement is also reliant upon the status of the moisture film around soil particles (Stirling 1991). Indirectly, plants obtaining an optimal water requirement will withstand a higher nematode damage threshold than plants under water stress. In a South African study it was found that as sugarcane yield increased in response to increasing rainfall, responses to nematode control decreased (Donaldson 1985).

However, the soil environment (i.e. water movement and clay content) is an important moderator of the effectiveness of nematicides, and can mislead estimates of nematode damage measured from the nematicide response. For instance, nematicides can be immobilised by adsorption onto clay particles (Abdellatif *et al.* 1967; Awad *et al.* 1984), which may account for some poor responses in heavy clay soils. When the crop suffers from water stress, greater damage from nematodes is expected, but paradoxically, nematicides are immobile in soils with low moisture. Hence, Donaldson (1985) has reported increased nematicide responses in very sandy

soils as rainfall increased. In Queensland, poor nematicide responses have also been attributed to planting sugarcane into dry soil (Chandler 1978). When assessing nematode damage from nematicide responses, thorough sampling of nematode populations are required to confirm that control has occurred.

1.7 Nematode control with biocides

In Australia, sugarcane production is based on a monoculture, typically requiring high external inputs (Thomason and Caswell 1987), such as fertiliser and pesticide applications, and mechanical cultivation. In sugarcane fields, the organic matter content of the soil is usually low, contributing to low microbial biodiversity (Thomason and Caswell 1987). As a result, nematode control by natural biological means does not stabilise the populations of nematode pests below damage levels, and an alternative method of control is required (Davies *et al*. 1991). In Australia, chemicals have been the primary candidates for use when a nematode problem is recognised. The large number of references to nematicides cited in this review, is testament to grower reliance upon agrochemicals.

1.7.1 Fumigants

Fumigant chemicals are broad-spectrum biocides that infiltrate the soil substrate as a gas. Members of this group of chemicals are methyl bromide, ethylene dibromide (EDB) and metham. The latter is a methyl isothiocyanate liberator. Fumigants are not widely used to treat sugarcane fields in Queensland because the increase in yield obtained from their use is not of an economic advantage when chemical costs and application costs are taken into account. Because these fumigant chemicals are also phytotoxic they are useful only as a pre-plant treatment.

In north Queensland in 1982, combinations of EDB and fenamiphos (a nematicide) were used to determine the importance of fungi relative to nematodes in granite gravels and alluvial loams (Chandler 1984). Despite fenamiphos being applied across the whole field via broadcasting, EDB was still able to give twice the nematode control in comparison. When fenamiphos and EDB were combined, nematode control was better than EDB alone. The largest responses occurred in the poorest crops (42-75 T/ha), with fenamiphos responses of 8-12% and in one case equivalent

to the EDB response. The results indicated that in poorly growing sugarcane, nematodes were responsible for a significant part of the fumigation response.

1.7.2 Non-volatile nematicides

Although not as effective as fumigants at controlling nematodes, non-volatile nematicides can be applied more economically, and that has led to their routine use in some sugarcane fields in Queensland. The non-volatile nematicides have the added advantage of not being phytotoxic at concentrations that inhibit nematodes. Therefore, applications can be made at any stage during the crop cycle, such as when the ratoon is tillering (Bull 1979). The non-volatile nematicides, aldicarb, fenamiphos, ethoprophos and oxamyl became available to the sugar industry in Australia in the mid 1970's, and provided a chemical means to control plant-parasitic nematodes on sugarcane. Historically, the vast bulk of field research involving nematodes on sugarcane in Australia has been through testing the economics of non-volatile nematicides.

In the Bundaberg district, the majority of experiments were conducted on plant crops between 1976 and 1979 (Bull 1979, 1981). Field trials were also conducted in the Rocky Point district from 1980-1987 and although unpublished, are also reviewed. In north Queensland, Chandler (1978, 1980) conducted the bulk of experiments from 1975-1978 on both plant and ratoon crops. Field sites were selected across the Mossman, Mulgrave, Babinda and Mourilyan mill areas, with some trials occurring further south in the Herbert Valley (Chandler 1978, 1980).

The economic viability of nematicide use was the main experimental focus and outcomes discussed were:

(a) whether yield responses were profitable,

(b) rates that were low but effective, thereby maximising profit,

(c) optimal placement methods and times of applications to maximise the effectiveness of the product.

1.7.2.1 Time of application and rate

Sugarcane is a crop with a low value per hectare, which has governed nematicide use. For the nematicide to be economic, nematodes have been targeted by treating narrow 30 cm bands of row (1/5 of the field area) at planting or early tillering of the ratoon when the root system is not extensive. Other crops are treated more judiciously by virtue of their higher returns per hectare. For example, banana crops are treated with fenamiphos and ethoprophos in bands 1.0-1.5 m wide, and 2-3 applications per growing season are recommended.

In north and south Queensland, the optimum time to apply nematicides was at early tillering (30-60 DAP). At this time, shoot roots are initiating and elongating and secondary tillers are emerging. Protection from nematodes at this important establishment phase has proven to be of major benefit to the crop. However, this may represent only a portion of the yield loss from nematodes because:

(a) nematode populations within the treatment zone had recovered or were only partially reduced by 60-120 days after treatment (The action of nematicides is biostatic rather than biocidal),

(b) the practice of mounding soil into the row contaminates treated soil with untreated soil from the inter-row,

(c) during summer, when most yield accumulation is occurring (Muchow *et al*. 1993), a large proportion of the root system has grown outside the treatment zone and is subjected to nematode attack.

Advice from the supplier and experimentation by Bull (1979, 1981) and Chandler (1978, 1980) determined the rate of aldicarb at 2.5-3.0 kg a.i./ha and fenamiphos at 4.0-6.0 kg a.i./ha to be effective and most economically viable. In some cases, higher rates of aldicarb produced exceptional yield improvement, either by additional nematode control, or by directly stimulating plant growth. For example, aldicarb rates of 4.0 and 5.0 kg a.i./ha improved crop yield from 69 to 130 T/ha and from 114 to 171 T/ha in two cases, the latter increase being an exceptional yield for the Bundaberg district and the loamy sand where the site was situated. In some other

crops aldicarb has been demonstrated to improve plant growth in the absence of nematodes (Barker and Powell 1988), but not in sugarcane (Spaull 1995). It is inconclusive whether aldicarb directly stimulates sugarcane growth, as higher rates did not produce additional responses in many other cases. In north Queensland, Chandler (1980) generally found that aldicarb at 2.8 kg a.i./ha produced superior growth improvements than aldicarb at 4.2 kg a.i./ha.

1.7.2.2 Effect of water availability

To activate nematicides and move them into the root zone, irrigation or rainfall is necessary. In the Bundaberg district, nematicides were applied under optimal conditions because supplementary irrigation was available. In north Queensland, unreliable rainfall limited effectiveness. In summary:

(a) When nematicide applications in plant crops coincided with no rainfall, subsequent nematode control was poor and responses were not significant (Chandler 1978). Despite this inadequacy, consistent responses of between 5-21% occurred, but were lower than Bundaberg responses.

(b) When nematicide applications in plant crops coincided with good rainfall or irrigation, populations of *Pratylenchus zeae*, *Achlysiella williamsi* and *Meloidogyne* spp. were still reduced at many of the sites 60-90 days after treatment. Significant responses (15-111%) occurred at four of six sites. Unresponsive crops were the most poorly yielding (25 and 42 T/ha) and linked to Ca and Mg deficiencies, which may have limited any benefit achievable using nematicides.

(c) Poor responses in ratoon crops coincided with poor moisture at treatment, and no follow-up rainfall.

During years of poor rainfall or inadequate irrigation, exposure of the crop to water stress heightens the impact of nematodes, and so greater nematicide responses are seen (Cadet and Spaull 1985). However, enough water has to be available to activate the nematicide.

1.7.2.3 Effect of soil type – south Queensland

In Bundaberg, the experimental use of nematicides against nematodes was confined to sand ridge, loamy sand and fine sandy loam soils. Root galls symptomatic of root-knot nematode were often used as a guide to identify damage and conclusions by Bull (1981) contained a recommendation to apply nematicides after symptoms of root galling had become evident. This recommendation has probably led to only root-knot infested areas being identified, and the perception that nematodes are confined to sandy soils, where root malformations are obvious.

The sand ridge soils of the Fairymead and Burnett Heads region are of poor fertility and characterised by a typical coarse sand, fine sand, silt and clay ratio of 50, 40, 5 and 5%, respectively. Experimentation with nematicide bands in those soils showed that very poor and uneconomic growth caused by nematodes could be alleviated. For example, yield was improved from 16 to 100 T/ha in one case. Other poor yielding plant crops (50-56 T/ha) were improved between 26-64%, resulting in more economically viable crops of around 90 T/ha, which was average for the region. Plant and ratoon crops yielding 65-85 T/ha showed less spectacular improvements of 13-20% possibly because of fewer nematodes, better soil fertility, better farming practices or a combination of those factors reducing nematode impact.

A large proportion (60%) of experiments were conducted on loamy sand soil in the Fairymead and Millaquin districts. These soils have a typical coarse sand, fine sand, silt and clay ratio of 40, 48, 5 and 7% respectively and their fertility varies, but typically is classed as poor. Trials were conducted where untreated yields ranged from 40-100 T/ha. When experimentation was conducted with nematicide bands in those soils, yield improvements of 2-90% were obtained and those improvements were usually significant (>25%). While nematicides increased yields of the poorest crops to 65-75 T/ha, these yields were still well below the district average. Poor fertility or an extreme lack of water may have been the major yield constraint in these cases, thereby limiting any response achievable using nematicides. Bull (1981) observed that no response to aldicarb in one field contrasting with a 54% response to the nematicide in a contiguous field where low P, K, Ca and pH had been amended. In better crops where the untreated yields were 70-100 T/ha, nematicides produced consistent increases. Therefore, some crops were improved to 130-140 T/ha, which

was an impressive yield for that soil type and indicative that nematodes were causing significant yield losses in crops considered to be growing acceptably.

Four experiments were conducted in fine sandy loams in the Millaquin mill district. Typically these soils consist of a coarse sand, fine sand, silt and clay content of 16, 60, 12 and 12% respectively, and are variable in fertility. The untreated sugarcane yielded above district average in three of four experiments (111-118 T/ha) and was poorly responsive to nematicides placed in bands. These findings have probably reinforced the perception that nematodes are damaging only on poor yielding sugarcane in sandy soils with <10% clay.

From 1980-1982, nematicides were used on poor yielding plant and ratoon crops growing on Rocky Point loams (BSES unpub.). Responses occurred in both plant and ratoon and were in the order of 10-45%, which generally lifted yields to above district averages.

1.7.2.4 Effect of soil type – north Queensland

Most trials (65%) were conducted in granitic gravel and sandy loam soils which are typically low in fertility and where nematode problems were perceived to be the most significant in the district. Although the crops were below average for the district, they were generally grown on more fertile soils than the beach sands and loamy sands in Bundaberg where nematicide trials were conducted. In comparison, nematicide responses were generally disappointing, but could have been confounded by poor placement of the product and dry soil conditions. Extremely poor yields (20-30 T/ha) were linked to serious nutrient deficiencies.

About 35% of trials were conducted in fertile silty loams to clay loams and schists, which typically yielded above the district average. Significant yield increases were observed if the nematicides were applied under optimal conditions, suggesting that nematode damage was occurring in crops considered to be growing acceptably.

Because nematicides are among the most toxic chemicals in use in agriculture today, there is pressure to find alternative methods of control (Stirling *et al*. 1992 a).

1.8 Host resistance/tolerance to nematodes

A program to detect and select for nematode-resistant cultivars has not been initiated in Queensland. However, the selection of new cultivars is based upon yield testing in the field. Therefore, the selection for nematode tolerance or resistance could be taking place unknowingly if nematodes are having a significant impact on crop yields in the field. The variable resistance of Hawaiian cultivars to *Meloidogyne incognita* has been observed (Holtzmann 1964). In Brazil, both resistant and tolerant cultivars have been reported against the lesion nematode (*P. zeae*) found in that country (Dinardo-Miranda and Ferraz 1991; Dinardo-Miranda *et al.* 1996). However, in Australia, six widely grown cultivars that were tested were all good hosts of *Meloidogyne* spp.. *Pratylenchus zeae* multiplied on all nine widely grown cultivars that were tested (Stirling *et al*. 1999 b).

Breeding for increased resistance to nematodes could present some difficulties due to the genetic profile of commercially grown *Saccharum* hybrids. There is a high heterozygous polyploidy of prospective parents and they have the ability to undergo parthenogenesis during reproduction (Raghavan and Govindaswamy 1956). Therefore, the transfer and expression of desirable genes from generation to generation is unpredictable. In addition, sugarcane is a perennial with a long crop cycle, so a major commitment of time and resources is needed to test that emerging clones have (a) inherited resistance, and (b) retained the other traits necessary to be an economic crop in the Queensland environment.

Nonetheless, there is no reason that nematode resistant traits cannot be located in Queensland or sourced from overseas germplasm, and hybridised with adapted cultivars. At the least, cultivars due for commercial release could be screened for evidence of resistance or tolerance to the nematode species deemed to be significant pathogens of Australian sugarcane. A glasshouse screening method for *Pachymetra* root-rot of sugarcane in Queensland has been developed to test the susceptibility of new clones that are becoming available to growers (Croft 1989). Susceptibility of sugarcane cultivars to *P. chaunorhiza* in the field (field rating) shows a corresponding susceptibility to *P. chaunorhiza* in glasshouse pots (glasshouse rating). However, at the time of this review, nematodes are not considered a serious enough pathogen to warrant such efforts.

1.9 Biological control

Natural enemies of nematodes include fungi that are both obligate parasites and opportunistic, bacteria, predatory nematodes and microarthropods such as mites and springtails. Attempts to use or encourage nematode trapping fungi (facultative fungi) as a control measure have rarely been effective (Stirling 1988). Few attempts have been made to utilise obligate fungi, predatory nematodes and microarthropods because of the difficulty in mass production (Stirling 1988).

Pasteuria penetrans Thorne, an obligate bacterial parasite of nematodes, has perhaps been the most intensively investigated. Naturally high infestations of *Pasteuria penetrans* on root-knot nematode have been recorded in South Africa and Mauritius (Davies *et al*. 1991). The effect on the nematode population was unclear. Control of *Meloidogyne javanica* Treub on tomatoes, using a powdered preparation of the bacterium, has been achieved (Stirling 1984). Further work is required to develop suitable mass production systems and to determine the effectiveness of obligate parasites in agricultural systems. The range of nematode biotypes that can be controlled also has to be investigated. The production of commercial biocontrol agents is probably some years away. Stirling (1988) stated that "*It is estimated that worldwide no more than 20 scientists spend a major part of their time actually engaged in research on antagonists of nematodes.*" It follows that progress in that area will be slow.

1.10 Cultural control

Rather than frequently broadcasting commercial preparations of a nematode parasite as a 'microbial nematicide', manipulation of the soil substrate to favour the multiplication of naturally occurring or introduced parasites may be a viable option. Organic amendments are one of the most common methods used to modify the soil environment to the detriment of nematodes. The decomposition of organic matter in the soil appears to restrict nematode populations (Rhoades and Fores 1986) and reduce their pathogenic effect on the plant host. This phenomenon has been attributed to production of nematicidal byproducts, stimulation of microorganisms harmful to nematodes, or improvement of the plant's growth substrate (Trivedi and Barker 1986; Sayre and Walter 1991). A decline in root-knot nematode levels on

pineapple during the decomposition of organic matter was attributed to the increased activity of nematode trapping fungi (Stirling 1988).

Sugarcane soils are typically low in organic matter due to a history of tillage and trash burning. However, the introduction of trash blankets via green harvesting has become increasingly popular in Queensland's sugarcane fields and must potentially be a source of organic carbon to the soil. An increase in the organic carbon content of soil has been recorded when sugarcane trash was incubated in combination with urea (Yadav and Prasad 1992). A significant increase in soil organic carbon from 0.55% to 0.85% has been recorded in the top 100 mm of soil following the adoption of a green trash system (Wood 1986). It remains to be seen whether trash blanketing will increase soil organic matter to a level that will affect nematode populations.

The potential exists to significantly reduce nematode numbers between sugarcane crop cycles by planting a non-host cover crop. Cowpea (*Vigna unguiculata*) is a popular inter-cycle crop in Queensland, but is listed as a host of *Meloidogyne* and *Pratylenchus* spp. and is not recommended as a cover preceding a crop that is susceptible to those nematode genera (Stirling and O'Brien 1991). The effect of cowpea on the levels of *Meloidogyne* and *Pratylenchus* spp. in sugarcane fields in Queensland is as yet unknown. However, other inter-cycle crops may be preferable.

1.11 Summary

Meloidogyne and *Pratylenchus* spp. are the most important nematodes that parasitise sugarcane roots in Queensland. *Achlysiella, Paratrichodorus, Tylenchorhynchus* and *Helicotylenchus* spp. are considered to be less important because they either have a restricted distribution, or are considered to be weak pathogens.

Nematodes reduce the plant crop yield by affecting sett root and/or early shoot root growth and associated fine root growth. This attack, in particular by endoparasitic nematodes, is manifested as poor and uneven tillering. Nematode attack on early ratoon root growth appears to be less important in terms of restricted tillering. The populations of endoparasites (*Pratylenchus* and *Meloidogyne* spp.) that decrease crop yield is probably over 1000/g of roots (dry weight) or over 1000/kg of soil, and even higher for ectoparasites.

In Australia, the bulk of nematode research on sugarcane has involved testing the economics of using nematicides. In the Bundaberg district, trials were confined mainly to sandy soils because root malformations due to nematodes were obvious there. The poor water holding capacity and low fertility of those soils would also have exacerbated nematode damage. Hence the findings by Bull (1981) that 22,000 ha of sugarcane soils could be responsive to nematicides. Currently only 2,000 ha are treated with nematicides in the Bundaberg district, and typically it is the most infertile and nematode prone (coastal sand ridges). Nematicide responses on higher yielding crops were also obtained, suggesting nematode losses may be more widespread. In north Queensland the reliance on rainfall to activate the nematicides was a major reason poor nematode control occurred in many cases. Compared to the south, trials were also placed in more fertile soils so responses were generally lower. A lack of chronic and obvious nematode damage, poor nematode control and lower responses have probably been responsible for growers being reluctant to invest in nematicides in north Queensland, and developed the industry perception that nematode problems are confined to sandy soils in Bundaberg.

Past research has been commercially focused to find nematicide rates that were inexpensive while offering a degree of nematode control. At early tillering, by controlling nematodes at an important stage in crop establishment, the nematicides were most effective. It is highly likely that total damage to nematodes is greater than the responses found by Bull (1979, 1981) and Chandler (1978, 1980), mainly because the treatment band is narrow and control has disappeared when most plant biomass is being accumulated during summer months. Responses to nematicides in north Queensland were often uneconomic, but uniform, suggesting that subtle but widespread nematode losses are occurring across the region. The feasibility of using commercial biocontrol agents appears to be some years away.

A program to detect or select for nematode resistant cultivars has not been undertaken in Queensland. The selection of new cultivars is based upon yield testing in the field and in this manner some selection for nematode resistance or tolerance may be occurring. However, few steps have been taken to verify this. The manipulation of soil health to be more antagonistic to nematodes appears to be the most practical approach for prolonged control.

CHAPTER 2

GENERAL INTRODUCTION

In Australia, sugarcane is grown over an area of 520,000 ha on coastal plains from Grafton in northern New South Wales to Mossman in far-north Queensland (Canegrowers 2000). Recently the industry has expanded onto the Atherton Tablelands in far-north Queensland and the Ord River irrigation area in Western Australia. Most farms range in size from 30-120 ha, and sugarcane yields currently average 80-110 T/ha, depending on the district and seasonal conditions (Briody 2000; Canegrowers 2000). Annually, the crop produces around 4.9 MT of sugar, which contributes over A$1 billion to the export economy (Briody 2000; Canegrowers 2000). Sugar production is one of the major primary industries in Queensland.

Sugarcane is a giant grass of the Gramineae family. The commercial cultivars in use today are descended mainly from interspecific hybrids of *Saccharum spontaneum* Brandes and Jesweit and *S. officinarum* Linn. The process of hybridization and back-crossing has combined the vigour and disease resistance of wild species with 'noble' cultivars that have thick stems and high sucrose but low vigour (Julien *et al.* 1989).

Sugarcane is grown as a perennial. The crop cycle is commenced vegetatively by planting stem lengths (billets) that support 2-3 nodes, each of which has a bud and band of root primordia. Sett roots grow from the primordia and support development of the bud into a primary shoot, which in turn initiates shoot roots from its base. About 30 days after planting (DAP), secondary shoots also emerge from the base of the primary shoot and subsequently initiate their own shoot roots, while the sett roots become senescent. This early establishment phase is impaired if sett roots are damaged (Bonazzi 1928), and the vigour of the primary shoot appears to affect the level of secondary shooting and ultimately the number of stems produced (Pankhurst *et al.* 2001). In field crops, the shoots emerging from the soil are often called tillers. Subsequent competition in the row usually causes mortality, and the dominant tillers that grow to maturity after 150 DAP are termed stalks or stems.

After about 12 months, the mature planted crop is harvested at ground level, the leafy trash remains in the field and the stems are transported to a central mill for processing. Axillary buds then tiller from the old stem bases (stool) that remain below ground, becoming the 1st ratoon, and then subsequent ratoon crops that are harvested annually (Julien *et al.* 1989). Typically, a plant crop and 2-4 ratoons are grown before a decline in vigour warrants plough-out of the stool.

In systems of intensive agriculture and tillage, there is usually a heavy reliance upon inorganic fertilizers, and levels of organic matter typically decline in the soil over time (Grace and Oades 1994). Australian sugarcane fields are no exception, with low levels of organic matter being documented from soil surveys and field studies (Wood 1986; Gillman and Sumpter 1986; Moody *et al.* 1999). Fields are often fallowed for about 250 days between sugarcane crop cycles, with either weed fallows becoming established or legumes being sown as green manure crops. However, in far-north Queensland the level of legume management is often poor and weeds tend to proliferate (Garside *et al.* 1996). Long-term rotations with other crops are very rare and plough-replant (no fallow) is increasing. Thus the cropping system is essentially one of continuous sugarcane monoculture and this has been maintained for 80 years or more in most districts. In agricultural systems based on monoculture, replant problems are documented in crops as diverse as apples and asparagus (Hoestra 1994; Blok and Bollen 1995).

In Australia, 'yield decline' (YD) is associated with the sugarcane monoculture and is defined as '*the loss of productive capacity of sugarcane-growing soils under long term monoculture'* (Garside *et al.* 1997 a). Sugarcane growing in these soils develops a poor root system characterised by lesions and rotted roots (Lawrence 1984; Egan *et al*. 1984). Root health and the root volume of sugarcane is restored by interventions such as fumigation, pasteurisation, long-term fallowing and rotation crops, suggesting that soil pathogens are associated with the monoculture (Croft *et al*. 1984; Garside *et al.* 1997 a). Numerous species of bacteria, fungi, actinomycetes and nematodes may parasitise sugarcane roots, and their relative abundance and importance probably varies both across districts and within fields (Lawrence 1984; Magarey *et al.* 1987). While pathogenic fungi are strongly implicated in the poor growth of roots,

pathogenicity tests have not identified specific causal agents and the biological cause(s) of YD remains a mystery (Magarey *et al*. 1995). Yield decline has probably affected productivity for over 65 years, as Bell (1935) achieved outstanding responses to soil fumigants and pasteurisation in continuously cropped sugarcane soils from that era.

Members of the Phylum Nematoda include nematodes from the Orders Tylenchida and Dorylaimida that inhabit soil and are obligate parasites of plant roots (Mai and Mullin 1996). Worldwide, more than 48 genera of plant-parasitic nematodes have been recorded from the roots and/or rhizosphere of sugarcane (Spaull and Cadet 1990). The mode of parasitism and life cycle varies between genera. Root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites. Vermiform juveniles disperse through the soil and locate and penetrate host roots. The host root is stimulated into producing giant nurse cells, which acts as a nutrient source for the nematode to become sedentary, develop into an obese adult and produce an egg mass. As a result, the tips of primary and fine roots develop terminal galls or clubs. Lesion nematodes (*Pratylenchus* spp.) are migratory endoparasites, and adults deposit eggs while moving through root tissue or the soil. Spiral nematodes (*Helicotylenchus* spp.), stubby-root nematodes (*Paratrichodorus* spp.) and stunt nematodes (*Tylenchorhynchus* spp.) are ectoparasites that tend to remain in the soil and parasitise the outer root cortex using a feeding spear (stylet). The above-mentioned genera are considered the most important pests because they are either widespread and/or clearly pathogenic to sugarcane roots, particularly *Meloidogyne javanica* Treub and *Pratylenchus zeae* Graham. In Australia, there are few detailed studies on the nematode association with sugarcane, nor their role in YD.

Historically, nematodes have been studied on Australian sugarcane as a consequence of non-volatile nematicides becoming available to the sugar industry in the late 1970's (Chandler 1978, 1980; Bull 1979, 1981). Field trials were done to evaluate the economics of controlling nematodes with nematicides. Effects of factors such as rate and time of application, placement method and environment on efficacy, were examined. In far-north Queensland, nematicides generally failed to improve yields significantly (Chandler 1978, 1980), generating perceptions that nematodes were not a cause of poor root growth. However, the nematicides were often inactive due to

unpredictable rainfall at most trial sites and, in some cases, crop growth was severely restricted by other factors, such as nutrient deficiencies. Nematicides require rainfall or irrigation to be activated and transported into the root zone. Economics also dictated that the nematicides were applied only once per year and in a narrow band, resulting in poor and very temporary control of nematodes. Non-volatile nematicides are nerve synapse inhibitors with a nemastatic action, so nematodes are temporarily paralysed rather than killed, and control rarely exceeds 80 days. However, at four of Chandler's sites (1978) where the nematicides were activated under favourable soil moisture, *P. zeae*, *Achlysiella williamsi* Siddiqi and *Meloidogyne* spp. were reduced for 90 DAP, and yields increased 15-111%.

In the Bundaberg district of south Queensland, nematicides improved yields more consistently because sugarcane was irrigated and trial sites were mainly on sandy soils (<12% clay) (Bull 1979, 1981). Nematicides are less effective in clay loam and clay soils due to adsorption onto clay particles (Abdellatif *et al.* 1967; Awad *et al.* 1984). Nematode damage was often diagnosed by the presence of root galls (Bull 1979), so trials were often placed in fields with serious root-knot nematode (*Meloidogyne* spp.) infestations. Sites with possibly more subtle damage by other nematodes were overlooked. In finer textured soils (>12% clay), nematicide responses were generally disappointing and, as a consequence, the sugar industry developed a perception that nematode problems were confined to sandy soils in south Queensland (Magarey and Croft 1995). However, these trials were a poor indicator of nematode damage because nematodes were rarely controlled for any extended length of time.

In the 1980's, soil fumigants provided better nematode control, and the responses obtained with ethylene dibromide suggested nematodes were contributing to YD in far-north Queensland (Chandler 1984). However, the fungal root pathogen *Pachymetra chaunorhiza* Croft and Dick was identified in 1984 and became a major focus of research efforts, perhaps with the expectation that it was the primary cause of YD. Indeed, *P. chaunorhiza* proved to be a serious root rotting pathogen, but resistant cultivars also respond to soil fumigants, indicating that other biota also contribute to YD (Magarey and Croft 1995).

This thesis reports work on the distribution, pathogenicity and importance of nematodes on sugarcane in Queensland. Specifically it covers the following topics:

(a) surveys of the major sugarcane growing regions of far-north Queensland to identify the species of nematodes present and their incidence (Chapter 3),

(b) microspatial dispersion of nematodes within sugarcane fields and its implications for sampling nematode populations (Chapter 4),

(c) the population dynamics of nematodes on sugarcane and its relationship to root growth and environmental factors (Chapter 5).

As a consequence of these descriptive studies (a-c) the pathogenicity of nematodes and particularly that of *P. zeae* to Australian sugarcane, was examined. The topics covered were:

(d) the effect of pasteurisation and/or nematicides on sugarcane growth in YD soils in short-term assays (1.5 L pots) (Chapter 6),

(e) the pathogenicity of axenic cultures of *P. zeae* to sugarcane in short-term assays (1.5 L pots) and 50 L field pots (Chapters 7 and 8),

(f) the role of sett roots, nematodes and general YD biota on shoot establishment under field conditions (Chapter 9),

(g) crop responses using non-volatile nematicides and relating to the nematode community and population densities controlled (Chapter 10).

CHAPTER 3

THE DISTRIBUTION OF PLANT-PARASITIC NEMATODES IN THE SUGARCANE FIELDS OF FAR-NORTH QUEENSLAND

3.1 Introduction

In far-north Queensland, about 75,000 ha of coastal plains and associated foothills from Tully to Cairns, are used to grow sugarcane. This farming system has typically involved intensive monoculture for over 80 years. Thus, 'yield decline' (YD) associated with long term monoculture, is prevalent in the sugarcane fields of far-north Queensland (Lawrence 1984; Magarey and Croft 1995). Nematode research in the region has mainly involved the experimental use of non-volatile nematicides and soil fumigants in the late 1970's (Chandler 1978, 1980, 1984).

Apart from the data from Chandler's trials and the occasional diagnostic sample, nothing is known of the nematode species and their distribution in the region. Reported here is a nematode survey of soils used for sugar production in the Tully, Mourilyan, South Johnston and Mulgrave mill zones. Densities of the most abundant nematodes (*Pratylenchus zeae* Graham, *Helicotylenchus dihystera* Cobb and *Criconemella curvata* de Grisse and Loof) were compared, (a) in different catchments, (b) in different soil types and (c) in crops of different age and fallow history.

3.2 Materials and Methods

In Survey Area 1 (Plate 3.2), surveyed in June and July 1993, nematodes were identified and counted in the soil from 135 sugarcane fields, randomly selected within the Tully, Mourilyan and South Johnston mill areas. Within each field, across an area of 0.2 ha, 16 soil cores were taken at approximately 10 m intervals in a grid pattern. Cores were collected with a steel soil-auger of 17 mm diameter. The cores were taken 20 cm from the stool, to a depth of 30 cm, and then combined to give one composite soil sample per site. This sample was mixed thoroughly and nematodes were extracted from 200 mL of soil using a Baermann tray (Whitehead and Hemming 1965). Nematodes were collected after 48 hours, concentrated by sieving

through a 20 μm sieve and counted by species. Preserved specimens were sent to the CSIRO Entomology Department in Canberra, Australia, for identification. At each site, the location was recorded and age of crop and fallow history was found from farm records. Soils from the same region were typed by soil texture (Northcote 1971) and soil particle size, as percent coarse sand, fine sand, silt and clay (Australian Standard AS 1289.3.6.3). Soils were then grouped by location and textural similarity to produce nine soil categories, as described in Table 3.2. Most soil categories were represented with 9-18 survey samples.

In Survey Area 1, mean densities of the most abundant nematodes were compared in different (a) catchments, (b) soil types and (c) crop ages and fallow histories using one-way analysis of variance (ANOVA). Where the F-Test found significant differences at the 5% level, means were compared via least significant difference (LSD).

To be valid, ANOVA models assume that data is normally distributed, and the variances of the nematode counts being compared are unrelated to the count means (Allsopp 1990). Transformations with log (x + c) are popular to normalise nematode counts, but can restrict ANOVA levels to the point where few differences are found. Thus, a cube root transformation of $(x + 0.5)^{1/3}$ was applied to nematodes in 200 mL of soil because (a) variances were adequately stabilised according to the Bartlett's test of equal variance (1% level), and (b) significant differences ($P<0.05$) between the transformed nematode counts were still discernable.

In Survey Area 2 (Plate 3.2), surveyed in January 1995, nematodes were identified and counted in the soil and roots from 29 sugarcane fields, selected randomly across part of the Mulgrave mill area. Within a 0.2 ha section of each field, 16 sub-samples of soil and roots were collected in a grid pattern about 20 cm from the edge of the stool, using a shovel. The samples were combined to give one composite sample of soil and roots per site. Soil and roots were separated and nematodes were extracted from 200 mL of soil and about 80 g of roots (fresh weight) using a Baermann tray. Nematodes were collected after 96 hours, concentrated by sieving through a 20 μm sieve and counted by species. In Survey Area 2, almost all samples were a fine, sandy clay-loam (Coom series) with a mean particle size of 7% coarse sand, 50%

fine sand, 15% silt and 28% clay. Three samples were sandy loam (Thorpe series) with a mean particle size of 40% coarse sand, 35% fine sand, 10% silt and 15% clay (Murtha 1986, Cannon *et al.* 1992).

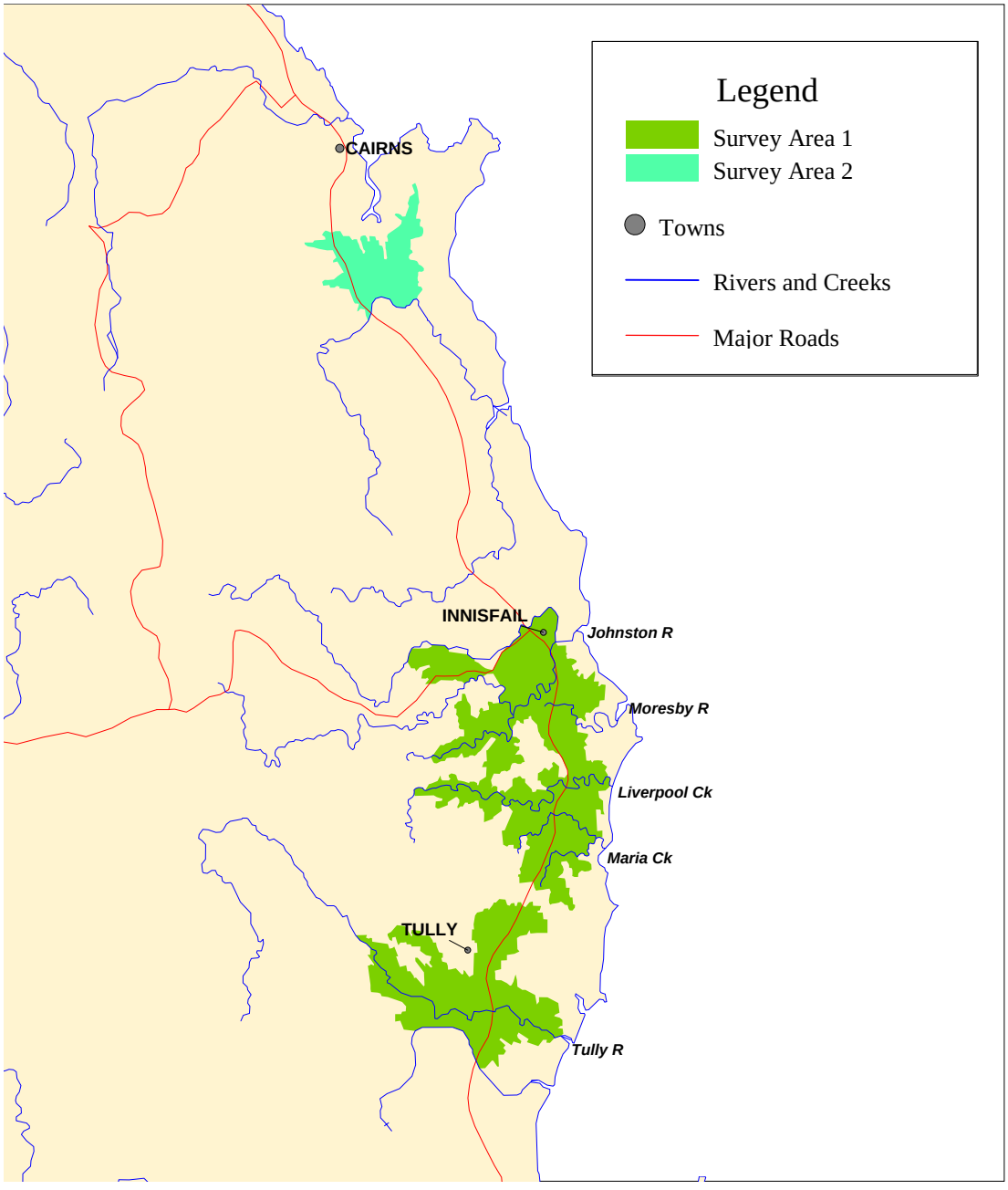


Plate 3.2: Regions (survey areas) of sugarcane production surveyed for nematodes.

3.3 Results

Eleven species of plant parasitic nematodes were detected from soil extractions (Tables 3.3.1 and 3.3.2). Additionally, *Meloidogyne* spp. and *Hoplolaimus* spp. were detected, but were identified only to the genus level. *Pratylenchus zeae* was ubiquitous, and generally at higher densities in soil and roots than other species. In Survey Area 1, *H. dihystera* and *C. curvata* were also common, whereas in Survey Area 2, *Meloidogyne* spp., *Tylenchorhynchus annulatus* Cassidy, *Achlysiella williamsi* Siddiqi, and *Xiphinema elongatum* Schuurmans, Stekhoven and Teunissen were common.

Table 3.3.1: Nematodes found in 135 sugarcane fields in far-north Queensland (Survey Area 1).

Nematodes		Nematodes/200 mL of soil		
Common name	Species	Incidence (%)	Mean[A]	Maximum
Lesion	*Pratylenchus zeae*	100	465	3020
	Pratylenchus brachyurus	1	40	40
Spiral	*Helicotylenchus dihystera*	80	129	1020
	Rotylenchus brevicaudatus	1	10	10
Ring	*Criconemella curvata*	67	85	610
Burrowing	*Achlysiella williamsi*	42	79	500
Dagger	*Xiphinema elongatum*	26	39	180
Stubby root	*Paratrichodorus minor*	21	82	300
Root-knot	*Meloidogyne* spp.	19	72	240
Stunt	*Tylenchorhynchus annulatus*	19	53	175
	Tylenchorhynchus claytoni	1	50	50
Reniform	*Rotylenchulus reniformis*	8	57	120
Lance	*Hoplolaimus* spp.	4	35	110

[A]Means were calculated from the samples only where nematodes were detected in Baermann tray extractions

Table 3.3.2: Nematodes detected from 29 sugarcane fields in the Mulgrave valley of far-north Queensland (Survey Area 2).

Nematodes			Nematodes/ 200 mL of soil		Nematodes/10 g root (fresh wt.)	
Common name	Species	Incidence (%)	Mean[A]	Maxi-mum	Mean[A]	Maxi-mum
Lesion	*Pratylenchus zeae*	100	1539	5000	1633	5000
Root-knot	*Meloidogyne* spp.	76	275	1270	229	960
Stunt	*Tylenchorhynchus annulatus*	83	174	880		
Dagger	*Xiphinema elongatum*	76	99	410		
Burrowing	*Achlysiella williamsi*	66	182	500	171	560
Spiral	*Helicotylenchus dihystera*	69	63	320		
Ring	*Criconemella curvata*	41	62	200		
Stubby root	*Paratrichodorus minor*	38	52	170		
Reniform	*Rotylenchulus reniformis*	27	137	270		

[A]Means were calculated from the samples only where nematodes were detected in Baermann tray or root misting extractions

Paratrichodorus minor Colbran and *Rotylenchulus reniformis* Linford and Oliveira were less common, particularly in Survey Area 1. *Pratylenchus brachyurus* Godfrey, *Rotylenchus brevicaudatus* Colbran, and *Tylenchorhynchus claytoni* Steiner were detected only at single sites.

While *P. zeae* and *C. curvata* occurred at a wide range of densities in all soils, an influence of location and soil type was evident. The mean soil densities of these nematodes in the Tully River catchment were significantly lower ($P<0.05$) than in other catchments (Table 3.3.3). Poorly draining clays (Category 5) in the Tully River catchment had a lower mean soil density of *P. zeae* than any other soil group ($P<0.05$). Poorly draining clays (Category 6) in the Maria/Liverpool catchments had a higher mean soil density of *C. curvata* than most other soil groups ($P<0.05$). Soil Categories 1, 3 and 7 contained fewer *H. dihystera* on average than soil Categories 2, 4, 6 and 8 (Table 3.3.4).

The mean soil density of *P. zeae* on crops planted after no fallow (replant), were higher ($P<0.05$) than nematodes in the rhizosphere of ratoon crops or plant crops that followed a fallow (6-12 months) (Table 3.3.4). Densities of *H. dihystera* and *C. curvata* were similar, regardless of crop age or prior fallow history ($P>0.05$, data not presented).

Table 3.3.3: Nematodes in 200 mL of soil, transformed[A] and compared in separate sugarcane growing catchments of far-north Queensland (Survey Area 1).

Catchment	*Pratylenchus zeae*	*Helicotylenchus dihystera*	*Criconemella curvata*
Tully river and Banyan creek basins and tributaries	6.34 b (255)	3.22 b (34)	1.98 b (8)
Maria/Liverpool creek basins and tributaries	7.64 a (446)	4.95 a (121)	3.13 a (31)
South Johnston river basins and tributaries	7.38 a (402)	3.53 b (44)	2.98 a (26)
Mourilyan sand belt and adjoining Moresby river basin and tributaries	7.57 a (433)	2.59 b (17)	3.26 a (35)
Average LSD[B] (P=0.05)	0.97	0.99	0.94

Values in the same column followed by the same letter are not significantly different at P=0.05
[A]Transformed to $(x + 0.5)^{1/3}$. Values in parentheses are back-transformed means
[B]Average LSD is shown, but exact LSD values were used for pair-wise testing

Table 3.2: Soil type categories used to describe soils in far-north Queensland sugarcane fields (Survey Area 1).

Soil category number and, (no. of sites sampled)	Description (Murtha 1986; Cannon *et al.* 1992)	Soil association	Mean particle size distribution[A]
1 (14)	Coarse sands to sandy loams formed on beach ridges.	Kurrimine, Brosnan	65:25:2:8
2 (15)	Sandy loams formed on granitic fans.	Thorpe	55:22:8:15
3 (16)	Well-drained, silty loam to silty clay loams formed on alluvial plains in the Tully River delta and tributaries.	Tully, Liverpool	5:40:20:35
4 (18)	Well-drained, silty loam to silty clay loams formed on alluvial plains in the Maria, Liverpool and Mena Creek deltas and tributaries.	Tully, Liverpool	22:30:15:33
5 (14)	Poorly drained, clay loam to light clays formed on alluvial plains in the Tully River delta and tributaries.	Coom, Bulgun, Hewitt, Banyan	15:20:20:45
6 (10)	Poorly drained, clay loam to light clays formed on alluvial plains in the Maria, Liverpool and Mena Creek deltas and tributaries.	Coom, Bulgun, Ramleh	10:20:25:45
7 (9)	Silty loam to light clays formed on alluvial plains in the Moresby and lower Johnston River deltas.	Innisfail, Coom, Timara	15:20:25:40
8 (11)	Clay loam to clays formed on gently undulating alluvial fans from Basalt and Metamorphic parents.	Mundoo, Galmara	10:17:15:58
9 (28)	Kraznozems (Basalt clays) formed *in situ* on sloping uplands and foothills.	Pin Gin Eugenangee	3:10:17:70

[A]Particle size distributions are expressed as % coarse sand:fine sand:silt:clay

Table 3.3.4: Nematodes in 200 mL of soil, transformed[A] and compared in different soil categories and crop stages in far-north Queensland sugarcane fields (Survey Area 1).

Soil category number (Table 3.2)	*Pratylenchus zeae*	*Helicotylenchus dihystera*	*Criconemella curvata*	Crop Stage	*Pratylenchus zeae*
1	7.37 ab (400)	2.27 c (11)	2.75 cd (20)	Replant	7.86 a (486)
2	6.91 b (330)	5.04 a (128)	2.59 cd (17)	Fallow plant	6.84 b (320)
3	7.17 b (369)	2.82 c (22)	2.08 cd (9)	Ratoon	6.88 b (326)
4	7.37 ab (400)	4.97 a (122)	2.62 cd (18)		
5	5.44 c (161)	3.37 bc (38)	1.83 d (6)		
6	8.69 a (655)	4.58 ab (96)	5.00 a (125)		
7	7.77 ab (469)	2.63 c (18)	4.67 ab (102)		
8	7.03 b (347)	4.34 ab (81)	3.37 bc (38)		
9	7.50 ab (422)	3.34 bc (37)	2.37 cd (13)		
Av. LSD[B] (P = 0.05)	1.90	2.25	1.25		0.81

Values in the same column followed by the same letter are not significantly different at P=0.05
[A]Transformed to $(x + 0.5)^{1/3}$. Values in parentheses are back-transformed means
[B]Average LSD is shown, but exact LSD values were used for pairwise testing

3.4 Discussion

The same nematode species were detected in far-north Queensland, as in sugarcane fields on the north, central and south Queensland coast (Blair *et al*. 1999 a, b), but the incidence of some species varied. As in other regions, *P. zeae* was ubiquitous and routinely found in the soil and roots at higher densities than other species. *Helicotylenchus dihystera* was also common. However in Survey Area 1, the incidence and densities of *Meloidogyne* spp., *T. annulatus*, *P. minor* and *R. reniformis*, were lower than in Survey Area 2, and other fields in Queensland (Blair *et al*. 1999 a, b). This may be because (a) this area was sampled late in the season when nematode populations tended to be low, whereas other surveys were conducted mid-season when nematodes are more numerous (Chapter 5), and (b) nematodes were extracted from soil over 48 hours, rather than 96 hours. The low incidence of *Meloidogyne* spp. in Survey Area 1 may also be due to the high number of clay soils in that region. In south Queensland, *Meloidogyne* spp. were found at a lower incidence in clay soils than sandy soils (Blair *et al*. 1999 a). In Survey Area 2 where sandy clay loams were predominant, the incidence of *Meloidogyne* spp. was comparable to populations in other areas of Queensland.

These surveys confirmed that *A. williamsi* is a widespread endoparasite of sugarcane roots in far-north Queensland. The common range of this nematode extends as far south as the Burdekin region (Blair *et al*. 1999 b). However its incidence is unknown in the Herbert River region because surveys have not been conducted there.

The extent that sugarcane yields are reduced by nematodes across Queensland is unknown. However, historically the sugar industry has perceived that nematode problems are confined to sandy soils in south Queensland (Magarey and Croft 1995), probably because nematode damage is the most obvious there and nematicides are used commercially there. In far-north Queensland, on the few occasions that nematicides and fumigants were applied under optimal conditions, they significantly reduced nematode populations and crop growth was improved (Chandler 1978, 1980, 1984). Specifically, the nematicides improved yield 15-111% at four of six sites. At those sites, nematode counts in untreated soil were equivalent to those found in these surveys, implying that crop losses from nematodes may be widespread in the far-north. Given that *P. zeae* is ubiquitous and an invasive root parasite, its pest status and role in YD of sugarcane requires further investigation. *Pratylenchus zeae* is the most important nematode pest of sugarcane worldwide and its pathogenicity to sugarcane has been demonstrated (Spaull and Cadet 1990).

The lower mean densities of *P. zeae* and *C. curvata* in the Tully River catchments cannot be attributed principally to soil type or texture. Densities of *P. zeae* were the lowest in poorly draining clays in the Tully catchment, but highest in equivalent poorly draining clays in the neighbouring Maria/Liverpool catchments. More *H. dihystera* were found in some soil types than others. However, texture of the soil did not appear to be the major factor affecting the distribution of this nematode.

To summarise, nematodes were detected at a wide range of densities across the sugarcane fields of far-north Queensland. Mean densities of *P. zeae* were similar rather than widely different between regions (catchments) and soil types, as were those of *C. curvata*. Apart from *Meloidogyne* spp., nematode densities in the soil are also largely independent of soil texture in other sugarcane growing regions (Blair *et al*. 1999 a, b). Generally, nematodes were equally common in plant or ratoon crops and crops with different fallow histories. An exception was *P. zeae*, which tended to be more abundant on sugarcane that was planted into non-fallowed (replant) soil.

Legume crops are typically poor hosts of *P. zeae* (Sundararaj and Mehta 1993 b), thereby reducing nematode densities during the fallow.

CHAPTER 4

WITHIN-FIELD DISTRIBUTION OF NEMATODES AND IMPLICATIONS FOR SAMPLING

4.1 Introduction

Most organisms are dispersed in an aggregated manner within the environment they occupy (Southwood 1978), and soil nematodes are no exception. Aggregated or clumped populations have a large variance to the mean ratio when sampled, and samples have skewed frequency distributions. In contrast, when organisms are distributed randomly, the variance is similar to the mean and the population has a 'normal' frequency distribution when repeatedly sampled. Various models have been used to describe clumped dispersion, such as the negative binomial, Iwao's patchiness regression and Taylor's power law (Southwood 1978).

Where densities of nematodes are to be estimated in field experiments, many sub-samples are taken from replicate plots to overcome the bias associated with dispersive patterns of nematodes. An unrealistic number of sub-samples are usually required to fully represent and account for nematode aggregates across fields. Thus at the sampling level used, the population data set requires a transformation have normality, which is an assumption of comparisons by ANOVA.

Nematodes are currently being researched in the Australian Sugar Industry within a multidisciplinary 'yield decline' program. To cater for all the research participants within the program, field experiments are large and cover whole fields, with many treatments and few replicates. These experiments must be sampled for nematodes, but there are few accounts describing the dispersive patterns of nematodes across sugarcane fields in Queensland (Allsopp 1990).

On other crops, work on dispersion has dealt chiefly with the sampling effort required to accurately assess nematode densities in diagnostic samples, or detect species in surveys with high confidence. Predictions vary widely, depending on the degree of nematode clumping. In alfalfa fields of up to 7 ha, Prot and Ferris (1992) found 10 bulked sub-samples provided an acceptable population estimate of

Pratylenchus neglectus Filipjev. However, under a tobacco-rye rotation, even 40 bulked sub-samples provided a poor estimate of the mean density of *Pratylenchus penetrans* Cobb in a 0.01 ha plot (Proctor and Marks 1974).

In this study, the horizontal distribution of nematodes was estimated in the roots and associated soil from crops of plant sugarcane. Nematode dispersions were related to plot size and nematode species, and the relevance to sub-sampling in field experiments was discussed. The negative binomial model was used to describe the distribution of nematodes, if clumped. Equations from the model were used to correlate sampling error with sub-sampling effort.

4.2 Materials & Methods

4.2.1 Site 1

The field selected was a sandy clay loam (Tully series, from Murtha 1986) with a coarse sand, fine sand, silt and clay content of 12, 34, 20 and 34% respectively. Sugarcane (cultivar Q117) was sampled in mid-March, at approximately 180 days from planting (DAP). From an area of 5 $\times$ 6 m (0.003 ha), 49 soil samples (in a regular 7 $\times$ 7 grid) were taken 0.8 m apart across rows and 1.0 m apart down rows. Thus with a row width of 1.6 m, all samples were collected about 20 cm from the edge of the sugarcane stool. This is an accepted zone to sample for nematodes around sugarcane roots (Allsopp 1990; Chapter 5). Samples were collected to a depth of 30 cm using a 3 cm diameter soil auger and nematodes were separated from 200 mL of soil using the Baermann tray method for 48 hours (Whitehead and Hemming 1965).

4.2.2 Site 2

The field selected was a sandy loam (Thorpe series, from Murtha 1986) with a coarse sand, fine sand, silt and clay content of 60, 12, 7 and 21% respectively. The field was planted to sugarcane (cultivar Q117) and was sampled in early-April at approximately 200 DAP. From an area of 220 $\times$ 120 m (3.36 ha), 84 samples were collected at 20 m intervals in a 12 $\times$ 7 grid down and across rows respectively. At each interval, a sample of soil and roots was collected with a spade at 20 cm from the edge of the sugarcane stool, and to a depth of 30 cm. Nematodes were separated from

200 mL of soil and 5 g of root (fresh weight) using the Baermann tray method for 48 hours.

4.2.3 Analyses

Nematode aggregation was described with the 'negative binomial model'. The number of samples taken (n) was related to the accuracy in estimating the mean field populations of nematodes, with high probability. The equations used were:

$$n = \frac{\frac{1}{\bar{x}} + \frac{1}{k}}{E^2} \quad where\ k = \frac{\bar{x}^2}{s^2 - x}$$

and 'k' is a measure of degree of nematode aggregation, 'x' is the sample mean, and 's^2' is the sample variance (Southwood 1978). From these equations, 'n' was plotted against the standard error/mean ratio (E) for each nematode species (McSorley and Parrado 1982). The model assumed that the mean density of nematodes recovered from a series of single samples, was the same as the density found if the samples were mixed as a composite sample and then nematodes extracted.

Transformations of $(x + 0.5)^{1/2}$, $(x + 0.5)^{1/3}$ and $\mathrm{Ln}(x + 1)$ were applied to the data sets from each site. The symmetry and spread of nematode counts around the mean were reported as skew and kurtosis respectively, with low values indicating adequate transformation of the data to a normally distributed set (Snedecor and Cochran 1989).

4.3 Results

4.3.1 Site 1

The dominant nematode at Site 1 was *Pratylenchus zeae* Graham with some *Helicotylenchus dihystera* Cobb, and mean densities were 636 and 46 nematodes/200 mL of soil respectively. Both nematode species had aggregated distributions across the plot. As an example, the clumping of *Pratylenchus zeae* is shown diagrammatically from contours (Figure 4.3.1.1).

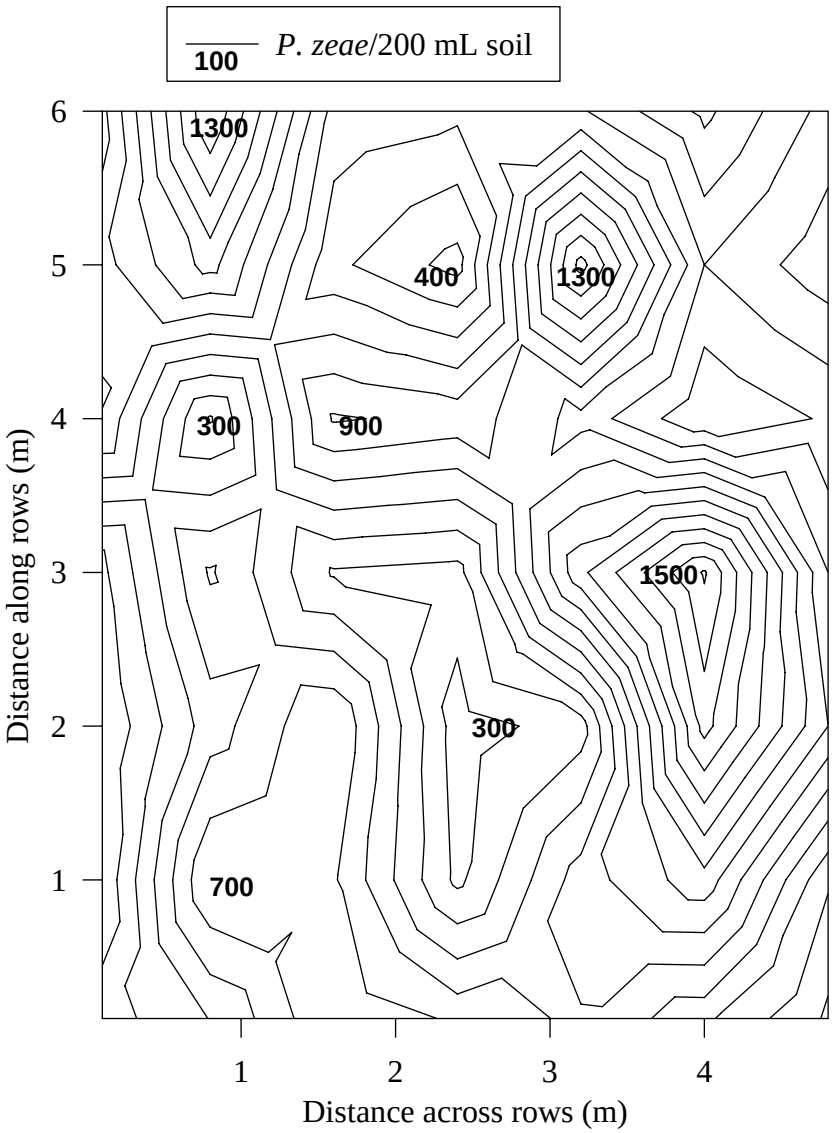
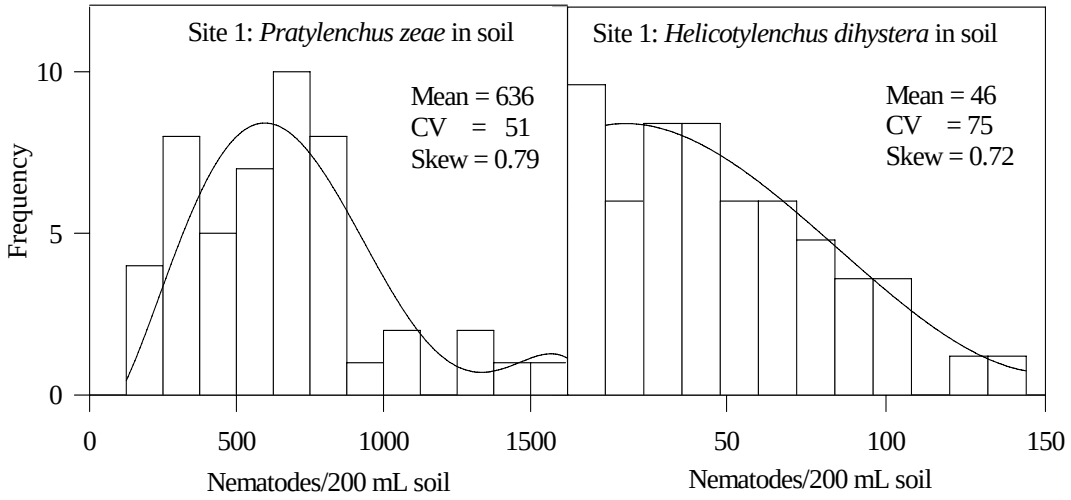


Figure 4.3.1.1: Diagrammatic representation of *Pratylenchus zeae* dispersed in the soil across Site 1, formulated from 49 points taken 20 cm from the edge of the stool to a depth of 30 cm. The isolines are drawn at population levels differing by 100.

Nematode frequency distributions and descriptive statistics also showed that both nematodes were distributed in clusters (Figures 4.3.1.2 and 4.3.1.3). The 'k' measures of aggregation were 3.8 for *P. zeae* and 1.9 for *H. dihystera*.



Figures 4.3.1.2 and 4.3.1.3: Nematode frequency distributions (histograms) and dispersion statistics in the soil at Site 1, generated from 49 points across the plot (5 × 6 m).

To estimate the mean field density of *P. zeae* with standard error to mean ratios of 20% and 10%, a composite sample of 7 and 27 sub-samples were required (Figure 4.3.1.4). Because *H. dihystera* was more clustered, a composite sample of 14 and 55 sub-samples were required to estimate the population mean with the same 20% and 10% precision (Figure 4.3.1.4).

While the Ln(x) transformation removed kurtosis in the data set, the power transformations removed skew more effectively (Table 4.3.1).

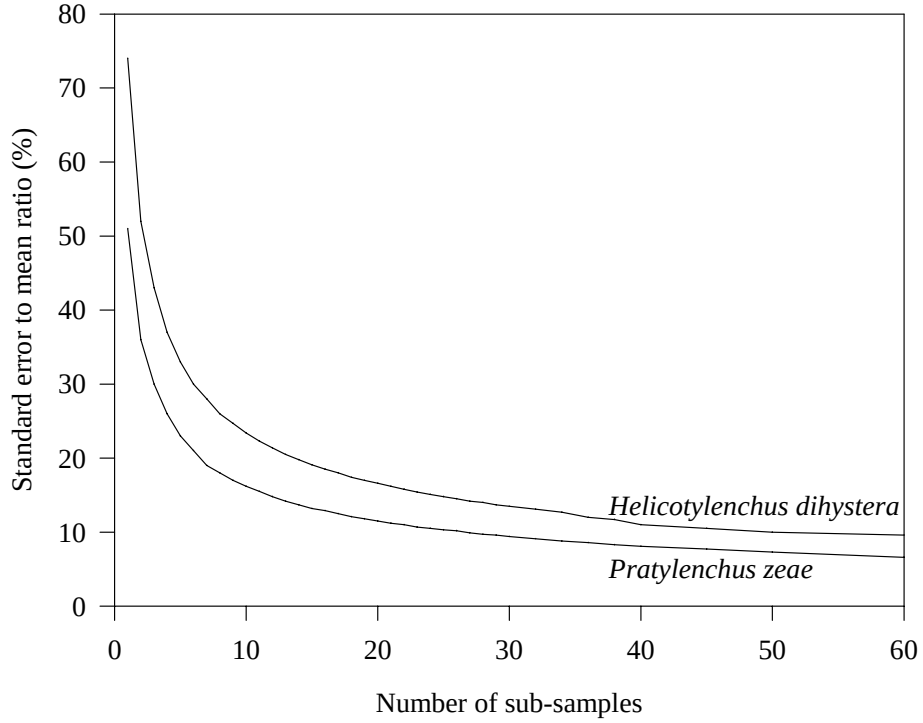


Figure 4.3.1.4: Correlations between precision achieved and sampling effort (sub-samples bulked) at Site 1.

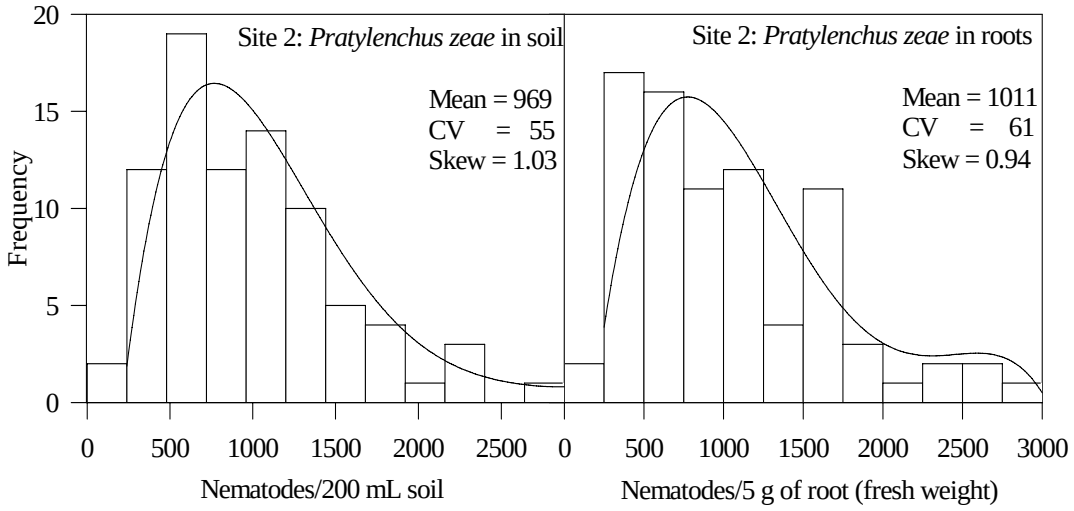
Table 4.3.1: Effect of transformations on the dispersion statistics of *Pratylenchus zeae* and *Helicotylenchus dihystera* in the soil at Site 1.

Statistic	*Pratylenchus zeae*				*Helicotylenchus dihystera*			
	Not transformed	$x^{1/2}$	$x^{1/3}$	Ln x	Not transformed	$x^{1/2}$	$x^{1/3}$	Ln x
Mean	636	24	8	3	46	6	3	3
CV	51	26	18	9	75	43	30	31
Skew	0.79	0.14	0.08	0.53	0.72	0.01	0.27	0.87
Kurtosis	0.42	-0.3	.034	0.01	-0.17	-0.87	-0.8	0.01

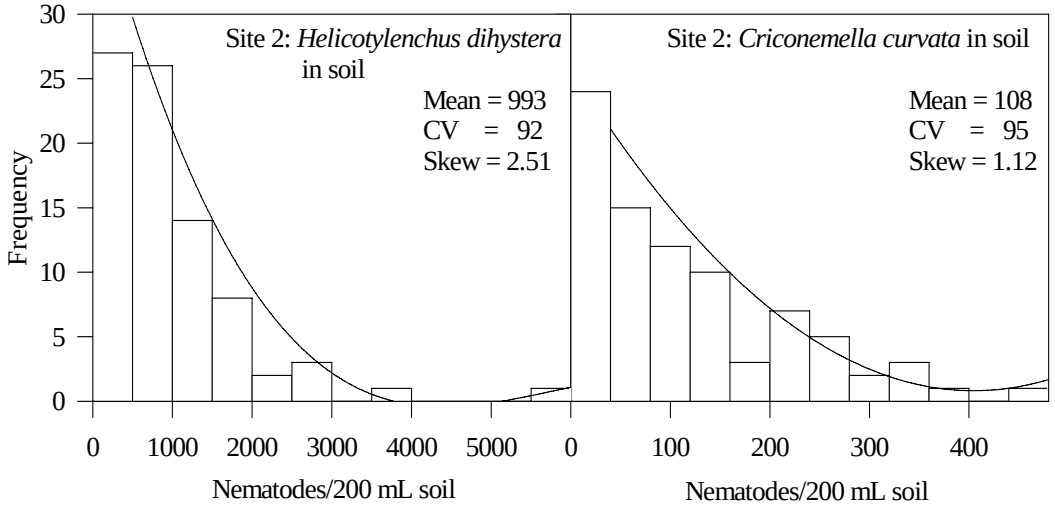
4.3.2 Site 2

At Site 2, *P. zeae* and *H. dihystera* were co-dominant with means of 968 and 993 nematodes/200 mL of soil respectively. *Criconemella curvata* de Grisse and Loof was also present at a mean density of 108 nematodes/200 mL of soil. Five g of root (fresh weight) contained an average of 1011 *P. zeae*. The frequency distributions and descriptive statistics indicate that all nematode species were clustered in their distribution (Figures 4.3.2.1-4.3.2.4). *Helicotylenchus dihystera* and *C. curvata* were

the most aggregated, as indicated by the high 'coefficient of variation' (CV) and highly skewed frequency distributions (Figures 4.3.2.1-4.3.2.4). Thus the 'k' value measures of aggregation were 3.41 for *P. zeae* in soil, 2.68 for *P. zeae* in roots, 1.17 for *H. dihystera* and 1.12 for *C. curvata*. To estimate the mean *P. zeae* density with standard error to mean ratios of 20% and 10%, a composite sample of 8 and 30 sub-samples of soil and 10 and 38 sub-samples of root were required (Figure 4.3.2.5). To estimate the mean of *H. dihystera* and *C. curvata* densities with the same 20% and 10% precision, composite samples of approximately 22 and 90 sub-samples were required (Figure 4.3.2.5).



Figures 4.3.2.1 and 4.3.2.2: *Pratylenchus zeae* frequency distributions (histograms) and dispersion statistic in soil and roots at Site 2, generated from 84 points across the plot (120 × 220 m).



Figures 4.3.2.3 and 4.3.2.4: Ectoparasite frequency distributions (histograms) and dispersion statistics in soil and roots at Site 2, generated from 84 points across the plot (120 × 220 m).

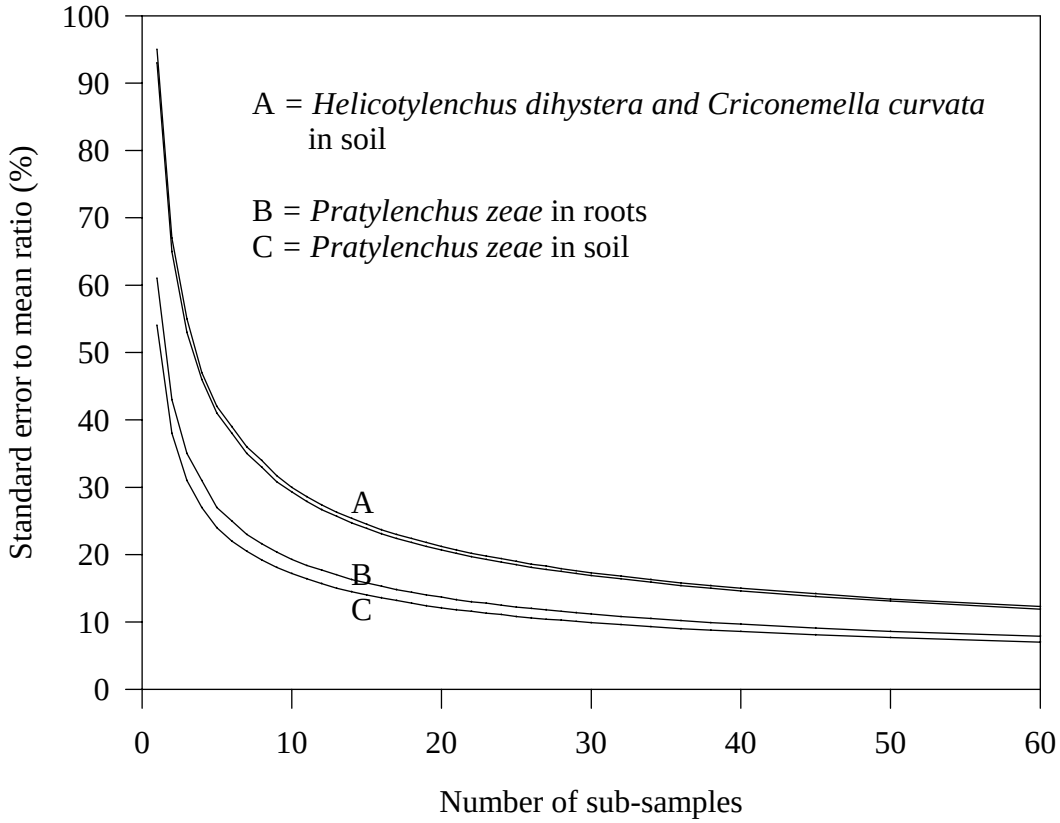


Figure 4.3.2.5: Correlation between precision achieved and sampling effort (sub-samples bulked) at Site 2.

The power transformation ($x^{1/3}$) most consistently lowered skew and kurtosis in the data sets of all nematode species in the soil and *P. zeae* in the roots (Table 4.3.2).

Table 4.3.2: Effect of transformations on the dispersion statistics of nematodes in the soil and *Pratylenchus zeae* in the roots at Site 2.

Statistic	*Pratylenchus zeae* in soil				*Pratylenchus zeae* in roots			
	Not transformed	$x^{1/2}$	$x^{1/3}$	Ln x	Not transformed	$x^{1/2}$	$x^{1/3}$	Ln x
Mean	968	30	10	7	1011	30	10	7
CV	54	28	19	9	61	31	21	10
Skew	1.03	0.32	0.07	-0.48	0.94	0.35	0.14	-0.27
Kurtosis	1.12	-0.09	-0.18	0.28	0.44	-0.55	-0.68	-0.64
	Helicotylenchus dihystera in soil				*Criconemella curvata* in soil			
Mean	993	29	9	6	83	9	4	4
CV	92	45	30	15	95	56	41	42
Skew	2.53	0.85	0.39	-0.41	1.16	0.21	-0.23	-1.26
Kurtosis	9.71	1.34	0.25	-0.29	0.84	-0.69	-0.63	0.85

4.4 Discussion

Across the two sites, all nematode species were distributed in a clumped pattern as illustrated in Figure 4.3.1.1, and described by descriptive statistics (Figures 4.3.2.1-4.3.2.4). Such patterns of distribution are common in other crops (Barker and Campbell 1981; Schmitt *et al.* 1990) and have previously been shown to occur on sugarcane (Allsopp 1990; Delaville *et al.* 1996). Because the level of nematode micro-distribution (metre by metre) is strongly linked to reproductive strategy and feeding habit (Ferris *et al.* 1990), the pattern of dispersion varied between species. *Pratylenchus zeae* was distributed the most uniformly, perhaps unexpectedly as its endoparasitic nature would suggest that populations may aggregate within roots.

Pratylenchus zeae was distributed more uniformly across Site 1 than Site 2, probably because samples were collected over a much greater area at Site 2. Gradients in edaphic factors, such as soil texture and moisture, were expected to further contribute to spatial variation in nematode dispersion at a macro-distributional level (hectare by hectare). These dispersions were also comparable to the aggregated pattern of *P. zeae* in 1.92 m^2 plots, between two rows of 9^{th} ratoon sugarcane in Martinique (Delaville *et al.* 1996). However, the density of roots and pore space (bulk density) varied between the two rows, and appeared to encourage gradients in nematode density. At

Sites 1 and 2, sampling a regular distance from the stool probably avoided these edaphic effects.

Compared to *P. zeae*, distributions of *H. dihystera* and *C. curvata* were more clumped, as has been found elsewhere (Delaville *et al.* 1996). This is perhaps due to the greater influence of micro- and macro-distributional factors on them. Criconematids tend to have a sedentary feeding habit (Hussey *et al.* 1991) and move sluggishly through the soil, thus decreasing their uniformity throughout the profile. Delaville *et al.* (1996) found that gradients in soil texture had a more pronounced effect on the distribution of *Criconemella oenensis* Luc than other nematodes, including *P. zeae*. *Helicotylenchus dihystera* is a relatively large nematode, with a feeding habit sometimes described as semi-endoparasitic (Luc *et al.* 1990 a). These characteristics may contribute to it becoming relatively aggregated in the soil profile.

At Site 2, *P. zeae* was distributed more uniformly in soil than roots, but the difference was not great. Obviously *P. zeae* transits roots and soil quite actively during its life cycle, unlike endoparasites such as *Meloidogyne* spp. (root-knot nematode) that feed and deposit their eggs in one location (Luc *et al.* 1990 a). *Pratylenchus zeae* may also feed ectoparasitically on the fine root network established by sugarcane. *Pratylenchus penetrans* juveniles feed ectoparasitically on the root hairs of various host plants (Zunke and Perry 1997). As *P. zeae* was distributed at similar levels in soil and roots, the implication for sampling is that one strategy will cater for both, at least in maturing crops of sugarcane.

Using Site 1 to represent a plot size of 30 m^2, a composite sample of about 10 sub-samples estimated the mean density of *P. zeae* in the soil with good precision. With a 40 plot field experiment, this is a realistic sampling effort. However, in subsequent field experiments (Chapter 11), a minimum of 90 m^2 was found necessary for destructive sampling and to providing an adequate sample of plant biomass. Because the aggregated habit of *P. zeae* did not vary much more over 3660 m^2 (Site 2) than 30 m^2 (Site 1), 10 sub-samples are probably still adequate to find the mean density of *P. zeae* in a 90 m^2 plot with good precision. Allsopp (1990) used composite samples of five sub-samples to construct a nematode data set from across sugarcane fields in

south Queensland. *Pratylenchus zeae* was distributed the most uniformly, and $x^{1/2}$ was the best transformation for counts from populations inside roots.

Helicotylenchus dihystera and *C. curvata* were less uniform in their distribution, and more extreme transformations would be needed to normalise data sets if there are limits on sub-sampling effort per plot. According to the data sets from Sites 1 and 2, power transformations and particularly $x^{1/3}$ are favoured over log transformations to normalise population counts of these nematodes. Allsopp (1990) recommended $x^{1/4}$ transformations to normalise *H. dihystera* and criconematid counts in the soil. However, the data set used was constructed from a number of different fields, and contrasting edaphic effects from field to field such as soil texture, root volume, root health, etc., probably stimulated a wider range of nematode densities. From a single field, $x^{1/3}$ transformations are likely to be adequate provided that sufficient numbers of sub-samples are bulked per experimental plot.

CHAPTER 5

THE POPULATION DYNAMICS OF PLANT-PARASITIC NEMATODES ON SUGARCANE CROPS

5.1 Introduction

Plant-parasitic nematodes are obligate parasites of plants, so densities of nematodes and crop vigour are directly related. Nematode populations may increase or decline depending on factors such as fallowing practice between crop cycles, host suitability of the crop species, and the growth rate of the crop as influenced by season. Heavily parasitised plants can ultimately become a limiting nutrient source, severely lowering the density of pest nematodes that are associated (McSorley and Phillips 1993).

On sugarcane in South Africa, populations of endoparasite nematodes fluctuated in response to successions from sett roots to shoot roots to ratoon shoot roots (Spaull and Cadet 1990). However, there have been no similar studies in Australia. Typically, single counts of nematode density have been reported simply to ascertain the level of nematode control obtained with a nematicide (Chandler 1980). Nevertheless, an understanding of nematode increase and decline is essential to:

(a) correlate yield loss with nematode density,

(b) predict yield loss from diagnostic counts, and

(c) evaluate management options (McSorley and Phillips 1993).

To better understand the dynamic in Australian sugarcane fields, densities of nematodes from row to inter-row were monitored at Tully during a cultivated fallow, a herbicide fallow and in the plant and 1st ratoon crop that followed. Additionally, densities of nematodes were monitored throughout fallows, plant crops and ratoon crops in a selection of sugarcane fields in far-north Queensland. During these studies, fallowing, season, root habit and crop stage were examined as influences on the population dynamics of nematodes throughout the sugarcane cropping cycle.

5.2 Materials and Methods

5.2.1 Spatial dynamics at Tully

The experiment was conducted in a field at the Tully BSES in a sandy clay loam soil (Tully series, from Murtha 1986) with a clay content of about 35%. Following the harvest of 4th ratoon sugarcane (cultivar Q117), most of the field was fallowed by ploughing out the old crop corm. This involved one ripper pass down to 40-50 cm and one disc plough pass down to 30 cm. This is the traditional method of implementing a fallow between sugarcane crop cycles in Queensland. In a section of the field the sugarcane was not ploughed out, but allowed to ratoon, and then sprayed with glyphosate at 3 L/ha when plants were 50 cm high. Thus:

(a) an untilled herbicide fallow, and

(b) a ploughed bare fallow were established in adjoining sections of the field.

In both fallow strategies, subsequent weed growth was controlled with glyphosate at 3 L/ha. After 240 days of fallowing (DAF) the entire field was disc ploughed down to 30 cm and rotary hoed down to 20-30 cm in preparation for sugarcane. Sugarcane (cultivar Q124) was then planted into these plots and grown according to accepted commercial practices.

At regular intervals during the cropping cycle, densities of nematodes were monitored in the rows where the two different fallow histories adjoined. Along 10 m lengths of crop row, 10 soil cores were taken to a depth of 30 cm, and combined into a composite sample. The row position sampled was either (a) in the centre at the row, (b) 30 cm from the centre of the row or (c) in the centre of the inter-row. Throughout this report these positions are referred to as (a) row centre, (b) near the row and (c) inter-row, respectively. From the composite samples taken at each row position, nematodes were extracted over 48 hours using a Baermann tray (Whitehead and Hemming 1965), concentrated with a 38 μm sieve, and counted. This process was repeated along five sections of row to produce five replications. In the tilled fallow, samples from the row to inter-row were taken according to the position of the previous crop. Sugarcane was planted in approximately the same row position as the

previous crop. Populations of nematodes (*P. zeae* or *H. dihystera*) were reported as a field mean, by combining counts from the row, near the row and inter-row (Figures 5.3.1.1a and 5.3.1.2a). Populations were also reported separately for each of the row positions (Figures 5.3.1.1b and 5.3.1.2b).

5.2.2 Dynamics at other sites

Sugarcane crops in various stages of development (fallow to 2nd ratoon) were monitored in 14 fields, from Tully to Gordonvale, in soil types that ranged from 8-55% clay. Across a 0.2 ha block of each field, 20 sub-samples of soil and roots were collected with a spade. Samples were collected 10-30 cm from the stool, to a depth of 30 cm. From the pooled sample, nematodes were extracted from all of the roots (washed) and 200 mL of soil, using a Baermann tray (48 hours). During fallows, sub-samples were collected in a grid pattern across the 0.2 ha of field, to a depth of 30 cm, using a soil auger. Nematode counts were presented as a sequential scatter plot of densities in fallow, plant, 1st ratoon and 2nd ratoon crops respectively. A generalised line of population dynamics was constructed by plotting the mean density of nematodes at bimonthly intervals.

5.3 Results

5.3.1.1 *Pratylenchus zeae* Graham at the Tully site

When fallowing commenced there were 220 *P. zeae*/200 mL of soil. The tilled fallow reduced levels of *P. zeae* more than the herbicide fallow initially, and so there were significantly fewer *P. zeae* in the tilled area for most of the fallow period ($P<0.05$). However, these initial differences had disappeared by 240 DAF. Thus, when the sugarcane crop was planted in July, both plots had about 50 *P. zeae*/200 mL of soil (Figure 5.3.1.1a). Throughout the crop cycle, the dynamics of *P. zeae* was similar between the two different fallow histories.

From 0-120 days after planting (DAP), densities of *P. zeae* in the soil did not change. However, *P. zeae* began to multiply rapidly on the plant crop during the monsoon season from December to May (150-300 DAP), and populations peaked at around 650 nematodes/200 mL of soil, at 330 DAP (Figure 5.3.1.1a). Nearing harvest of the plant crop (390 DAP) and from 0-180 days of the next ratoon (DAR), populations of

P. zeae declined steadily to 300 nematodes/200 mL of soil. Populations began to increase on the ratoon crop when the monsoon was well advanced in February (180 DAR), and attained densities of around 600 nematodes/200 mL of soil. This density carried through to harvest (420 DAR) and coincided with steady, higher than average rainfall throughout that season (Figure 5.3.1.1a). In both the plant and ratoon crop, densities of *P. zeae* peaked about 150 days after summer temperatures reached a maximum (Figure 5.3.1.1a).

Prior to fallowing, lower populations of *P. zeae* were in the row centre than the inter-row, and this difference was generally significant for the duration of the herbicide fallow ($P<0.05$). In the tilled fallow, cultivation removed population differences across the row profile (Figure 5.3.1.1b). In the plant crop, consistently more *P. zeae* were found near the row rather than in the row centre and inter-row. These differences were sometimes significant ($P<0.05$) in the crop that followed the herbicide fallow. However, the 1st ratoon developed relatively even populations of *P. zeae* across the row profile. In contrast, the tilled fallow history was associated with consistently fewer nematodes in the ratoon inter-row than near the row. This difference was significant ($P<0.05$) when the population of *P. zeae* peaked at 300 DAP (Figure 5.3.1.1b).

5.3.2.1 *Pratylenchus zeae* at other sites

While other sites often had higher densities of *P. zeae* than the Tully site, the populations generally increased and declined by the same dynamic (Figure 5.3.2.1). Inside roots, populations reflected trends that occurred in the soil (Figure 5.3.2.1). While data for individual sites are not presented, the following observation were made: (a) at planting, replanted sugarcane (no fallow) had relatively high densities of *P. zeae* at two sites (Figure 5.3.2.1). These sites subsequently developed the highest densities of *P. zeae* on plant crops but not on ratoons, (b) lower densities of *P. zeae* occurred in the roots and associated soil of 2nd ratoons compared to plant crops and 1st ratoons (Figure 5.3.2.1).

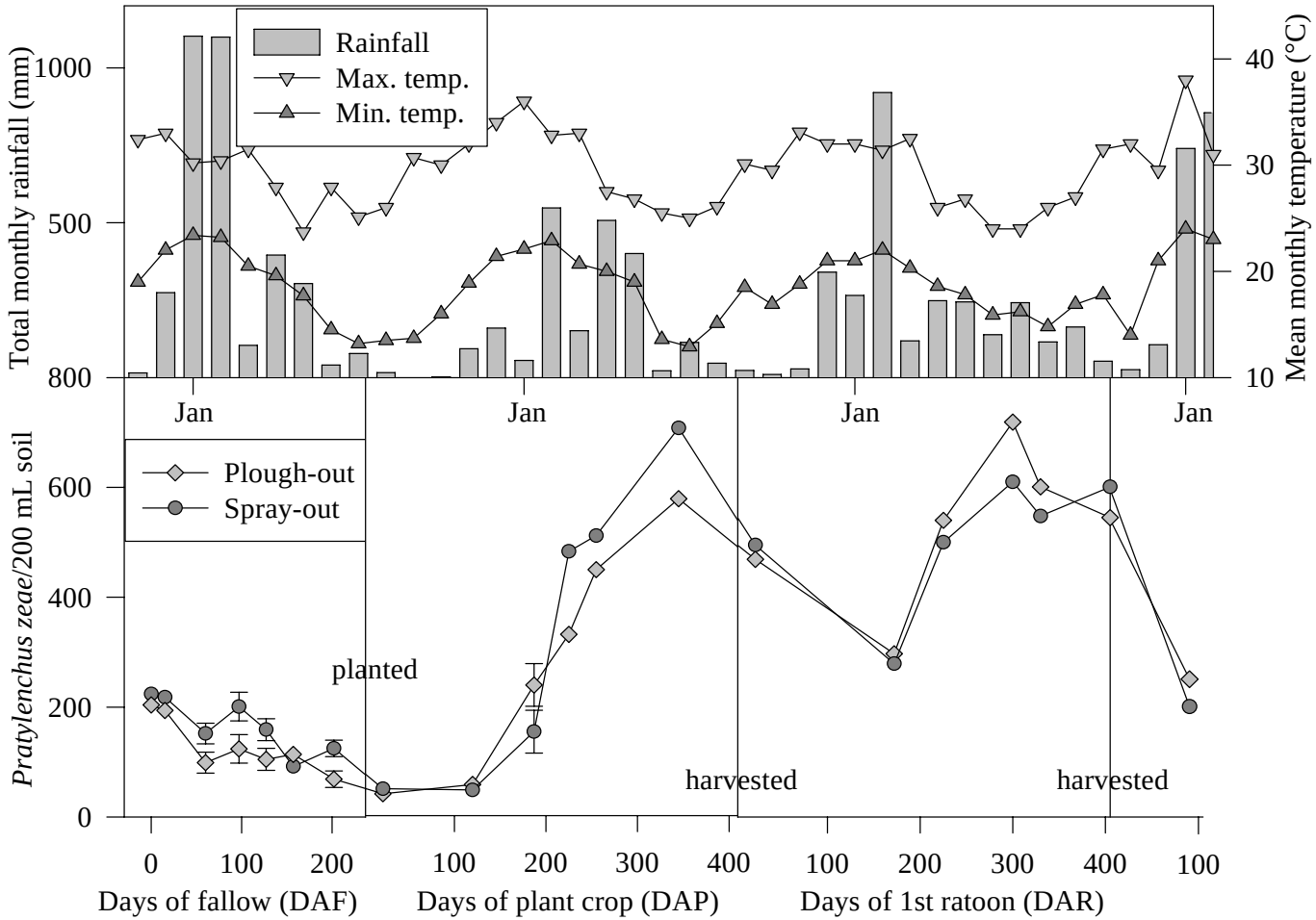


Figure 5.3.1.1a: *Pratylenchus zeae* on a sugarcane crop after a ploughed-out fallow and a herbicide fallow (bottom), and environmental conditions (top) at the site at Tully (LSD bars shown when P<0.05).

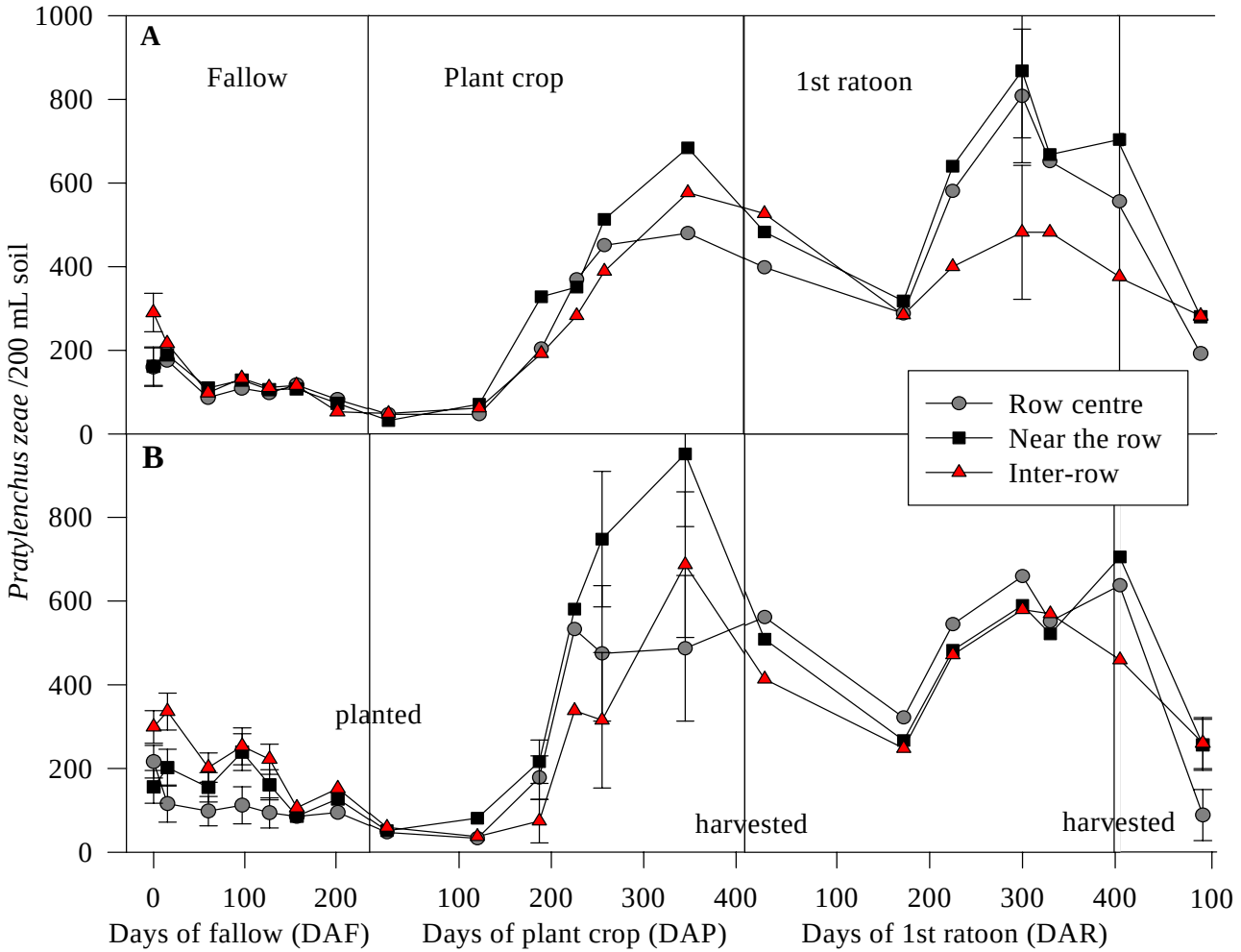


Figure 5.3.1.1b: *Pratylenchus zeae* in the row centre, near row and inter-row of a sugarcane crop after a ploughed-out fallow (A) and a herbicide fallow (B) (LSD bars shown when P<0.05).

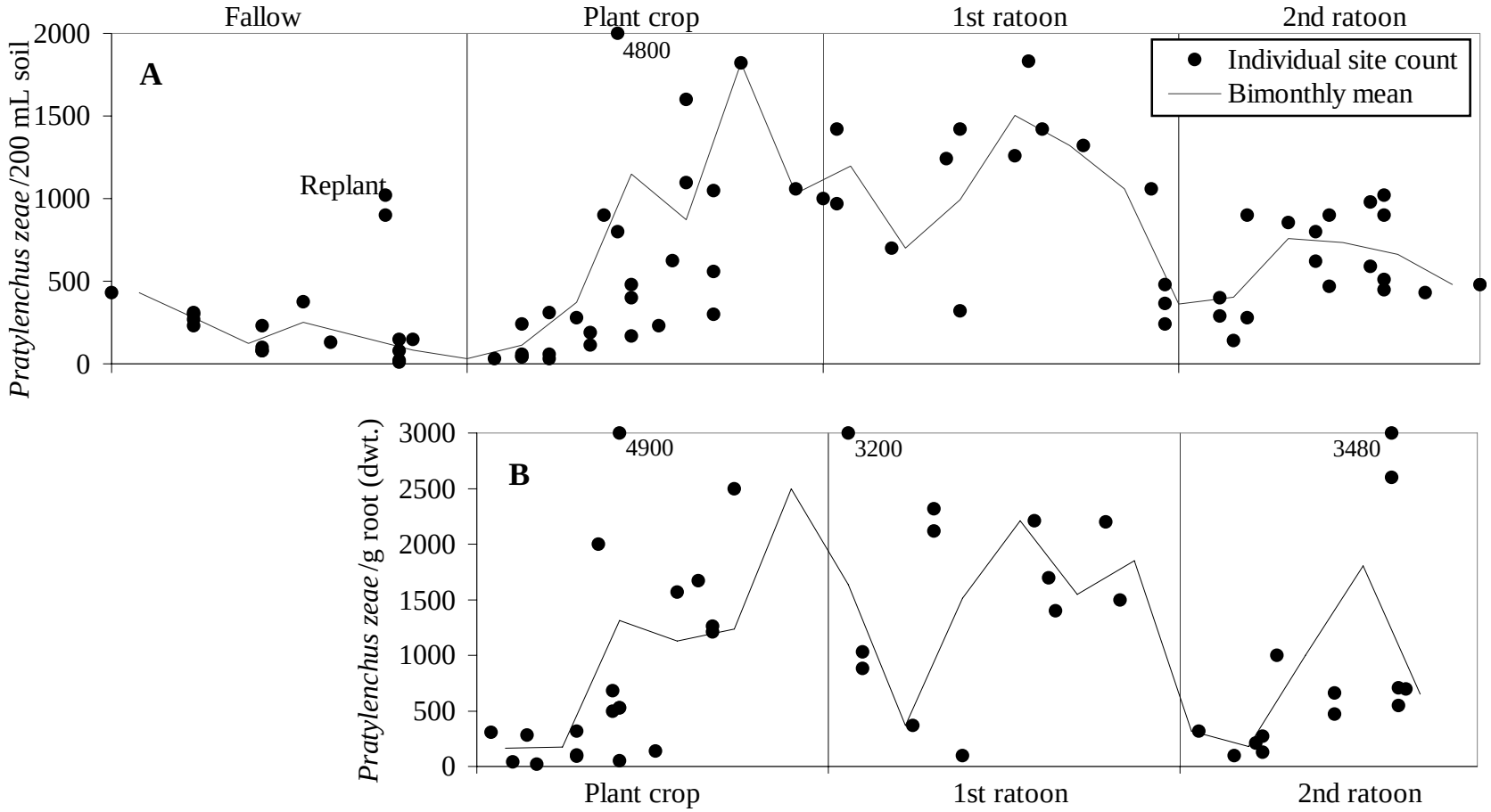


Figure 5.3.2.1: Lesion nematode (*Pratylenchus zeae*) in the soil (A) and roots (B) through progressive crop stages, in a selection of sugarcane fields in north Queensland.

5.3.1.2 *Helicotylenchus dihystera* Cobb at the Tully site

When fallowing commenced at the Tully site, *H. dihystera* was present at densities of about 400 nematodes/200 mL of soil, with fewer nematodes in the section of field to be tilled (Figure 5.3.1.2a). A sharp decline in the density of *H. dihystera* in the row was associated with plough-out of the stool, whereas a high density was maintained in the row of the herbicide fallow (Figure 5.3.1.2b). Shortly after planting to sugarcane, these differences had been maintained. Thus, the plot fallowed with herbicide contained 400 nematodes/200 mL of soil, whereas the ploughed-out plot contained 210 nematodes/200 mL of soil, which was significantly less ($P<0.05$, Figure 5.3.1.2a).

In the ploughed-out plot, populations of *H. dihystera* rarely increased above 300 nematodes/200 mL of soil in the plant and 1st ratoon crop that followed (Figure 5.3.1.2a). From row to inter-row, populations of *H. dihystera* remained similar in the plant and 1st ratoon crop (Figure 5.3.1.2b). This population was usually less ($P<0.05$) than in the plot fallowed with herbicide, with a population peak of 700 nematodes/200 mL of soil in both the plant and 1st ratoon. However this peak was only short lived in the plant crop. A more persistent peak in levels of *H. dihystera* late in the 1st ratoon coincided with more prolonged rainfall throughout that season (Figure 5.3.1.2a).

Prior to fallowing, higher populations of *H. dihystera* were in the row centre rather than near the row, and inter-row, and this difference was maintained throughout the herbicide fallow ($P<0.05$, Figure 5.3.1.2b). However, shortly after planting, row populations plummeted and for the entire plant and 1st ratoon crop cycle, fewer *H. dihystera* were usually found in the row (Figure 5.3.1.2b). More specifically, populations in the row were sometimes significantly less ($P<0.05$) than populations in the inter-row. Under fallow, populations of *H. dihystera* declined more slowly and not as much as *P. zeae* (Figures 5.3.1.1a and 5.3.1.2a).

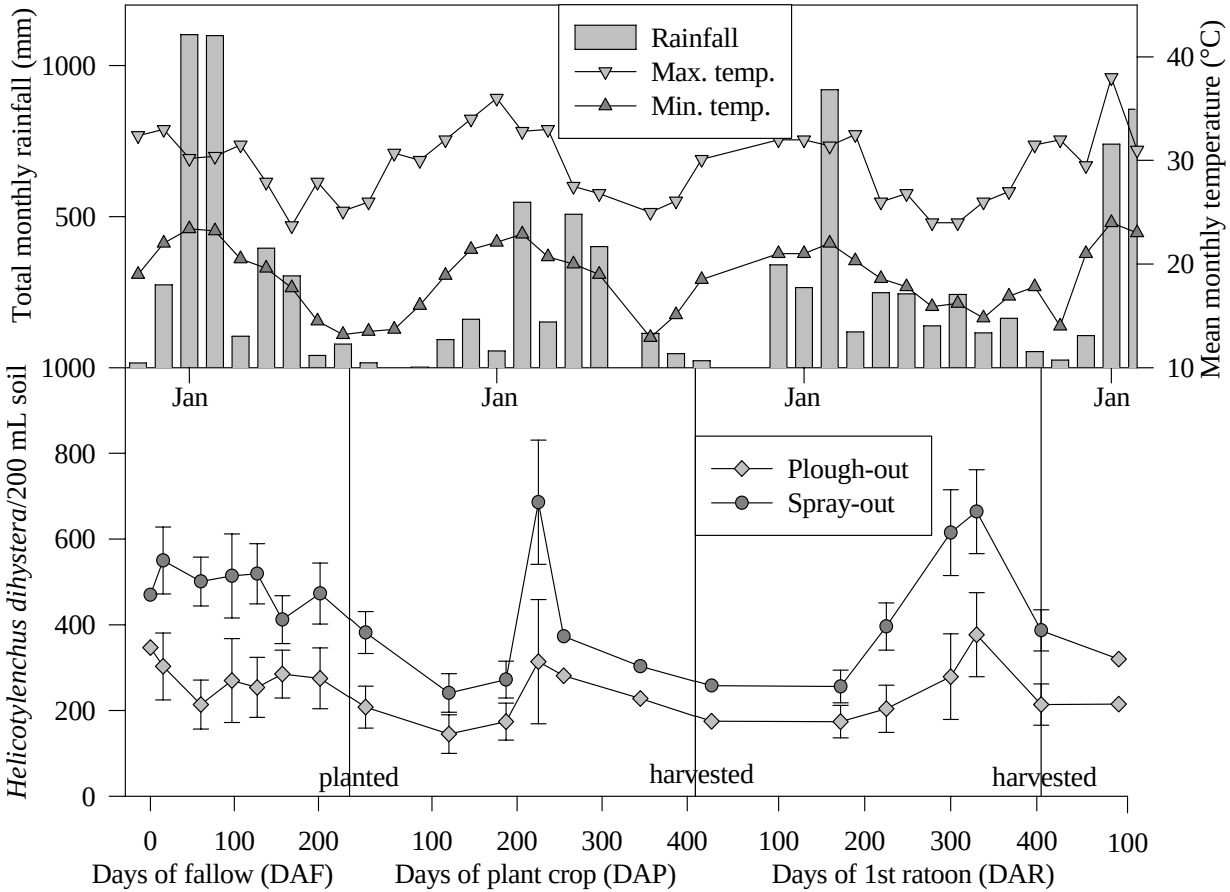


Figure 5.3.1.2a: *Helicotylenchus dihystera* on a sugarcane crop after a ploughed-out fallow and a herbicide fallow (bottom), and environmental conditions (top) at the site at Tully (LSD bars shown when P<0.05).

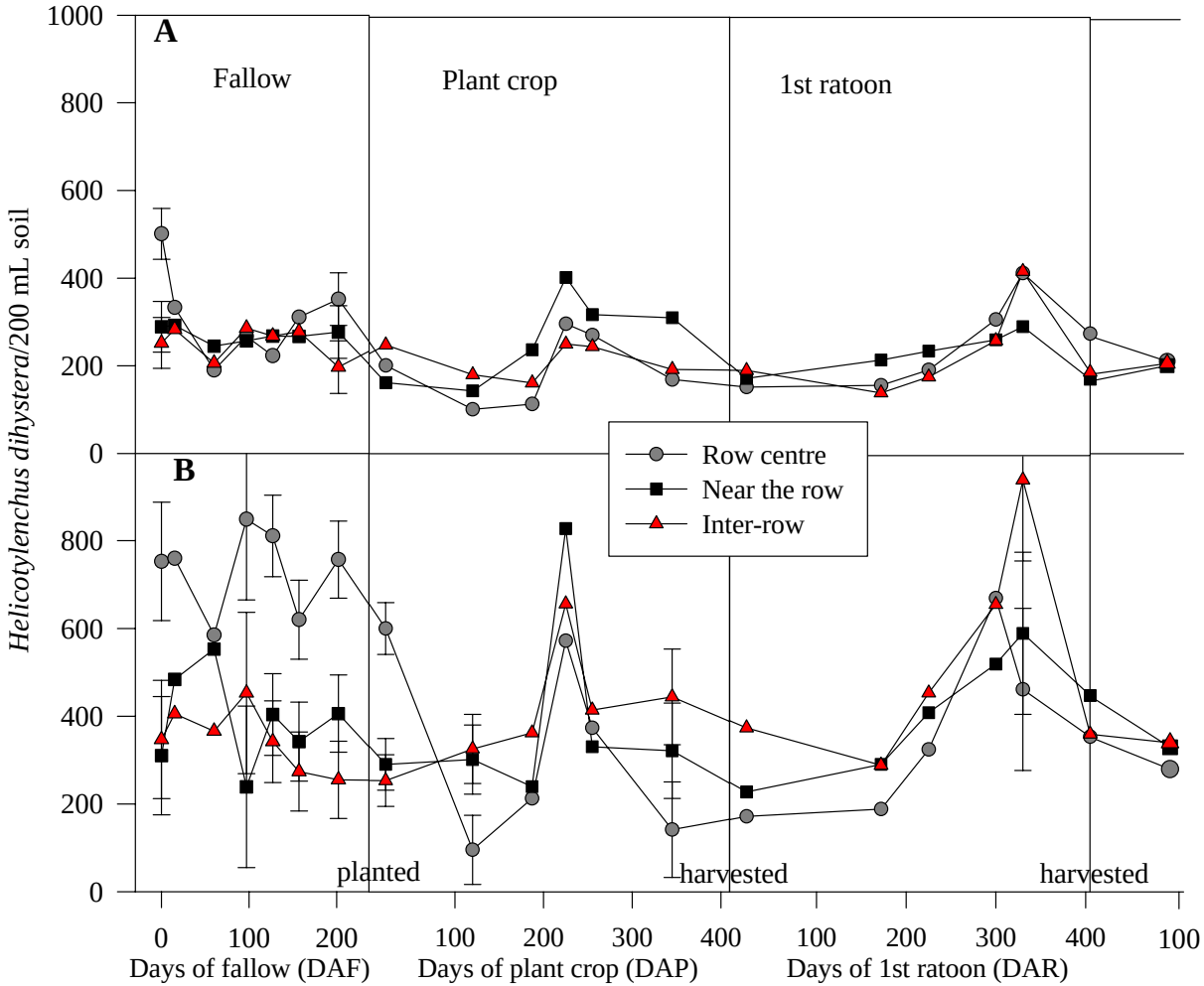
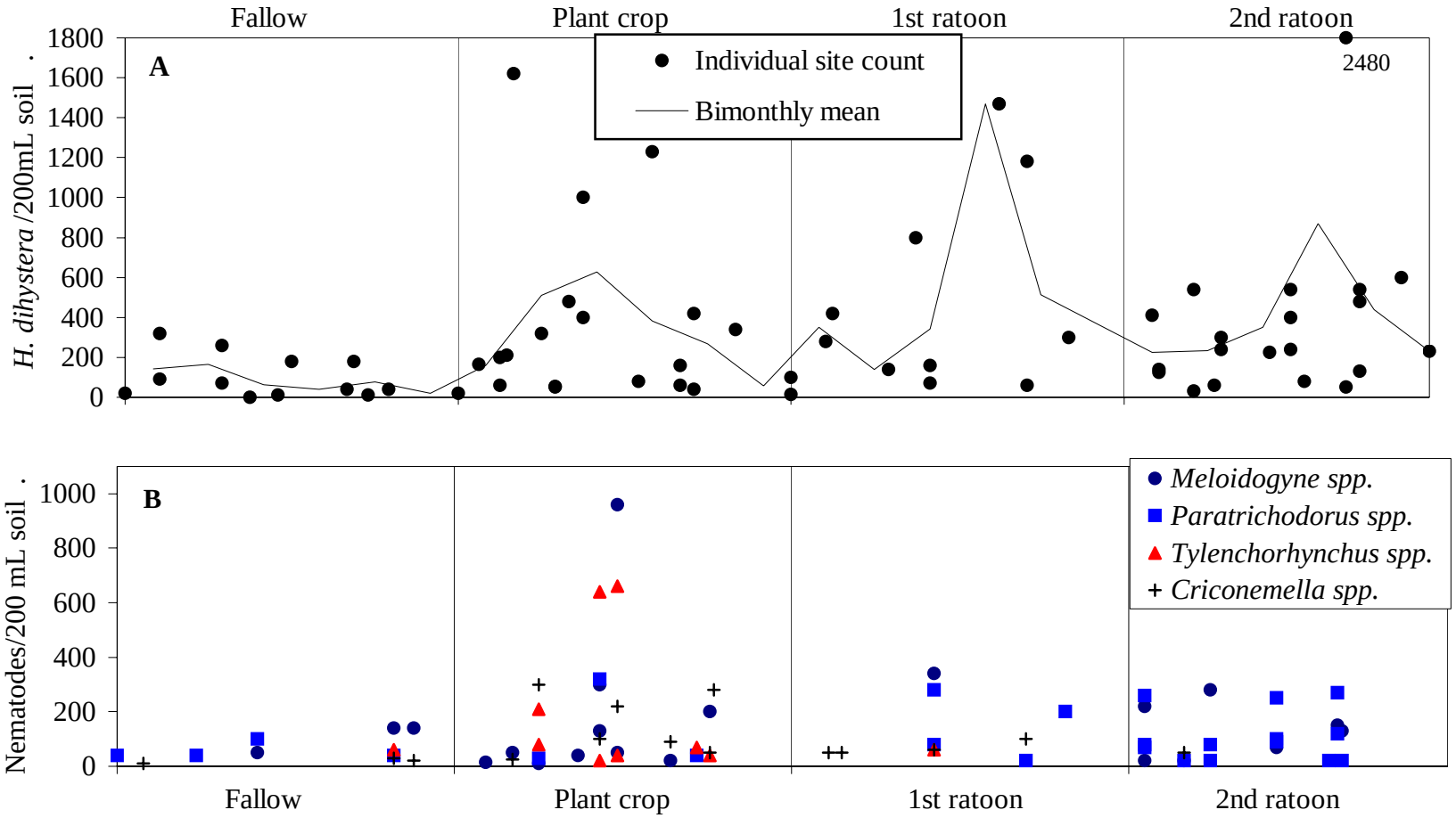


Figure 5.3.1.2b: *Helicotylenchus dihystera* in the row centre, near row and inter-row of a sugarcane crop after a ploughed-out fallow (A) and a herbicide fallow (B) (LSD bars shown when P<0.05).



Figures 5.3.2.2 and 5.3.2.3: Spiral nematode (*Helicotylenchus dihystera*) in the soil (A) and other nematodes in the soil (B) through progressive crop stages, in a selection of sugarcane fields in north Queensland.

5.3.2.2 *Helicotylenchus dihystera* at other sites

Populations tended to be lowest early and late in the growing season, with a peak mid-season. Densities of *H. dihystera* tended to be low at the time that the fallow was implemented (Figure 5.3.2.2).

5.3.2.3 Other nematodes at other sites

At a site where *Ageratum* spp. was the dominant weed in the fallow, populations of *Meloidogyne* spp. increased and then established at high densities on the following plant crop of sugarcane. Thus there were 2300 *Meloidogyne* spp./10 g of fresh root compared to only 50 *P. zeae*/10 g of fresh root. On a nearby replant crop, 1210 *P. zeae*/10 g of fresh root developed, and fewer *Meloidogyne* spp. were present. At other sites, the incidence of *Tylenchorhynchus* spp. and *Criconemella* spp. was higher in plant crops than ratoons, while the reverse occurred with *Paratrichodorus* spp. (Figure 5.3.2.3).

5.4 Discussion

A break in continuous monoculture, either by alternate cropping or fallowing, is the most common practice used in agricultural systems to manage nematodes (McSorley and Phillips 1993). Thus it was not surprising that at the Tully site the removal of host plants, either by tillage or herbicide, reduced populations of *P. zeae* by 80% by the end of the fallow. Densities of *P. zeae* were lowered by the tillage associated with plough-out of the old crop stool, and likely causes are mechanical disturbance and exposure of the soil to drying. However, in the long term (240 DAP), the herbicide fallow was equally effective in lowering densities of *P. zeae*. At the other sites monitored, densities of *P. zeae* were comparatively high at planting in replant (no fallow) situations, illustrating the benefit of a fallow. However, in far-north Queensland the fallows often become dominated with graminaceous weeds and remnant (volunteer) sugarcane from the previous crop (Garside *et al.* 1996), so fallowing is being under-utilised as a tool to manage *P. zeae* because a wide range of graminaceous plants are hosts (Luc *et al.* 1990 b). Better management to establish monocultures of legumes (eg. *Vigna unguiculata* cv. Meringa and *Glycine max* cv. Leichart) in the fallow have been recommended (Garside *et al.* 1996) because of

their poor host status (Sundararaj and Mehta 1993 b) and benefit to the soil from nitrogen and organic matter inputs. Stirling *et al.* (2001) reported fewer *P. zeae* following a legume monoculture than a fallow with a mixture of legume and pasture grass.

Weed fallows not only maintain populations of *P. zeae*, but also other nematode species. For example, populations of *Meloidogyne* spp. were high in a fallow dominated by *Ageratum* spp.. Continuous cropping of sugarcane (replant) has been reported as more profitable than fallow crop cycles in far-north Queensland (Muchow *et al.* 1998). Perhaps this situation would change if fallows were managed at a standard that reduced populations of soil pathogens and nematodes more thoroughly. The upsurge in herbicide fallowing is perhaps testament to its greater practicality in the north Queensland situation. Compared to cultivation, removing the crop without tillage renders the field less vulnerable to erosion and weed establishment and more accessible to management equipment in the monsoon season. While tillage significantly reduced levels of *H. dihystera* at the Tully site in the short term, excessive tillage is linked to reduced soil carbon and less stable soil aggregates in sugarcane soils (Blair 2000). By reducing tillage, conserved soil structure and organic matter may encourage a biological diversity that is antagonistic to pathogens, including nematodes, in the long term.

It is argued that tillage is an opportunity to reduce pathogen levels, by diluting heavily infested soil from the row with the relatively pathogen free soil of the inter-row. In particular, this practice is relevant to the root rotting fungus *Pachymetra chaunorhiza*, that targets newly emerging primary roots, and can develop 20 times higher spore loads in the row than the inter-row (Magarey 1999). However, at the Tully site, *P. zeae* was evenly distributed from row to inter-row under sugarcane, and tillage had no diluting benefit. Although *H. dihystera* is not a serious pest of sugarcane, these data found more nematodes surviving in the row centre of the untilled fallow. Thus, novel systems of minimum tillage (Bell *et al.* 2003) need to be monitored to detect unexpected changes in pathogen levels. At Tully, the section of field to be tilled had fewer *H. dihystera* than the sprayed-out section, which was evident prior to fallowing, and was very persistent throughout the sugarcane crop cycle. Possibly *H. dihystera* was sensitive to subtle differences in the soil

environment (eg. soil texture) between the two sections of field, and this favoured the persistence of more *H. dihystera* in the sprayed-out section.

In the wet tropics of north Queensland, the sugarcane crop is either planted or ratooned in the dry season, then grows through the wet season, to maturity. These two seasons have a major influence on the growth and activity of roots, which in turn appear to influence population densities of the plant-parasitic nematodes. In the first 120 DAP, nematode densities did not increase significantly in the soil and few *P. zeae* were found in roots. However, this is a period of early crop establishment when sett roots and then shoot roots are emerging, and are not extensive. In the dry period after planting, low rainfall (<100 mm/month at Tully) probably also inhibited root growth, thus giving nematodes a limited resource to multiply upon. The dry soil conditions would also have inhibited nematode movement, because nematodes can only migrate through the soil to infect new roots when a moisture film is present around soil particles (Jones 1978).

In West and South Africa, *P. zeae* multiplied to 750-3000 nematodes/g sett root by 90 DAP (Cadet and Spaull 1985), and infection levels in Australia are similar (Chapter 9). However, the temporary sett root system is not extensive and would contribute less than 150 *P. zeae*/200 mL of soil to the row area, if calculated from findings by Pankhurst *et al.* (2001). The agronomic practice of mounding up the row, from 30-100 DAP, probably also influenced the samples collected. In Queensland, sugarcane is planted in a row trench, and then filled with inter-row soil as the shoots establish, until a row mound is produced. This process buries older and more infected roots and rhizosphere soil below the sampling zone, further reducing the number of nematodes recovered. In this study, mostly new shoot roots would have been collected from 30-120 DAP. Because these roots are initiated after 30 DAP, and associated fine roots take further time to develop, nematodes were expected to take a few months to multiply, as observed in shoot roots in the African studies (Cadet and Spaull 1985). In the sprayed-out plots at Tully, *H. dihystera* declined from 750 to 100 nematodes/200 mL of soil between 30-120 DAP, perhaps due to being buried deeper in the profile under less infested soil, rather than perishing. The distribution of nematodes deeper in the soil profile requires further study.

An increase in *P. zeae* in the crop coincided with the onset of the monsoon season. This season is the main period of biomass accumulation in the crop (Muchow *et al.* 1994 a) and when the root system develops rapidly. From 90-150 DAP, five-fold increases in root weight have been recorded from sugarcane grown in the tropics (Roxas and Villano 1930), thereby providing an abundant resource for nematodes to multiply upon. Towards harvest, the nematode levels declined inside and around the root system as found elsewhere (Spaull and Cadet 1990), and coincides with the root system losing vigour and suberising, thereby becoming a less attractive food source. However, in the 1st ratoon at the Tully site, nematodes tended to persist through to harvest. Extensive winter rainfall in that season possibly kept stimulating the growth of new roots, thus maintaining a food source for nematodes. After harvest, old roots from the previous crop cease to grow (Glover 1968). Therefore, nematode levels remained low until new ratoon roots became extensive in the monsoon season that followed. Dry soil conditions probably also limited nematode multiplication over this period, as described previously for the plant crop.

Environmental temperatures probably had an indirect affect on nematode populations. Low temperatures around 300-400 DAP or DAR contributes to the crop ceasing vegetative growth in favour of sugar accumulation (maturation). Root growth and renewal also slows, eventually affecting populations of obligate root-parasites such as nematodes. During the plant and 1st ratoon crop at Tully, populations of *P. zeae* peaked in the winter months, probably because soil temperatures did not decline to a level that directly inhibited nematode activity. Thus the situation in the tropics is different to those observed in temperate climates (Brodie *et al.* 1993).

The crop cycle had fundamentally similar effects on the population dynamics of *P. zeae* and *H. dihystera*. Notable differences were perhaps reflective of different strategies of survival employed by the two species. *Pratylenchus zeae* declined rapidly when host plants were removed, suggesting that this nematode relies for survival on a high potential to reproduce. Thus, high populations quickly developed when roots became available. Because *P. zeae* parasitises fine roots (Chapter 8), which constitute about 88% of the total root length (Magarey and Grace 1998), this nematode always had an abundant source of nutrients. *Helicotylenchus dihystera* appeared less adept at multiplying on the roots when they became available. Brief

spikes in densities of *H. dihystera*, only at the apex of crop growth, suggested only a limited type and age of roots were exploited by this nematode. *Helicotylenchus dihystera* was more adept than *P. zeae* in surviving the fallow period.

Lower populations of *P. zeae* were found in 2nd ratoon crops, and fewer *P. zeae* on ratoons have also been detected in south Queensland (Chapter 10; Blair *et al.* 1999 a). This finding is attributed principally to a decline in the volume of fine roots as the crop cycle progresses (Glover 1970). The decline in older ratoons is associated with pests and diseases, soil compaction and harvester damage deteriorating the crop over the years. Alternatively, fewer *P. zeae* may be due to nematode predators, declining under fallow and planting operations, re-establishing in older ratoons and suppressing nematodes, as documented in other perennial crops (Stirling 1991). There have been no studies in Australia examining levels of natural suppression in sugarcane crops of different age.

Across the row to inter-row soil profile, significant differences between nematode levels were very transitory and trends in the plant crop rarely persisted in the ratoon. This uniformity in nematode distribution was reflective of the extensive shallow and fibrous root network established by sugarcane, especially during the monsoon season. Studies in South Africa have found about 64% of the root system exploits the top 20 cm of soil, and some single roots extend horizontally up to 1.8 m (Wolters 1929). Studies in Australia indicate Queensland cultivars have a similar habit (Reghenzani 1993). Because high densities of both *P. zeae* and *H. dihystera* were most regularly recovered from near the row (20-30 cm from the stool), this zone is recommended for consistent sampling. Samples taken early in the crop cycle (up to 150 DAP or DAR) and then mid-season (240-300 DAP or DAR) were required to capture the temporal extremes in nematode densities that are likely to be found on plant and ratoon crops.

CHAPTER 6

GLASSHOUSE EXPERIMENTS TO EVALUATE NON-VOLATILE NEMATICIDES AS A RESEARCH TOOL TO ASSESS NEMATODE DAMAGE TO SUGARCANE

6.1 Introduction

Sub-optimal yields associated with the poor health of roots are typical of replant problems in perennial crops as diverse as apples, pineapples and sugarcane (Croft *et al.* 1984; Brown *et al*. 1999; Stirling *et al.* 1999 c). Soil pathogens are strongly implicated because root health and vigour is restored when the soil is fumigated or pasteurised, and sugarcane soils are no exception (Croft *et al.* 1984). Heat and fumigation treatments affect a whole suite of microflora and soil fauna, from bacteria through to insects, and it is difficult to determine which organisms are involved in the response. Certain chemical biocides are useful at this level, because they control specific groups of soil pathogens, thereby revealing their impact on root growth. For example, dithio-carbamate fungicides (eg. mancozeb) improve root health in north Queensland sugarcane fields, implicating pathogenic fungi in yield decline (YD) in that region (Magarey *et al.* 1997 a).

Non-volatile nematicides such as aldicarb and fenamiphos are nerve-synapse inhibitors that suppress nematode activity, thereby providing a means to assess the impact of nematodes on the crop. In Australia, researchers have used nematicides experimentally on wheat for this purpose. However, results must be interpreted with caution, as nematicides have stimulated plant growth in some crops in the absence of nematodes (Barker and Powell 1988). Also, nematicides may move systemically within the plant, and important insect pests can also be controlled on the roots and above ground. Nonetheless, the specificity of non-volatile nematicides is unrivalled when compared to other chemicals or processes that are nematicidal.

To assess the importance of nematodes as a root pest of sugarcane in Queensland, nematicides are likely to be employed as a future research tool. Thus, there is a need to clarify their efficacy, and their effect on sugarcane growth. A series of pot experiments in the glasshouse were established to:

(a) test the efficacy of nematicides at different rates, and measure plant responses when nematodes were suppressed in YD soils,

(b) determine whether sugarcane was stimulated by nematicides in the absence of nematodes,

(c) assess whether sorghum and sugarcane were equivalent in their response to pasteurised and nematicide-treated soil. [Previous work has identified that YD biota may also inhibit sorghum growth in sugarcane soils (Garside *et al*. 1995)].

6.2 Materials and Methods

6.2.1 General methods

Mature stalks of sugarcane cultivar Q114 were selected from the field and single-bud nodes (setts) were cut from them. This cultivar was used because of its resistance to *Pachymetra chaunorhiza* Croft and Dick, an important pathogenic fungus of roots in the region. The setts were immersed in water at 50°C for 3 hours as a treatment against ratoon stunting disease (*Leifsonia xyli* subspecies *xyli* Davis) and chlorotic streak (unknown agent). Buds and sett roots were activated by planting the setts into steam-sterilised UC potting mix (Baker 1957), and at 2-3 leaf initiation, plants of even size were selected for the experiment. Excess UC mix was washed from the sett roots before replanting into the soils under test.

Soil was collected beside sugarcane stools, to a depth of 30 cm, in fields that had grown sugarcane for at least 15 years. The soil was sieved to remove roots, extraneous matter and large clods, and homogenised by being tumbled on a large plastic sheet. About 1.4 kg of soil (dry wt.) was placed in 1.5 L terracotta pots of 15 cm diameter with a single drainage hole. At planting and after 30 days, pots were fertilised by applying an aqueous solution of $NH_4H_2PO_4$ (0.1 g) and urea (0.15 g) to the soil surface. For the duration of the experiment, pots sat in a temperature-controlled bench at 26 $\pm$ 3°C. Pots were surface-watered daily to field capacity. This was the point where water was being lost from the bottom drainage hole. Treatments were imposed in randomised complete block designs.

At 20 days from planting, the leaves of plants were sprayed with an aqueous mist of pyrethrin based pesticide (0.25 g/L pyrethrin and 1.0 g/L piperonyl butoxide) to control foliar insect pests.

At harvest, soil was gently shaken and washed from the root system. The dry weight of roots and shoots, the length of the primary shoot to the top leaf collar, and number of shoots, were measured. One-way analysis of variance (ANOVA) was used to compare the size of plants in different soil treatments. To comply with assumptions of ANOVA, nematode counts were transformed to $(x + 0.5)^{1/3}$ for analysis. However, counts in Experiment 6.2.4 did not require a normalising transformation. Nematodes were extracted from 200 mL of soil using a Baermann tray for 48 hours (Whitehead and Hemming 1965). The extracted nematodes were concentrated by sieving through a 20 μm sieve, and counted. In this series of experiments, nematicides were incorporated throughout the soil prior to planting, by lightly mixing individual volumes (1.4 kg) of soil in a 10 L bucket.

6.2.2 Fenamiphos experiment

A sandy loam (Thorpe series, from Murtha 1986) of 15% clay content was collected from a sugarcane field in far-north Queensland and prepared as described above. The treatments imposed in replicates of four were:

(a) untreated soil,

(b) untreated soil + fenamiphos at concentrations of 5, 10, 20 and 40 mg/kg of soil,

(c) pasteurised soil (70° C for 90 minutes),

(d) pasteurised soil + fenamiphos at concentrations of 5, 10, 20 and 40 mg/kg of soil.

The experiment ran for 60 days.

6.2.3 Aldicarb experiment

The same sandy loam was used as above. However prior to use, the soil was stored for 30 days pending completion of other tasks. The soil was then prepared for the glasshouse as described above. The treatments imposed in replicates of four were:

(a) untreated soil,

(b) untreated soil + aldicarb at concentrations of 5, 10, 20 and 40 mg/kg of soil,

(c) pasteurised soil (70° C for 90 minutes),

(d) pasteurised soil + aldicarb at concentrations of 5, 10, 20 and 40 mg/kg of soil.

The experiment ran for 80 days.

6.2.4 Sorghum and sugarcane susceptibility to YD

A sandy clay loam (Marian series, from Holz and Shields 1985) of 20% clay content was collected from a central Queensland sugarcane field and prepared as above. The treatments imposed in replicates of five were:

(a) untreated soil,

(b) untreated soil + fenamiphos at 20 mg/kg of soil,

(c) pasteurised soil (70° C for 90 minutes).

The treatments were duplicated for the separate growth of sugarcane and sorghum (*Sorghum bicolour* × *sudanensis* cv. Jumbo). Sorghum seeds were germinated on moist cotton wool. Healthy plants of even size were then transplanted into the treatment pots. Sorghum was harvested after 70 days and sugarcane after 60 days.

6.3 Results

6.3.2 Fenamiphos experiment

At harvest, untreated soil contained *Pratylenchus zeae* Graham, *Paratrichodorus minor* Colbran and *Criconemella curvata* de Grisse and Loofe at 1093, 270 and 64 nematodes/200 mL of soil respectively. Fenamiphos at all rates (5-40 mg/kg soil) significantly reduced densities of *P. zeae* and *P. minor* ($P<0.05$, Figure 6.3.2.1). Densities of *C. curvata* were not significantly reduced, but few nematodes were extracted from untreated or treated pots. Fenamiphos increased root weight ($P<0.05$) between 66-115% at all rates, except at 40 mg/kg of soil. All rates of fenamiphos significantly increased shoot weight by 41-60%. Most rates significantly increased the length of the primary shoot by 11-18%, but only 20-40 mg/kg soil significantly increased the number of shoots (125% and 150%) at $P=0.05$ (Table 6.3.2.1).

In pasteurised soil, nematodes were not detected. Plants in pasteurised soil had a 98% larger root mass, a 92% larger shoot mass, a 20% longer primary shoot and 200% more shoots, than plants in untreated soil ($P<0.05$, Table 6.3.2.1). Adding fenamiphos to pasteurised soil generally had no effect on plant growth compared to pasteurisation alone ($P<0.05$). Significant effects were observed with 5 mg/kg soil, reducing the weight of roots by 29%, and 20 mg/kg soil, reducing the weight of shoots by 23% (Table 6.3.2.1).

Because different rates of fenamiphos had similar effects on nematode densities and plant yields, the rates were grouped and further analysed as a single treatment (Table 6.3.2.2). In the grouped treatments, the overall size of plants varied in the order, untreated < fenamiphos ≤ (pasteurised + fenamiphos) ≅ pasteurised.

When fenamiphos was added to untreated soil, shoot/root ratios were consistently lower regardless of the fenamiphos concentration, but this was not significant ($P>0.05$, Table 6.3.2.1). However, when the data was grouped, untreated soil had a greater shoot/root ratio than untreated soil + fenamiphos ($P<0.05$, Table 6.3.2.2).

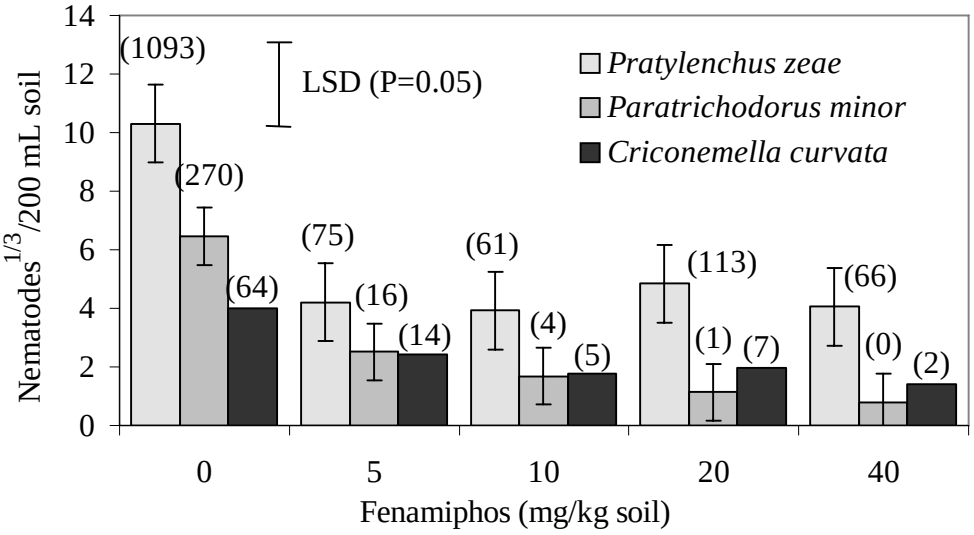


Figure 6.3.2.1: Nematodes in untreated and fenamiphos-treated soil in glasshouse pots after 60 days (at harvest). (Values in parentheses are back-transformed means. LSD compares treatment differences between the same nematode species).

Table 6.3.2.1: Sugarcane growth in pots in untreated and pasteurised sugarcane soil at different rates of fenamiphos.

Treatment	Plant yield				
	Root dry wt. (g)	Shoot dry wt. (g)	Length of primary shoot (cm)[A]	Number of shoots	Shoot/root ratio
Untreated soil (U)	1.63 a	5.47 a	21.75 a	1.00 a	3.81
U + fenamiphos –					
5 mg/kg dry soil	2.95 b	7.74 b	24.25 b	1.25 ab	2.74
10 "	2.82 b	8.14 b	24.37 b	1.75 abc	3.09
20 "	3.50 b	8.39 b	23.87 ab	2.25 bc	2.49
40 "	2.70 ab	8.73 b	25.62 b	2.50 c	3.16
LSD (P=0.05)	1.16	1.92	2.22	1.05	ns
Untreated soil (U)	1.63 a	5.47 a	21.75 a	1.00 a	3.81
Pasteurised soil (P)	3.22 c	10.51 c	26.12 bc	3.00 b	3.29
P + fenamiphos –					
5 mg/kg dry soil	2.28 ab	10.54 c	27.37 c	2.75 b	4.65
10 "	3.28 c	10.94 c	27.62 c	2.75 b	3.48
20 "	2.38 abc	8.10 b	27.12 c	1.75 ab	3.46
40 "	2.99 bc	9.34 bc	24.00 ab	3.00 b	3.19
LSD (P=0.05)	0.93	2.33	2.81	1.32	ns

[A] Length of the shoot from soil level to the top leaf collar

Values in the same column followed by the same letter are not significantly different at P=0.05

Table 6.3.2.2: Effect of fenamiphos (grouped rates) on sugarcane growth in pots in untreated and pasteurised sugarcane soil.

Treatment	Plant yield				
	Root dry wt. (g)	Shoot dry wt. (g)	Length of primary shoot (cm)[A]	Number of shoots	Shoot/root ratio
Untreated soil (U)	1.63 a	5.47 a	21.75 a	1.00 a	3.81 a
U + fenamiphos	2.99 b	8.25 b	24.53 b	1.94 ab	2.87 b
Pasteurised soil (P)	3.22 b	10.51 c	26.12 bc	3.00 c	3.29 ab
P + fenamiphos	2.73 b	9.73 c	26.53 c	2.56 bc	3.70 ab
Average LSD (P=0.05)[B]	0.79	1.70	2.07	0.98	0.92

[A] Length of the shoot from soil level to the top leaf collar

[B] Average LSD values are shown, but exact LSD values were used for pairwise testing

Values in the same column followed by the same letter are not significantly different at P=0.05

6.3.3 Aldicarb experiment

At harvest, untreated soil contained *P. zeae*, *P. minor* and *C. curvata* at 396, 94 and 7 nematodes/200 mL of soil respectively. Aldicarb at all rates (5-40 mg/kg soil) significantly reduced densities of *P. zeae* and *P. minor* ($P<0.05$, Figure 6.3.3.1). Densities of *C. curvata* were not significantly reduced, but few nematodes were extracted from untreated or treated pots. All rates of aldicarb significantly increased shoot weight by 18-39%, except at 20 mg/kg of soil, and the numbers of shoots were increased significantly by 100-140%, except at 5 mg/kg soil ($P<0.05$). Although all aldicarb rates increased root weight and length of the primary shoot, differences were not significant ($P>0.05$, Table 6.3.3.1).

In pasteurised soil, nematodes were undetectable. Pasteurising the soil increased plant growth, but only the 30% increase in shoot weight and 120% increase in number of shoots, were significantly greater than untreated plants ($P<0.05$, Table 6.3.3.1). Adding aldicarb to pasteurised soil did not further increase plant growth. An exception was the significantly greater weight of roots obtained with 5 mg/kg soil (Table 6.3.3.1).

Because different rates of aldicarb had similar effects on nematode densities and plant yields, the rates were grouped and further analysed as a single treatment (Table 6.3.3.2). In the grouped treatments, the overall size of plants varied in the order, untreated < aldicarb ≅ pasteurised ≅ (pasteurised + aldicarb).

In the aldicarb experiment, all treatments had similar shoot/root ratios (Tables 6.3.3.1 and 6.3.3.2).

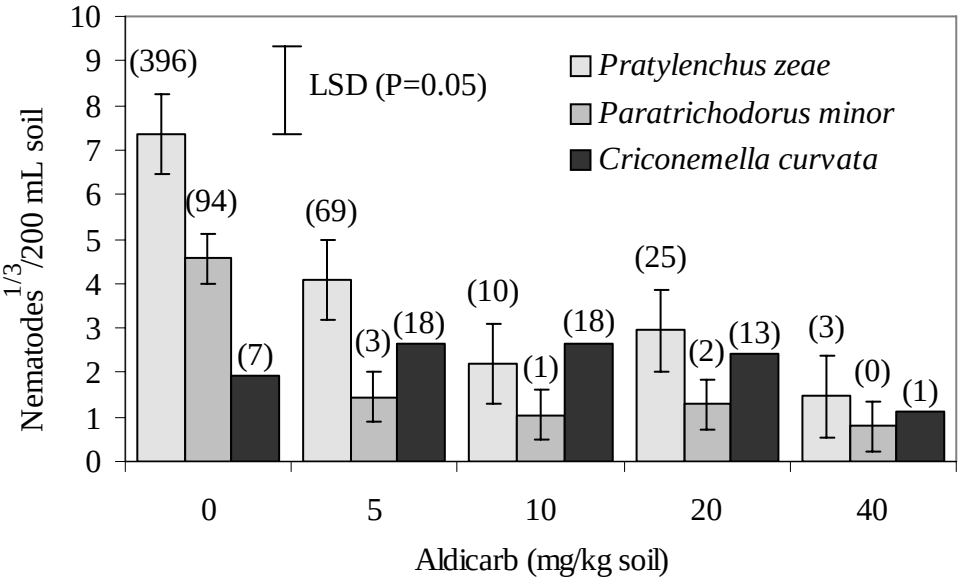


Figure 6.3.3.1: Nematodes in untreated and aldicarb-treated soil in glasshouse pots after 80 days (at harvest). (Values in parentheses are back-transformed means. LSD compares treatment differences between densities of the same nematode species).

Table 6.3.3.1: Sugarcane growth in pots in untreated and pasteurised sugarcane soil at different rates of aldicarb.

Treatment	Plant yield				
	Root dry wt. (g)	Shoot dry wt. (g)	Length of primary shoot (cm)[A]	Number of shoots	Shoot/root ratio
Untreated soil (U)	6.10	16.33 a	30.06	1.25 a	2.77
U + aldicarb –					
5 mg/kg dry soil	8.14	22.17 bc	34.87	1.50 a	2.75
10 "	8.28	20.46 bc	32.12	2.50 b	2.51
20 "	7.26	19.32 ab	31.00	2.50 b	2.69
40 "	8.88	22.64 c	33.00	3.00 b	2.62
LSD (P=0.05)	ns	3.04	ns	0.93	ns
Untreated soil (U)	6.10 a	16.33 a	30.06	1.25 a	2.77
Pasteurised soil (P)	7.24 ab	21.25 b	34.50	2.75 b	2.96
P + aldicarb –					
5 mg/kg dry soil	10.23 c	22.91 b	34.75	2.75 b	2.28
10 "	8.53 bc	22.46 b	35.50	3.00 b	2.65
20 "	8.28 b	19.93 b	32.81	3.00 b	2.42
40 "	8.17 b	21.41 b	33.31	3.25 b	2.66
LSD (P=0.05)	1.82	3.55	ns	1.26	ns

[A] Length of the shoot from soil level to the top leaf collar
Values in the same column followed by the same letter are not significantly different at P=0.05

Table 6.3.3.2: Effect of aldicarb (grouped rates) on sugarcane growth in pots in untreated and pasteurised sugarcane soil.

Treatment	Plant yield				
	Root dry wt. (g)	Shoot dry wt. (g)	Length of primary shoot (cm)[A]	Number of shoots	Shoot/root ratio
Untreated soil (U)	6.10 a	16.33 a	30.06	1.25 a	2.77
U + aldicarb	8.14 b	21.15 b	32.75	2.37 b	2.64
Pasteurised soil (P)	7.24 ab	21.25 b	34.50	2.75 bc	2.96
P + aldicarb	8.80 b	21.65 b	34.09	3.00 c	2.50
Average LSD (P=0.05) [B]	1.48	2.51	ns	0.88	ns

[A] Length of the shoot from soil level to the top leaf collar
[B] Average LSD values are shown, but exact LSD values were used for pairwise testing
Values in the same column followed by the same letter are not significantly different at P=0.05

6.3.4 Sugarcane and sorghum susceptibility to YD

At harvest, the untreated soil growing sugarcane contained *P. zeae*, *P. minor* and *Tylenchorhynchus annulatus* Cassidy at 400, 480 and 330 nematodes/200 mL of soil respectively. Fenamiphos at 20 mg/kg soil significantly reduced *P. zeae* and *P. minor*, but not *T. annulatus* (Figure 6.3.4.1). Fenamiphos significantly increased the weight of shoots by 29% and the number of shoots by 180% ($P<0.05$, Table 6.3.4.1). The mean weight of fenamiphos-treated roots was 35% more than untreated roots, but this difference was not significant. In pasteurised soil, nematodes were undetectable. This treatment significantly increased root weight by 135%, shoot weight by 74% and the number of shoots by 200% ($P<0.05$, Table 6.3.4.1).

At harvest, the untreated soil growing sorghum contained *P. zeae*, *P. minor* and *T. annulatus* at 1000, 3250 and 990 nematodes/200 mL of soil respectively. Fenamiphos at 20 mg/kg soil significantly reduced densities of *P. zeae* and *P. minor*, but *T. annulatus* became more numerous compared with levels in untreated soil (Figure 6.3.4.2). Fenamiphos increased sorghum growth, but only a 36% increase in the number of shoots was significant ($P<0.05$, Table 6.3.4.1). In pasteurised soil, nematodes were undetectable. This treatment significantly increased root weight by 133%, shoot weight by 56% and the number of shoots by 55% ($P<0.05$, Table 6.3.4.1).

Shoot/root ratios did not differ significantly between soil treatments or plant species (data not shown).

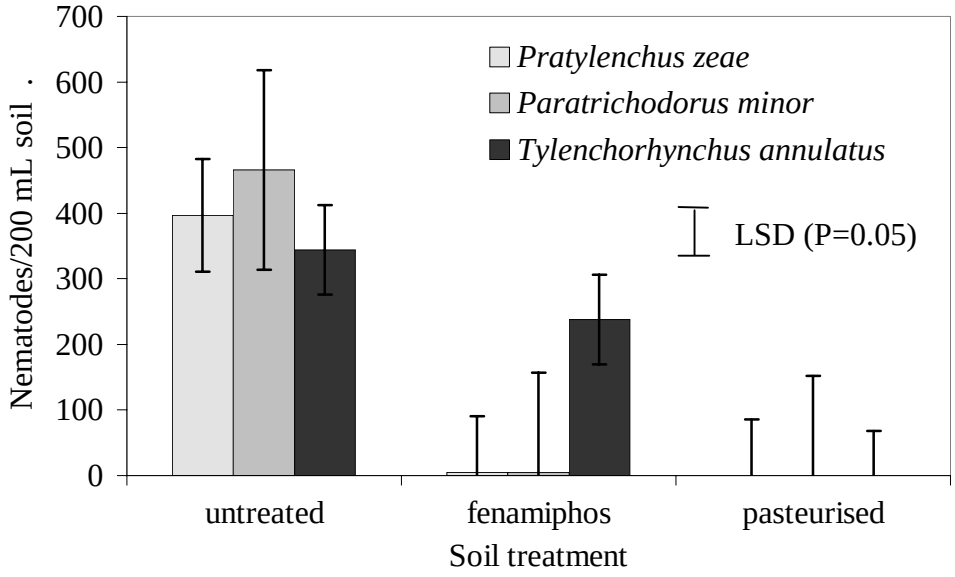


Figure 6.3.4.1: Nematodes present at harvest (60 days) around sugarcane roots following different soil treatments (LSD compares treatment differences between densities of the same nematode species).

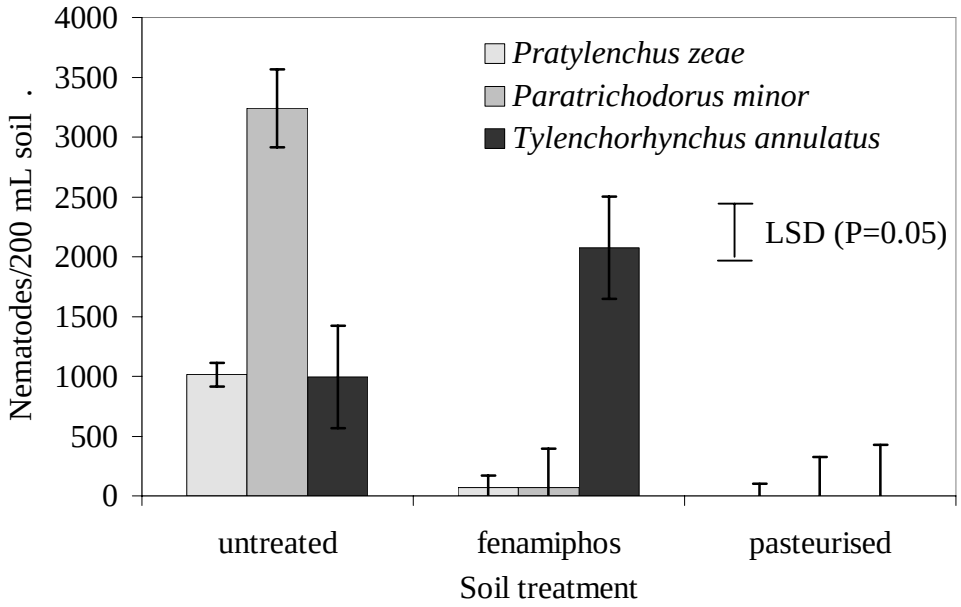


Figure 6.3.4.2: Nematodes present at harvest (70 days) around sorghum roots following different soil treatments (LSD compares treatment differences between densities of the same nematode species).

Table 6.3.4.1: Sugarcane and sorghum growth in pots following soil treatment with biocides.

Soil treatment	Sugarcane (dry weights)			Sorghum (dry weights)		
	Roots (g)	Shoots (g)	Number of shoots	Roots (g)	Shoots (g)	Number of shoots
Untreated	3.38 a	6.83 a	1.0 a	3.96 a	10.09 a	2.2 a
Fenamiphos (20 mg/kg soil)	4.56 a	8.83 b	2.8 b	4.64 a	11.14 a	3.0 b
Pasteurised soil	7.93 b	11.87 c	3.0 b	9.21 b	15.70 b	3.4 b
LSD (P=0.05)	1.55	1.43	0.87	0.81	2.35	0.79

Values in the same column followed by the same letter are not significantly different at P=0.05

6.4 Discussion

Where the use of nematicides is economic in high value vegetable crops such as tomato, ginger and cucurbits, fenamiphos is recommended at about 10 mg/kg of soil. In these glasshouse experiments, the efficacy of nematicides was optimised by soil mixing and controlled irrigation, so in the two sugarcane soils used, *P. zeae* and *P. minor* were controlled at all rates of fenamiphos, including 5 mg/kg of soil. *Pratylenchus zeae* and *P. minor* are both demonstrated pathogens of sugarcane (Apt and Koike 1962 b; Sundararaj and Mehta 1994) and in particular *P. zeae* is an important pest worldwide (Spaull and Cadet 1990). In Experiment 6.2.4, *T. annulatus* was not controlled on sugarcane using fenamiphos, but the population density of 250 nematodes/200 mL of soil, was probably too low to be of importance. *Tylenchorhynchus annulatus* is considered a mild pathogen of sugarcane. For example, 21000 nematodes/plant were recovered from pot experiments in Louisiana, without significantly reducing the size of roots or shoots (Birchfield and Martin 1956).

Coinciding with the control of *P. zeae* and *P. minor*, root volume, shoot size and shoot numbers were increased. The improved growth of sugarcane can probably be attributed solely to the suppression of nematodes. In saying this, it is noted that fenamiphos failed to reduce total bacteria, fungi or actinomycetes on sugarcane grown in glasshouse pots (Magarey and Bull 1996), nor the general microbial population on wheat grown in the field (Thompson *et al*. 1980). In these experiments (Chapter 6), root-browsing insects were not observed and foliar sucking insects were controlled on all plants. Fenamiphos did not stimulate sugarcane growth in the

absence of nematodes in pasteurised soil. In fact, when data was grouped (Table 6.3.2.2), adding fenamiphos to nematode-free soil appeared to inhibit plant growth. However, within treatments, variable plant growth mitigated against finding a significant trend. In Experiment 6.2.2, shoot/root ratios were typically the lowest in untreated soil where fenamiphos was added. This finding alludes to shoot growth not fully responding to increased root weight when nematodes were controlled. Perhaps there is some phytotoxic effect of fenamiphos on shoot growth. However, this phenomenon was not observed when fenamiphos was used in Experiment 6.2.4.

The composition and density of soil biota varies with location, season, crop age and cropping practice in YD soils, so that crop responses to soil fumigants or heat treatments are variable from site to site (Magarey and Croft 1995). However, by comparing plant growth between treatments, about 50% of the pasteurisation responses were attributed to the control of nematodes in these experiments (6.2.2 and 6.2.4), whereas sugarcane has been found less responsive to fenamiphos previously (Magarey *et al*. 1995; Magarey and Bull 1996). In the past, soils from the Tully River delta have frequently been used in YD studies in the glasshouse. These soils typically have low densities of nematodes (Chapter 3), which may partly account for the lack of fenamiphos responses in past experiments. However, the soil in Experiment 6.3.2 was more responsive to fenamiphos than soil taken from the same site previously (R Magarey pers com.). In experiments by Magarey, the watering system (Chapter 7), and rate of fenamiphos used (40 mg/L soil) may have inhibited both the multiplication of nematodes and plant growth respectively, contributing to lower nematicide responses.

Experiment 6.2.3 ran for longer than Experiment 6.2.2, but fewer nematodes were recovered at harvest. Thus, storing the soil for 30 days, prior to use in Experiment 6.2.3, appears to have lowered the densities of nematodes in the soil. So even though the nematicide (aldicarb) suppressed nematodes, sugarcane growth did not increase to the extent seen in the previous experiment (6.2.2) where the same soil was used, but not stored. In Experiment 6.2.3, the response to pasteurisation was also greatly reduced, which suggests that storage of the soil also reduced other biota involved in YD. Also, running the experiment for longer (80 days) than usual, may have inhibited the pasteurisation response, due to pot size limiting the growth of larger

plants. In pots of 2.4 L capacity, sugarcane growth has been significantly restricted after 135 days (Smith *et al.* 1999).

Sorghum was a good host of *P. zeae*, *P. minor* and *T. annulatus*, which are among the most common nematodes on sugarcane in Queensland (Blair *et al*. 1999 a, b). Thus, this crop could be useful in multiplying sugarcane nematodes for research purposes. Sorghum also showed promise as a short bioassay for YD, being as responsive as sugarcane to soil pasteurisation. Fenamiphos failed to control *T. annulatus* on sorghum, and at the density found (2000 nematodes/200 mL of soil) the nematode was probably damaging (Swarup and Sosa-Moss 1990). Thus, it is inconclusive as to whether sugarcane and sorghum share a similar response to nematicides when nematodes are suppressed in YD soils.

Although there is no evidence that aldicarb reduces the total number of bacteria and fungi around the roots of wheat crops (Thompson *et al*. 1980), aldicarb has increased growth parameters in some other crops in the absence of nematodes (Barker and Powell 1988; Barker *et al*. 1988). When sugarcane was grown in nematode-free soil in my experiments, aldicarb increased root weight and number of tillers, but these responses were too minor to be significant at the level of replication used. Similar experiments using sugarcane have also alluded to some growth promotion with aldicarb in the absence of nematodes. However, despite using more replications, responses were not statistically significant (Spaull 1995; Stirling *et al*. 1999 b). In contrast, when root-parasitic nematodes were present and then suppressed by fenamiphos or aldicarb in the two YD soils used in my experiments, sugarcane growth was often significantly improved. This indicates that nematodes are components of YD in these soils.

It is concluded from these experiments that non-volatile nematicides are suitable as a research tool to quantify nematode damage to sugarcane crops from the yield responses observed. At concentrations up to 40 mg/kg or soil, growth promotion by aldicarb and fenamiphos that is independent of nematodes, is likely to be very minor.

CHAPTER 7

PATHOGENICITY OF LESION NEMATODE (*PRATYLENCHUS ZEAE*) TO SUGARCANE IN SHORT-TERM POT EXPERIMENTS

7.1 Introduction

Lesion nematode (*Pratylenchus zeae* Graham) is a common plant-parasitic nematode on sugarcane worldwide (Spaull and Cadet 1990). Pathogenicity has been demonstrated by inoculation on different sugarcane cultivars in different countries (Harris 1974; Valle-Lamboy and Ayala 1980; Sundararaj and Mehta 1994). *Pratylenchus zeae* is ubiquitous to sugarcane fields in Queensland (Chapter 3; Blair *et al.* 1999 a, b), and occurs at densities considered damaging on other crops such as rice and sorghum (Chevres-Roman *et al*. 1971; Plowright *et al.* 1990). Nonetheless, the symptoms of root damage by *P. zeae*, and effect on the plant, have not been examined on any cultivars of sugarcane grown commercially in Queensland, despite the fact that the nematode can be cultured readily (Moody *et al*. 1973). For a short-term pot bioassay to be developed, such as to screen for cultivar resistance, the relationship between *P. zeae* inoculum, abiotic factors and sugarcane growth, needs to be explored.

A series of experiments were conducted with an isolate of *P. zeae* to test pathogenicity to plant sugarcane during early growth (up to 70 days) in glasshouse pots (1.5 L). Influences on pathology under test were, (a) mode of inoculum, (b) density of inoculum and (c) effect of different watering regimes.

7.2 Materials and Methods

7.2.1 General methods

Mature stalks of sugarcane cultivar Q114 were selected from the field and single-bud nodes (setts) were cut from them. This cultivar was used because of its resistance to *Pachymetra chaunorhiza* Croft and Dick, an important pathogenic fungus of roots, in the region. The setts were immersed in water at 50°C for 3 hours as a treatment against ratoon stunting disease (*Leifsonia xyli* subspecies *xyli* Davis) and chlorotic streak (unknown agent). Growth of the bud and sett roots was stimulated by planting

the setts in steam-sterilised UC potting mix (Baker 1957), and at 2-3 leaf initiation, plants of even size were selected for the experiment. Excess UC mix was washed from the sett roots before replanting into experimental pots.

Soil was collected beside sugarcane stools, to a depth of 30 cm, in fields that had grown sugarcane for at least 15 years. The soil was sieved to remove roots, extraneous matter and large clods, and homogenised by being tumbled on a large plastic sheet. About 1.4 kg of soil (dry wt.) was placed in 1.5 L terracotta pots of 15 cm diameter with a single drainage hole. Pots and soil were autoclaved in batches of six where this treatment was required.

At planting and after 30 days, pots were fertilised by applying an aqueous solution of $NH_4H_2PO_4$ (0.1 g) and urea (0.15 g) to the soil surface. For the duration of the experiment, pots sat in a temperature-controlled bench at 26 $\pm$ 3°C. Treatments were imposed in randomised complete block designs.

Pratylenchus zeae was grown and maintained aseptically on carrot discs (Moody *et al*. 1973). The parent population was isolated from sugarcane roots at El Arish in far-north Queensland. Adults, juveniles and eggs were harvested by thinly slicing and gently macerating the carrot tissue and separating nematodes using a Baermann tray (Whitehead and Hemming 1965). The nematodes were then resuspended in distilled water as an inoculant.

At harvest, soil was gently shaken and washed from the root system. The dry weight of roots and shoots, the length of the primary shoot to the top leaf collar and number of shoots were measured. To comply with assumptions of ANOVA, nematode counts were transformed to $(x + 0.5)^{1/3}$ where this analysis was used. Nematodes were extracted from 200 mL of soil and 10 g of roots (fresh wt.) using a Baermann tray for 48 hours (Whitehead and Hemming 1965). The extracted nematodes were concentrated by sieving through a 20 μm sieve, and counted. Where the multiplication rates of *P. zeae* are reported, they were calculated as; the number of nematodes recovered from soil and roots at harvest (P_f) divided by the number of nematodes inoculated at planting (P_i).

7.2.2 Mode of inoculation

A clay loam (Tully series, from Murtha 1986) of 35% clay content was prepared as described above. The treatments imposed in replicates of five were:

(a) autoclaved soil (121°C for 20 minutes),

(b) autoclaved soil + 16000 nematodes/pot. (Nematodes were stirred through the soil with a large fork prior to planting the sett),

(c) autoclaved soil + 16000 nematodes/pot. (Nematodes were introduced around the roots via a straw after the sett was planted),

(d) untreated soil.

The experiment ran for 70 days, and average minimum and maximum glasshouse temperatures were 18 and 29°C, respectively. Pots were surface-watered daily to field capacity. This was the point where water was being lost from the bottom drainage hole.

7.2.3 Inoculum density

A sandy loam (Thorpe series, from Murtha 1986) of 15% clay content was prepared as described above. The treatments imposed in replicates of six were:

(a) autoclaved soil (121°C for 20 minutes),

(b) autoclaved soil + nematodes stirred through the soil at densities equivalent to 200, 500, 1000, 2000 and 3500 nematodes/200 mL of soil,

(c) untreated soil.

The experiment ran for 70 days, and average minimum and maximum glasshouse temperatures were 16 and 25°C, respectively. Pots were surface watered daily to field capacity.

7.2.4 Influence of watering regime

A sandy loam (Thorpe series, from Murtha 1986) of 15% clay content was prepared as described above and the pots and soil were autoclaved at 121°C for 20 minutes. Where required, *P. zeae* was introduced around the sett roots via a straw. Assuming the nematodes dispersed evenly in the pot, the initial nematode density was 1000 nematodes/200 mL of soil.

The sub-irrigated treatment was imposed by standing pots in terracotta saucers that were continuously filled with water. Soil in the pots graded from saturated in the bottom of the pot, to field capacity mid-pot, and below field capacity at the top of the pot. This is the standard method used to irrigate plants in pots in YD studies at the BSES Tully (Magarey *et al*. 1995). Surface watered pots were watered daily to maintain field capacity. This was the point where water was being lost from the bottom drainage hole. The experiment ran for 60 days, and average minimum and maximum glasshouse temperatures were 21 and 29°C, respectively. The treatments generated were, $\pm$ *P. zeae*, by two watering regimes, by five replicates. The relationship between nematodes and watering regime on plant growth was evaluated with two-way ANOVA.

7.3 Results

7.3.2 Mode of inoculation

Nematode levels were measured in the soil and roots only at harvest (Figure 7.3.2.1). From the autoclaved treatment, no nematodes were detected in the soil and very few *P.zeae* were detected in the roots. Because autoclaving was expected to totally remove nematodes, minor contamination is the likely explanation, and probably occurred when nematodes were being recovered from the samples at harvest. This contamination was too low to be a confounding issue.

Although two very different methods of were used to inoculate *P. zeae*, populations at harvest were similar, namely about 800 *P. zeae*/200 mL of soil and 550-750 *P. zeae*/g of root. These densities were higher than in untreated field soil ($P<0.05$). Inoculated plants also had more *P. zeae* in their roots than plants from untreated field

soil, but differences were significant (P<0.05) only with direct inoculation (Method B). Untreated soil contained *P. zeae*, *Criconemella curvata* de Grisse and Loofe and *Helicotylenchus dihystera* Cobb.

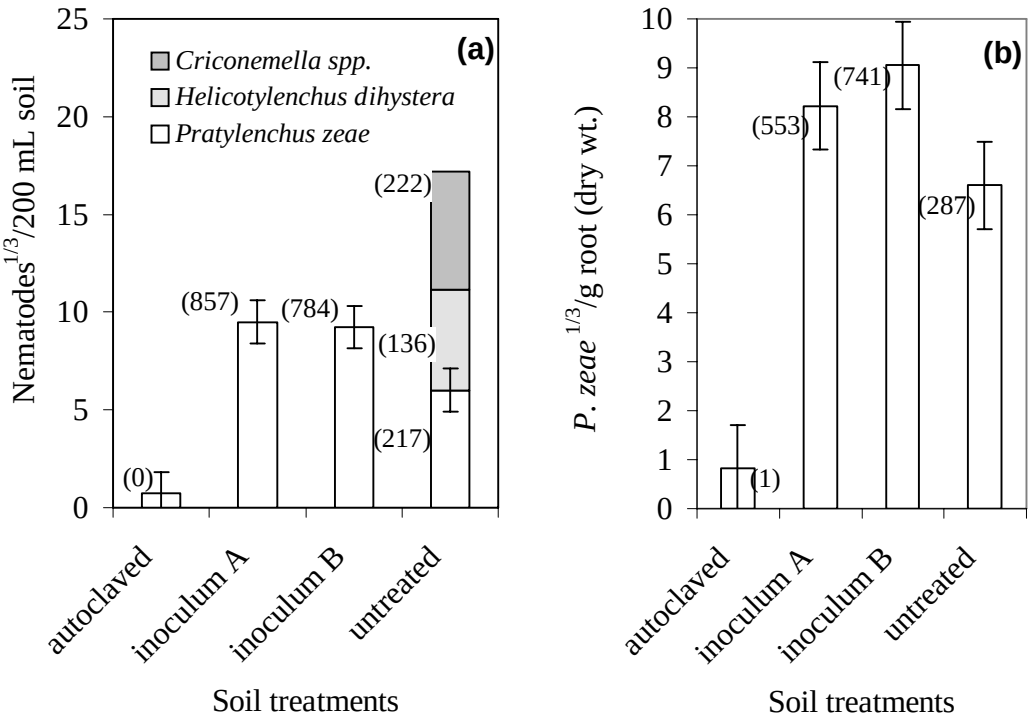


Figure 7.3.2.1: The effect of soil treatments and mode of inoculation on (a) nematode density in the soil and (b) *Pratylenchus zeae* density in the roots (Values in parentheses are back-transformed means. LSD bars represent P=0.05).

Inoculating *P. zeae* into autoclaved soil by mixing (Method A) significantly reduced the weight of roots by 36%. Adding *P. zeae* around the sett in autoclaved soil (Method B) reduced root weight (55%) more severely than mixing nematodes in the soil (Method A, P<0.05). All measurements of shoot biomass from inoculated plants were lower on average than plants from autoclaved soil. However, the only significant effect was shorter shoots (16%) using Method B to add *P. zeae* to the soil (P<0.05, Table 7.3.2.1).

From untreated field soil, root weight, shoot weight and shoot length were significantly less by 62%, 51% and 20% respectively, compared to plants from autoclaved soil (P<0.05). There were also 45% fewer shoots on average, but this was not significant (P>0.05, Table 7.3.2.1).

The shoot/root ratios of plants inoculated with *P. zeae* were significantly higher than plants from autoclaved or untreated field soil. The shoot/root ratios of plants inoculated by Method B were significantly higher than those inoculated by Method A (P<0.05, Table 7.3.2.1).

Table 7.3.2.1: Sugarcane growth in a clay loam soil, autoclaved and inoculated with *Pratylenchus zeae*.

Soil treatment	Dry root wt.– g	(%[C])	Dry shoot wt.– g	(%)	Length of primary shoot– cm	(%)	Number of shoots	(%)	Shoot/root ratio
Autoclaved (A)	7.98 a	(0)	11.55 a	(0)	21.80 a	(0)	1.80	(0)	1.45 a
A + *P. zeae* mixed[A]	5.11 b	(36)	10.35 a	(10)	20.85 ab	(4)	1.60	(11)	2.11 b
A + *P.zeae* added[B]	3.61 c	(55)	9.64 a	(17)	18.31 bc	(16)	1.25	(31)	2.73 c
Untreated	3.07 c	(62)	5.66 b	(51)	17.40 c	(20)	1.00	(45)	1.85 ab
LSD (P=0.05)	1.24		1.94		3.11		ns		0.59

[A]2000 *Pratylenchus zeae*/200 mL of soil, mixed through the soil prior to planting
[B]equivalent of 2000 *Pratylenchus zeae*/200 mL of soil (16,000/pot) inoculated around the roots after planting
[C] % reduction compared to sugarcane growth in autoclaved soil
Values in the same column followed by the same letter are not significantly different at P=0.05

7.3.3 Inoculum density

Nematode levels were measured in the soil and roots only at harvest (Figure 7.3.3.1). From the autoclaved treatment, no nematodes were detected in the soil and very few *P.zeae* were detected in the roots. This contamination was too low to be a confounding issue. When *P. zeae* was established at a wide range of densities in autoclaved soil prior to planting, soil and root populations were similar by harvest. An exception was that fewer nematodes were detected at the lowest inoculation density (P<0.05). At harvest, inoculated plants generally had more *P. zeae* in and around their roots than plants from untreated soil (P<0.05). In untreated soil the nematodes present, in order of abundance, were *Paratrichodorus minor* Colbran, *H. dihystera*, *Tylenchorhynchus annulatus* Cassidy and *P. zeae*.

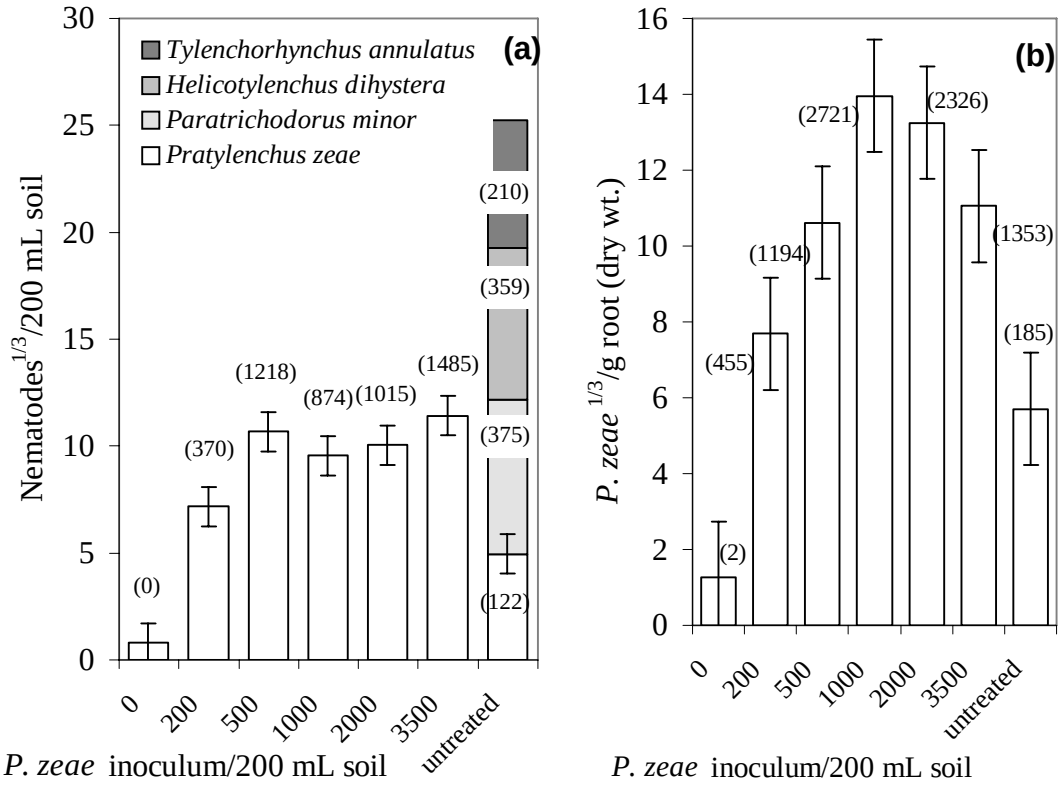


Figure 7.3.3.1: The effect of soil treatments and inoculum density on (a) nematode density in the soil and (b) *Pratylenchus zeae* density in the roots (Values in parentheses are back-transformed means. LSD bars represent P=0.05).

In autoclaved soil alone, root weight, shoot weight and shoot length was significantly better (96%, 68% and 25% respectively) than plants in untreated field soil ($P<0.05$). There were also more shoots on average (114%), but this was not significant at the level of replication used (Table 7.3.3.1).

The densities of *P. zeae* inoculated at planting (P_i) were negatively correlated with root weight, shoot weight and length of the primary shoot, but the correlation slopes were not pronounced (Figure 7.3.3.2). There was no correlation between nematode densities and the number of shoots produced (data not shown). However, the multiplication rate of *P. zeae* declined sharply as the inoculum density increased.

The root and shoot weights of plants from untreated field soil were significantly lower than plants from autoclaved soil inoculated with *P. zeae* (Table 7.3.3.1).

Table 7.3.3.1: Sugarcane growth in a sandy loam soil, autoclaved and inoculated with varying densities of *Pratylenchus zeae*.

Soil treatment	Dry root wt.–		Dry shoot wt.–		Length of primary shoot–		No. of shoots	
	g	(%[B])	g	(%)	cm	(%)		(%)
Autoclaved (A)	3.83 a	(0)	13.24 a	(0)	28.18 a	(0)	2.14	(0)
A + 200 nematodes/200 mL soil[A]	3.48 a	(9)	13.41 a	(+1)	27.67 a	(2)	1.83	(15)
A + 500 nematodes	3.16 a	(18)	12.55 a	(5)	27.04 ab	(4)	1.83	(15)
A + 1,000 nematodes	3.46 a	(10)	12.90 a	(3)	26.96 abc	(5)	2.67	(+25)
A + 2,000 nematodes	3.18 a	(17)	11.82 a	(11)	25.50 bcd	(10)	1.83	(15)
A + 3,500 nematodes	3.15 a	(18)	11.70 a	(12)	25.10 cd	(11)	1.80	(16)
Untreated	1.95 b	(49)	7.90 b	(40)	23.75 d	(16)	1.00	(53)
LSD (P=0.05)	0.98		1.79		1.83		ns	

[A]nematodes were mixed through the soil prior to planting

[B] % reduction compared to sugarcane growth in autoclaved soil

Values in the same column followed by the same letter are not significantly different at P=0.05

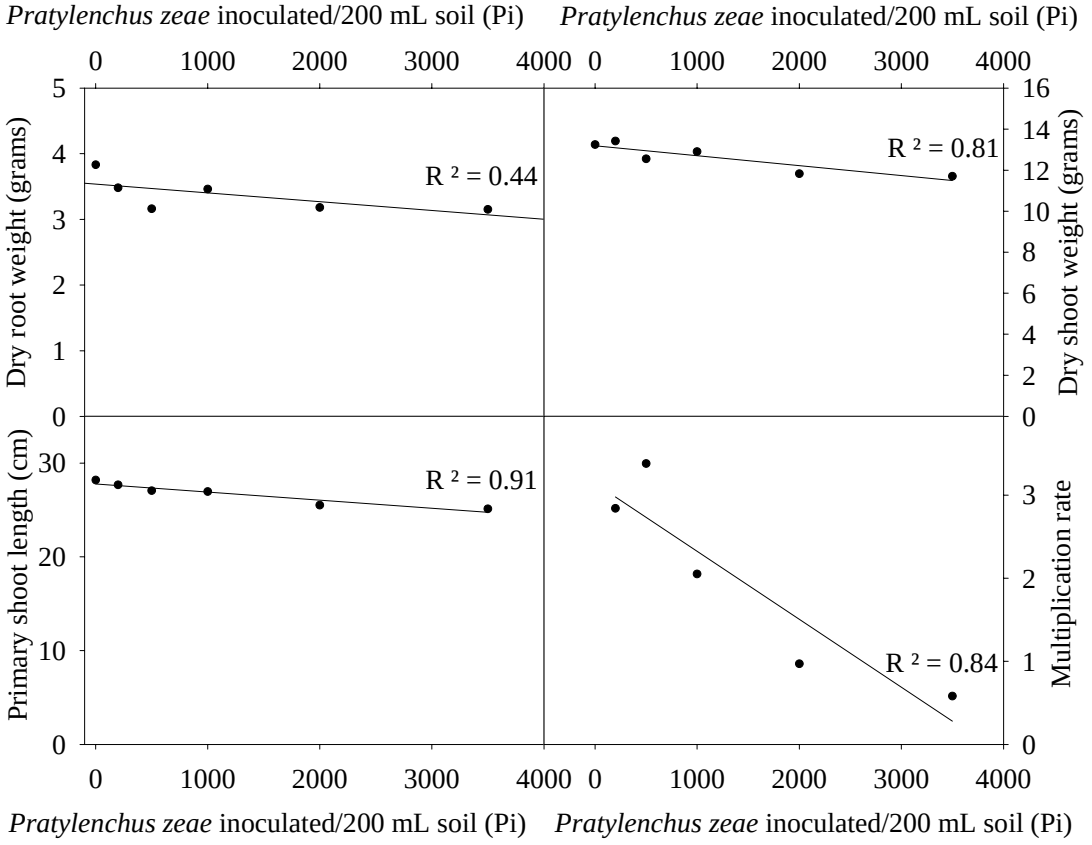


Figure 7.3.3.2: Relationship between the mean inoculum density (Pi) of *Pratylenchus zeae*, mean root and shoot growth, and nematode multiplication.

7.3.4 Influence of watering regime

After 60 days, sub-irrigated plants were bigger than plants that were surface watered (Table 7.3.4.1). However, at the level of replication used, only a 20% difference in shoot length was significant ($P<0.05$). In pots inoculated with *P. zeae*, the weight of roots was 33% less with 24% fewer shoots ($P<0.05$). Shoot weight and length was also less on average, but not significantly ($P>0.05$). Thus plants inoculated with *P. zeae* had a significantly higher shoot/root ratio than uninoculated plants (Table 7.3.4.1).

No interaction was found between 'watering regime' and the effect of *P. zeae* on growth. Nematodes multiplied nearly six-fold in surface watered pots compared to a two-fold multiplication in sub-irrigated pots (Figure 7.3.4.1).

Table 7.3.4.1: Effect of *Pratylenchus zeae* on sugarcane growth in glasshouse pots at two watering regimes.

Treatment	Dry root wt– g (%[A])	Shoot dry wt.– g (%)	Length of primary shoot– cm (%)	No. of shoots (%)	Shoot/root ratio (%)
Water regime A	7.30 (0)	8.80 (0)	28.19 (0)	3.00 (0)	1.29 (0)
Water regime B	6.21 (15)	7.79 (11)	22.56 (20)	2.50 (17)	1.30 (0)
LSD (P=0.05)	ns	ns	3.32	ns	ns
Autoclaved (A)	8.09 (0)	8.85 (0)	26.62 (0)	3.12 (0)	1.11 (0)
A + *P. zeae*	5.42 (33)	7.74 (13)	24.12 (9)	2.37 (24)	1.48 (+33)
LSD (P=0.05)	1.92	ns	ns	0.74	0.26

[A] % reduction in sugarcane biomass

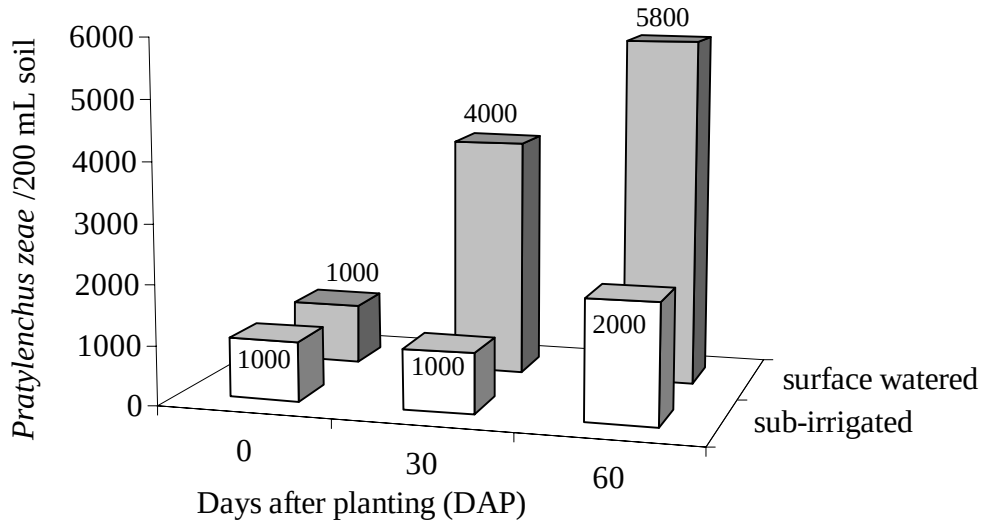


Figure 7.3.4.1: Multiplication of *Pratylenchus zeae* on sugarcane in glasshouse pots at two watering regimes

7.4 Discussion

The pathogenicity of *P. zeae* to a Queensland cultivar (Q114) of sugarcane was demonstrated in this series of pot experiments. Inoculated plants had either significantly lower root weights (Experiments 7.2.2 and 7.2.4) or there was some decline in root weight as inoculum density increased (Experiment 7.2.3). Compared to the healthy white/light brown roots in autoclaved soil, inoculated roots were darker in colour and lesions were visible on new shoot roots. Fine roots appeared to be shorter, but no measurements were taken to confirm this. In South Africa, *P. zeae* reduced root weight by 28% and fewer feeder roots and root hairs had developed in 12 L pots after 60 days (Harris 1974).

In untreated field soil the poor growth of roots induced similarly poor shoot growth, so plants from untreated and autoclaved soil had equivalent shoot/root ratios. In contrast, when *P. zeae* was the sole pathogen present on inoculated plants, shoot/root ratios were generally higher because root damage did not induce similarly lower shoot growth. Thus, the multi-pathogen complex in yield decline (YD) soil impacted more severely on shoot biomass, perhaps by hindering root function, than when the nematode was acting alone.

Despite pre-shooting buds on the stem nodes and selecting plants of even size, sugarcane grew variably in these experiments and so differences in sugarcane growth of less than 20% between treatments was found not to be significant ($P<0.05$). With this type of experimental system, more than six replicates are required to demonstrate the more subtle effects of single pathogens.

The inoculation method markedly influenced damage caused by *P. zeae*, probably because different nematode densities (P_i) were initially established at planting. Many nematodes either perished or were distributed away from newly developing roots by the physical process of mixing. Nematode counts were not done at planting to confirm whether these differences were established. However, in a subsequent experiment (Chapter 8) where *P. zeae* was established by mixing, after seven days only 10-20% of the inoculated nematodes were recoverable using a process that relies upon nematode movement (Whitehead tray). Thus, in the experiments where mixing was used, while 200-3500 *P. zeae*/200 mL of soil were added at planting, a much lower population would have been viable to infect roots. In contrast, adding *P. zeae* directly to the roots probably established a high local population of viable nematodes around emerging shoot roots. Hence, delivering *P. zeae* directly to the roots was more damaging to the plant, significantly reducing both the root system and growth above ground. In subsequent experiments, nematodes should be allowed to naturally disperse through the soil rather than through physical mixing. After dispersal, counts are required to find the density of nematodes that are actually viable at planting.

Given that densities of *P. zeae* at planting (P_i) have been inadequately reported in pathogenicity tests in other countries, and pot sizes and duration of experiments differ, comparisons are difficult to make. In South Africa and Louisiana, low populations of *P. zeae* were inoculated at planting and recovered at harvest (5-15 nematodes/g of root), but yield in large pots was still significantly reduced (Khan 1963; Harris 1974). In Puerto Rico, similar densities of *P. zeae* were inoculated and recovered as in the experiments reported here, but plant weights were reduced by greater margins in Puerto Rico (Valle-Lamboy and Ayala 1980). Because experiments ran for more then 60 days, potentially the time for a pathogenic impact to develop, was greater. Alternatively, the variable tolerance of different cultivars to

P. zeae may account for lower damage thresholds overseas. Cultivar selection in Queensland is based on field performance and it is possible that this process has selected for some tolerance to a nematode that is ubiquitous.

When inoculated directly onto the roots, final densities of *P. zeae* were high, except where severe damage limited the amount of roots available for subsequent nematode generations. This phenomenon is often observed in experiments with nematodes, as nematode densities can increase to a point where damage to the host leads to a loss in its capacity to sustain the population (McSorley and Phillips 1993). Thus, the highest direct inoculum did cause the most damage to roots, but relatively low densities of *P. zeae* were recovered at harvest (Experiment 7.2.2). Densities of *P. zeae* in untreated field soil were also relatively low at harvest, and coincided with poor root growth and an association of other plant-parasitic nematodes (*C. curvata*, *H. dihystera*, *T. annulatus* and *P. minor*) and unknown pathogens. A limited root resource and/or antagonism between pathogens was the probable cause of relatively low *P. zeae* densities in YD soil in these experiments. Competition and antagonism between nematode species and with plant-pathogenic fungi, has also been demonstrated on sugarcane in pots (Valle-Lamboy and Ayala 1980).

Although 200-3500 *P. zeae*/200 mL of soil were inoculated in Experiment 7.2.3, densities in the soil were similar at harvest. The rate of nematode multiplication significantly declined as inoculum densities increased, but root weights were similar and probably not a limiting resource. The high mortality of introduced nematodes, particularly at high inoculum densities, perhaps reduced differences in the infective population of *P. zeae*. Ten-fold, rather than two-fold increments in the population, established at planting, would have been more useful in correlating nematode density with reduced plant size.

In sub-irrigated pots, roots grew well in the saturated soil, so it appears that anaerobic conditions were not induced in Experiment 7.2.4. Fewer nematodes developed on sub-irrigated plants, probably because the movement of nematodes was inhibited in saturated soil. In saturated soil, nematodes move forward inefficiently due to a lack of purchase on soil particles from tension forces (Jones 1978). The two watering regimes did not stimulate major differences in plant size. Thus, the surface watered plants were not stressed enough to induce a greater impact of the nematode

on plant biomass as demonstrated on other crops (McSorley and Phillips 1993) and despite greater nematode multiplication on surface watered plants.

To conclude, *P. zeae* damaged the roots of a Queensland cultivar (Q114) of sugarcane. However, a unit decrease in root weight generally did not confer a corresponding unit decrease in shoot weight, contrasting with plants in YD soil with a combination of root pathogens. Improved shoot and root growth (about 100%) from autoclaving the soil, was largely reversed when a high nematode density (16000 *P. zeae*/plant) was established around the root zone at planting. Because YD develops early in the growth of planted crops (Garside *et al*. 1999), the results of these short-term experiments suggests that *P. zeae* may contribute to the syndrome. However, field crops of sugarcane are grown for >360 days in a range of environments, and in any year about 3/4 of the crops are ratoon. Thus, further work is required to assess the impact of lesion nematode in these situations.

CHAPTER 8

PATHOGENICITY OF LESION NEMATODE (*PRATYLENCHUS ZEAE*) TO SUGARCANE IN FIELD MICROPLOTS

8.1 Introduction

Lesion nematode (*Pratylenchus zeae* Graham) is probably the most important plant-parasitic nematode on sugarcane worldwide (Spaull and Cadet 1990). The pathogenicity of *P. zeae* to sugarcane has been demonstrated in pots in a number of countries (Khan 1963; Harris 1974; Valle-Lamboy and Ayala 1980; Sundararaj and Mehta 1994), and an isolate of *P. zeae* inhibited the growth of sugarcane cultivar Q114 in Australia (Chapter 7). The relationship between nematodes and sugarcane is certain to be more complex in the field than is found in pots, because the crop is a relatively large perennial that develops an extensive root system. When sugarcane is planted, shoot roots take about 100 days (DAP) to become extensive, and a stable population of shoots is established at about 140 DAP (Garside *et al.* 2000; Pankhurst *et al.* 2001). However, pot experiments typically run for only 60 days.

Compared to pots, microplots more closely simulate field conditions, allow roots to grow unconfined for longer periods, and increase the exposure time to the pathogen being studied. Microplots also allow growth conditions, plant measurements, nematode levels and other pathogens and pests to be regulated or controlled in a relatively large system. This experiment examined the effect of *P. zeae* on sugarcane grown for 140 days in field microplots of 50 L capacity.

8.2 Materials and Methods

The experiment was established at an outdoor site at the QDPI Bundaberg Research Station in Spring 1998. Holes of 0.5 m diameter, 0.75 m apart and 1 m deep were excavated across a 12 × 12 m clay platform. Circular PVC microplots, 0.4 m in diameter and 0.5 m deep, with a steel mesh bottom, were seated in the holes on 0.5 m of gravel screenings to aid drainage. The upper 5 cm of pipe protruded above the surrounding clay platform. The site was covered with black plastic sheeting and fumigated with an equivalent of 1200 kg/ha methyl bromide to kill potential plant

pathogens. Approximately 4 m^3 of sandy loam soil with 60% coarse sand, 21% fine sand, 8% silt and 11% clay, was spread over an area of 10 × 10 m to a depth of 4 cm and sealed inside black plastic sheeting. The soil was exposed to an equivalent of 1200 kg/ha methyl bromide for 72 hours, aired for a further seven days, and poured into the microplots.

Pratylenchus zeae was isolated from a sugarcane field in south Queensland (Bundaberg) and multiplied aseptically on carrot discs (Moody *et al.* 1973). Adults, juveniles and eggs were harvested by thinly slicing and gently macerating the carrot tissue and separating nematodes using the Whitehead tray technique (Whitehead and Hemming 1965). The nematodes were suspended in water and added to the microplots through a straw inserted about 30 cm deep, to create five treatment densities. After the nematodes had been allowed seven days to disperse through the soil, five sub-samples per microplot were removed down to 30 cm and combined. The nematode density in the composite sample of soil was estimated by Whitehead tray extraction (Whitehead and Hemming 1965). The mean densities of *P. zeae* at planting (P_i) in the five treatments were estimated to be 0, 9, 37, 250 and 350 nematodes/200 mL of soil. These populations were established in five replicate microplots, set out in a randomised block design.

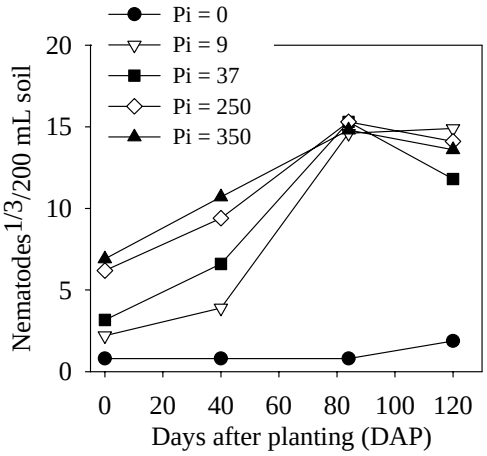
Short nodal pieces of sugarcane cultivar Q124 with a single bud were planted in a seedling mix of sand, peat and vermiculite. At 2-3 leaf initiation, plants of even size were selected and planted into the microplots in mid-spring. The fertilizer program was based on soil nutrient analysis and commercial recommendations. At planting, nitrogen and phosphorus were added at 22.5 and 25 kg/ha respectively, in the form of di-ammonium phosphate (125 kg/ha). At 30 DAP, nitrogen, phosphorus and potassium were added at 136, 12 and 103 kg/ha respectively as GrowForce 506® (600 kg/ha). The crop was also supplemented with sodium molybdate (applied foliar at 0.24 g/plant), magnesium sulphate (300 kg/ha) and potassium sulphate (100 kg/ha). At 80 DAP, nitrogen was added at 92 kg/ha, as urea. A total of 146, 100, 140 and 158 mm of water (equivalent to a total of 5.44 ML/ha) was applied at 30, 60, 90 and 120 DAP, respectively.

During the experiment and at harvest, the number of shoots, length of the primary shoot and number of leaves per microplot, were measured. The nematode density in the soil was estimated regularly during the experiment. At harvest (140 DAP), shoot weight, root weight and nematodes in the roots were measured. The length of roots and their surface area in longitudinal cross-section (area/g of root sample) was estimated, using a desktop scanner and Sci-scan root imaging computer program. Root diameters were grouped as <0.7 mm, 0.7-1.0 mm and >1.0 mm, to represent ratios of tertiary, secondary and primary roots respectively. To comply with assumptions of analysis of variance (ANOVA), root lengths, root surface areas and nematode densities were transformed to $(x + 0.5)^{1/3}$ prior to analysis.

8.3 Results

Differences in densities of *P. zeae*, established at planting, were maintained to 40 DAP. However, by 90-120 DAP, densities had peaked at about 2700 nematodes/200 mL of soil and were not significantly different, except in uninoculated (control) microplots (Figure 8.3.1). At harvest, about 2500 nematodes infested each g of root in inoculated microplots. In some uninoculated microplots, the soil and roots were contaminated with nematodes, but the populations were very low.

By 20 DAP, the density of nematodes at planting (P_i) was negatively correlated with the number of shoots initiated (Figure 8.3.2a) and with the number of leaves initiated (Figure 8.3.2c). These trends were still present at 35 DAP and 60 DAP, but shoot numbers became equivalent between treatments thereafter. Leaf numbers also became equivalent between treatments after 90 DAP. A negative correlation between P_i and primary shoot length had developed by 20 DAP, and was maintained for the entirety of the experiment (Figure 8.3.2b). Shoot biomass was the highest in uninoculated and lightly inoculated plants (9 *P. zeae*/200 mL of soil), but only the latter was significantly heavier than plants inoculated with 37-350 *P. zeae*/200 mL of soil ($P<0.05$, Table 8.3.1).



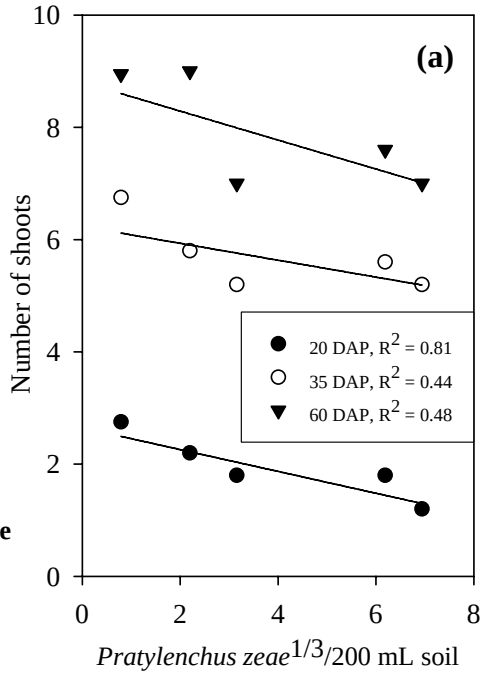
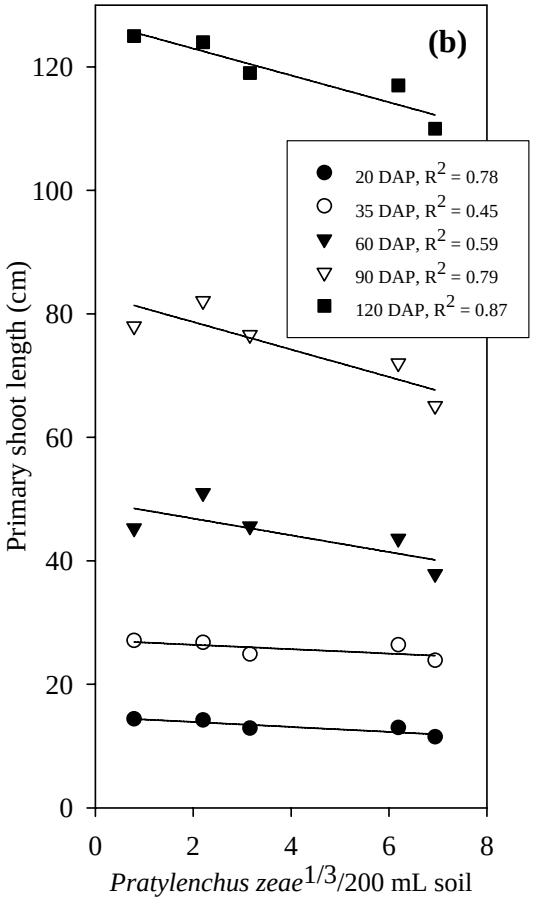
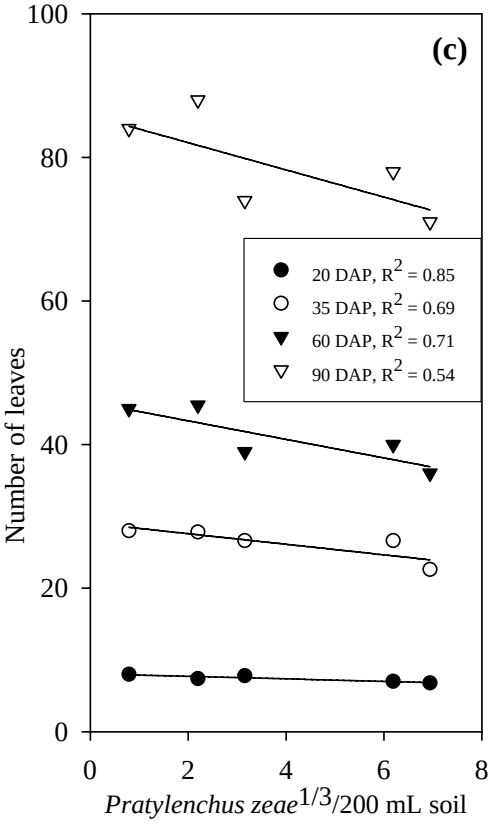


Figure 8.3.1: Multiplication of *Pratylenchus zeae* on sugarcane in microplots after inoculation at five different population densities.





Figures 8.3.2a-8.3.2c: Effect of the mean inoculum density (Pi) of *Pratylenchus zeae* on mean (a) number of shoots, (b) length of the primary shoot and (c) number of leaves.

At harvest, root weights were not affected by treatment (Table 8.3.1). However, from fumigated soil the root system was a healthy light brown colour with a dense mat of fine roots, whereas inoculated root systems were dark brown to black with fewer fine roots (Plate 8.3.2). Newly formed primary roots had discrete dark red lesions. On all inoculated plants, the length and surface area of tertiary roots were reduced about 58% and 47% respectively. Treatment 4 had more primary roots than most other treatments (Table 8.3.2).

Table 8.3.1: Effect of *Pratylenchus zeae* on root weight and shoot growth of sugarcane at harvest.

Treatment	Nematodes/200 mL soil (P_i)	Root weight (g)	Shoot weight (g)
1	0	89	865 ab
2	9	104	999 a
3	37	105	777 b
4	250	95	781 b
5	350	92	688 b
	LSD (P=0.05)	ns	200

Values in the same column followed by the same letter are not significantly different at P=0.05

Table 8.3.2: Effect of *Pratylenchus zeae* on root length and surface area of sugarcane at harvest.

Pratylenchus zeae/200 mL of soil (P_i)	Root length[A] (mm/g of dry root)		
	Primary	Secondary	Tertiary
0	8.1b (533)	(518)	20.1a (8168)
9	8.3b (563)	(434)	14.9b (3287)
37	9.1ab (760)	(483)	15.7b (3844)
250	10.2a (1056)	(515)	14.7b (3196)
350	8.7b (668)	(466)	14.9b (3281)
LSD (P=0.05)	1.14	ns	2.55
	Surface area[A] (mm^2/g of dry root)		
	Primary	Secondary	Tertiary
0	(3444)	(1355)	15.8a (3931)
9	(3756)	(1131)	12.6b (1998)
37	(5139)	(1278)	13.1b (2247)
250	(5730)	(1353)	13.0b (2208)
350	(4079)	(1237)	12.4b (1925)
LSD (P=0.05)	ns	ns	2.01

[A]Transformed to $(x + 0.5)^{1/3}$

Values in parentheses are back-transformed means

Values in the same column followed by the same letter are not significantly different at P=0.05

Plate 8.3.2: Roots of sugarcane (cultivar Q124) from fumigated soil (A) without nematodes and (B) inoculated with 350 *Pratylenchus zeae*/200 mL of soil.

8.4 Discussion

Although every effort was made to prevent contamination of uninoculated microplots, eventually a few *P. zeae* appeared. The variable ontogeny of plants also meant that there was considerable variability within treatments despite selecting plants of even size prior to transfer into the test plots. Nevertheless, the pathogenicity of *P. zeae* was demonstrated in a situation where sugarcane was grown for an extended time, and root growth was initially not limited by available soil volume.

The pathology exhibited by *P. zeae* was typical of that seen previously in glasshouse experiments (Harris 1974). These symptoms were also similar to those that develop on field roots in yield decline (YD) soils (Lawrence 1984). However the length and surface area of tertiary (fine feeder) roots were reduced significantly, which is an important added observation. Fewer fine roots have also been reported in YD soils (Croft *et al*. 1984) and when poor root syndrome has been transmitted in the glasshouse (Magarey *et al*. 1995). While later studies report that different components of the root system are reduced equally in YD soils (Magarey and Grace 1997, 1998), different pathogens could be affecting each component. In the microplots, *P. zeae* damaged roots at all inoculum densities because it was able to multiply quickly, even from densities as low as 9 nematodes/200 mL of soil. The densities used in this experiment are typical of pre-plant fallows throughout the industry and nematodes multiplied at rates typical of the field situation. Hence it is concluded that the root damage observed in this experiment, would occur in the sugarcane fields of Queensland.

There were significant relationships between *P.zeae* density at planting and lower shoot biomass (shoot numbers, shoot length and leaf numbers) as early as 20 DAP, supporting findings that *P. zeae* affects early establishment of sugarcane (Chapter 9). Poor early growth is recognised as a primary cause of poor sugarcane yields associated with YD (Garside *et al.* 1999). The plants had already grown sett roots prior to planting into the test soils (to ensure even plant size), so *P. zeae* must have damaged developing shoot roots mostly, and affected initiation of secondary shoots. Had ungerminated setts been used, perhaps *P. zeae* would have restricted early growth more severely. The effect of *P. zeae* on sett roots and subsequent shoot growth requires further investigation. However, varied bud shooting and bud

dormancy introduces great variability when single nodes are planted without prior screening for even plant size.

Apart from consistent shorter primary shoots due to *P. zeae*, all treatments had similar shoot and leaf numbers by harvest. Thus final yield was not markedly affected, perhaps because sugarcane has a large root biomass relative to shoot biomass from 0-100 DAP (Smith *et al.* 1999) and plants were grown with an excess of water and fertilisers. If the environment had been less conducive to growth (eg. with less available water or added pressure from other pathogens), *P. zeae* may have impacted more severely on yield.

CHAPTER 9

THE ROLE OF SETT ROOTS AND SHOOT ROOTS IN THE ESTABLISHMENT OF SUGARCANE PLANTED INTO YIELD DECLINE SOILS

9.1 Introduction

The sugarcane crop is propagated by planting stem cuttings (setts or billets) end on end in rows, 1.4-1.8 m apart. The stem cuttings have 2-3 nodes, each with an associated bud and ring of sett root primordia. Under favourable conditions, sett roots grow from the primordia, and the associated buds begin growth to become a stand of regularly spaced primary shoots. From the primary shoot bases, shoot roots and secondary shoots are then initiated (tillering), and appear at around 30 days after planting (DAP). This combination of primary and secondary shoots then competes for light and space in the row to become the final stand of mature stalks.

When few buds shoot at planting, the resulting gap between primary shoots may be too large to be filled by secondary shoots, resulting in a row gap. Row gaps are responsible for a significant loss in productivity, especially as they are inherited by subsequent ratoon crops. The growing bud can source water and nutrients from the associated sett roots, or from the parent stem cutting. The primary shoot also relies on the sett roots in the period before functional shoot roots develop. For example, when sett roots are physically removed, or damaged with an inoculum of *Pythium* spp., the developing primary shoot is inhibited (Ryker and Edgerton 1931). Improved shoot numbers following treatments that reduce soil pathogens (eg. long fallow, crop rotation or soil fumigation), is also associated with improved sett root volume (Garside *et al*. 2002 a; Pankhurst *et al.* 2002). On the other hand, growers have also reported established primary shoots without sett roots.

The dependence of buds and shoots on sett roots may also vary according to the planting material used. Stem cuttings and buds from the bottom of the sugarcane stalk may be up to 250 days older than those newly formed at the stalk apex, and also have differing sugar content. Bud viability varies with position on the stalk (King 1965), and with cultivar.

The two experiments reported in this chapter assess the role of sett roots on the development of buds and shoots following the planting of sugarcane in the field. By physically damaging sett root primordia, the growth of sett roots was inhibited. This effect was examined in concert with soil treatments to manipulate nematode numbers and the general community of yield decline (YD) organisms. The influence of bud age and two different cultivars (Q117, Q138) on early establishment was also examined.

9.2 Materials and Methods

9.2.1 General methods

The two field sites were located approximately 200 m apart on a granitic sandy loam (Thorpe series, from Murtha 1986) of clay content 15-20%. This field had been cropped to sugarcane for at least 30 years. Experiment 1 was planted with Q117 in mid-spring 2002 and harvested 100 days after planting (DAP), while Experiment 2 was planted with Q117 and Q138 in mid-spring 2003 and harvested 70 DAP.

Individual plots were 3.0 m long by 1.5 m wide. Four replicates of nine treatments were arranged in randomised block designs. Each plot consisted of two furrows (rows) of 10 cm depth, 50 cm apart and 3 m long. In those furrows, 20 stem cuttings (3 bud billets) were planted and covered with 8 cm of soil to reflect standard industry practice. At 7 DAP, 10 samples of soil per plot were collected to a depth of 20 cm with an auger, and combined to assess nematode species and numbers in selected treatments. Nematodes were extracted from 200 mL of soil using a Baermann tray for 96 hours (Whitehead and Hemming 1965). Nematodes were concentrated by sieving twice through a 38 μm sieve, and counted. This process was repeated at 50 DAP in Experiment 1. At harvest, nematode populations were estimated in rhizosphere soil shaken from the root system using the extraction process described above. Endoparasite nematodes were extracted from sett roots and a sample of shoot roots by misting roots for 96 hours (Seinhorst 1950).

The numbers of shoots appearing above ground were counted regularly during the experiments. To standardise measurements, shoot numbers were reported per m^2 assuming a 1.5 m row spacing, as is standard for industry planting. At harvest, the

number of activated buds, established primary shoots, and associated secondary shoots (tillers) were counted. The dry weights of primary shoots, secondary shoots, sett roots and shoot roots were also measured. The number of activated buds and primary shoots established, were reported as a percentage (%) of total buds planted. From selected treatments, the stem cuttings (billets) were split and compared visually for degree of tissue discoloration (i.e. degree of bacterial/fungal invasion). In Experiment 2, the health of sett and shoot roots was rated by the method described in Chapter 10 (Table 10.2.4 and Appendix Plate 10.2.4).

GENSTAT Version 6, Rothamstead Experiment Station, U.K. (statistical program) was used for all analyses. Where tests revealed significant differences at the 5% level, means were compared using least significant difference (LSD) at P=0.05.

9.2.2 Experiment 1

Fenamiphos was used to control nematodes, while methyl bromide was used as a broad-spectrum biocide. Fifty days prior to planting, fenamiphos was broadcast at 2 g/m^2 and lightly raked into the soil. The following day, 55 mm of rain fell. Since subsequent nematode counts showed that control was only 30%, fenamiphos was broadcast again at 2 g/m^2, 10 days prior to planting. An equivalent of 20 mm of rainfall was applied as overhead irrigation at 7 days prior to planting. Plots to be fumigated were sealed under black plastic sheeting 25 days prior to planting, and gassed with methyl bromide at 1200 kg/ha.

Axenic cultures of lesion nematode (*Pratylenchus zeae* Graham) were multiplied on carrot callus (Moody *et al.* 1973). Seven days prior to planting, 650 000 nematodes and eggs were applied in a 1 m band down the centre of some fumigated plots. Plots were then watered with 20 mm of irrigation. Another 478 000 nematodes and eggs were added to the bottom of the furrow when it was excavated at planting.

Stem lengths were selected from a mature crop of first ratoon sugarcane (cultivar Q117). Portions of the stem with damaged buds were discarded. The old lower lengths of stem were divided from the relatively new upper lengths of stem, thereby providing a source of ‘old’ and ‘new’ buds respectively (Appendix Plate 9.5). Ten old and 10 new stem cuttings, each with three nodes and buds, were planted per plot.

By shaving off bands of root primordia, 0, 10 or 40% were left intact on the sett surface, along with unshaved treatments. Prior to planting, the stem cuttings were soaked for 5 minutes in 0.15 g/L methoxy ethyl mercuric chloride (Shirtan®), to protect from fungal invasion. The combined soil and shaving treatments were:

0% sett root primordia in untreated soil,
10% sett root primordia in untreated soil,
40% sett root primordia in untreated soil,
100% sett root primordia in untreated soil (industry norm),
100% sett root primordia in nematicide-treated soil,
10% sett root primordia in fumigated soil,
40% sett root primordia in fumigated soil,
100% sett root primordia in fumigated soil,
100% sett root primordia in fumigated soil, reinoculated with lesion nematode.
Plots were harvested at 100 DAP.

The following treatments were duplicated in an adjacent randomised block design of four replicates:

10% sett root primordia in untreated soil,
40% sett root primordia in untreated soil,
100% sett root primordia in untreated soil (industry norm),
100% sett root primordia in nematicide-treated soil,
10% sett root primordia in fumigated soil,
100% sett root primordia in fumigated soil,
In these plots, shoot numbers were monitored from 100-150 DAP.

9.2.3 Experiment 2

Soil treatments were modified from Experiment 1 to provide more effective control of soil biota. To fumigate plots, methyl bromide (1200 kg/ha) was applied after row furrows had been excavated. The nematicide treatment consisted of aldicarb broadcast at 1 g/m^2 in a 30 cm band in the bottom of the furrows at planting. At row

fill, fenamiphos was applied at 2 g/m^2 in a 1 m band over the two rows. Where required, fumigated plots were reinoculated with 1.2 million lesion nematodes/plot within the row as it was filled with soil.

Stem lengths of the cultivars Q117 and Q138 were selected from mature crops of first ratoon sugarcane growing in the same field. Many of the buds and sett root primordia were damaged on the lower 25% of the stems, so this portion was discarded. However, the remaining 75% of stem used was considered adequately aged to be typical of planting material used by growers, and was not overly biased with new buds. By shaving with a scalpel, 0 and 25% of the sett root primordia were left on the stems, along with unshaved (100%) treatments. Thus the combined soil and sett shaving treatments were:

0% sett root primordia in untreated soil,
25% sett root primordia in untreated soil,
100% sett root primordia in untreated soil (industry norm),
25% sett root primordia in nematicide-treated soil,
100% sett root primordia in nematicide-treated soil,
0% sett root primordia in fumigated soil,
25% sett root primordia in fumigated soil,
100% sett root primordia in fumigated soil,
25% sett root primordia in fumigated soil, reinoculated with lesion nematode.
Plots were harvested at 70 DAP.

9.3 Results

Within 10 DAP, rain fell on both experiments. However, from 10-50 DAP, only 42 mm of rain fell on Experiment 1, and within a single week (Table 9.3). Conditions were much wetter in Experiment 2, where 234 mm of rainfall fell during the same period. Adequate rainfall after 50 DAP provided adequate moisture in both experiments (Table 9.3).

Table 9.3: Rainfall during the two experiments.

Days after planting (DAP)	Experiment 1 (mm)	Experiment 2 (mm)
0 – 10	64	32
10 – 20	0	0
20 – 30	0	58
30 – 40	42	157
40 – 50	0	19
50 – 60	44	0
60 – 70	28	145
70 – 80	250	
80 – 90	93	
90 – 100	72	

At harvest, the shaving had left scars on the surface of the stem cutting. However, when the stem cutting was split open, there was no obvious sign of increased tissue rotting (Appendix Plate 9.3.5).

9.3.2 Experiment 1

The weight of sett roots was significantly reduced by shaving the sett root primordia both in untreated and fumigated soil ($P<0.05$, Table 9.3.2.1, Appendix Plate 9.3.6). Unshaved sett roots grew significantly better in fumigated soil, and new stem cuttings produced more sett roots than old stem cuttings ($P<0.05$, Table 9.3.2.1). Across all of the treatments, 68% of new buds commenced growth, which was significantly more ($P<0.05$) than the 49% of old buds that commenced growth (Table 9.3.2.1). About 10% of activated buds failed to become established shoots (i.e. died) regardless of cutting age or treatment. An exception was the 33% death of new buds with 100% root primordia shaved, that were activated in untreated soil ($P<0.05$, data not shown).

The percentage of primary shoots that established was related to the range of sett root weights created from shaving the primordia and/or different soil treatments (Figure 9.3.2.1). Best-fit relationships were curvi-linear and differed between old and new buds. Severe sett root shaving (>60%) of old stem cuttings greatly reduced the primary shoots established in untreated soil (Table 9.3.2.1). The sett roots and primary shoots on new stem cuttings were stimulated more by fumigation and the nematicide than those on old stem cuttings (Table 9.3.2.1).

Table 9.3.2.1: Effect of soil treatment and root primordia shaving on sett root weight, buds activated and primary shoots established at 100 DAP.

Primordia remaining (%)	Soil treatment	Sett root wt./plot (g)		% buds activated	% primary shoots established	
		New buds	Old buds	Mean	New buds	Old bud
0	Untreated	0.00 a	0.00 a	51 b	43 ab	19 a
10	Untreated	0.70 a	0.10 a	34 a	31 a	8 a
40	Untreated	1.54 a	0.76 a	57 b	55 abcde	42 b
100	Untreated	3.49 b	2.96 b	60 bc	50 abcd	40 b
100	Nematicide	4.40 bc	3.12 b	66 bcd	71 de	42 b
10	Fumigated	1.49 a	0.86 a	53 b	45 abc	38 b
40	Fumigated	1.30 a	1.27 ab	55 b	56 bcde	43 b
100	Fumigated	6.85 d	5.69 c	78 d	76 e	55 bc
100	Fumigated + lesion nematode	6.00 cd	5.82 c	74 cd	69 cde	62 c
	LSD (P=0.05)	1.90	1.94	15	24	16
Mean of treatments						
	New buds	2.87 a		68 a	55 a	
	Old buds	2.28 b		49 b	39 b	
	LSD (P=0.05)	0.47		8	8	

Values in the same column followed by the same letter are not significantly different at P=0.05

Industry standard

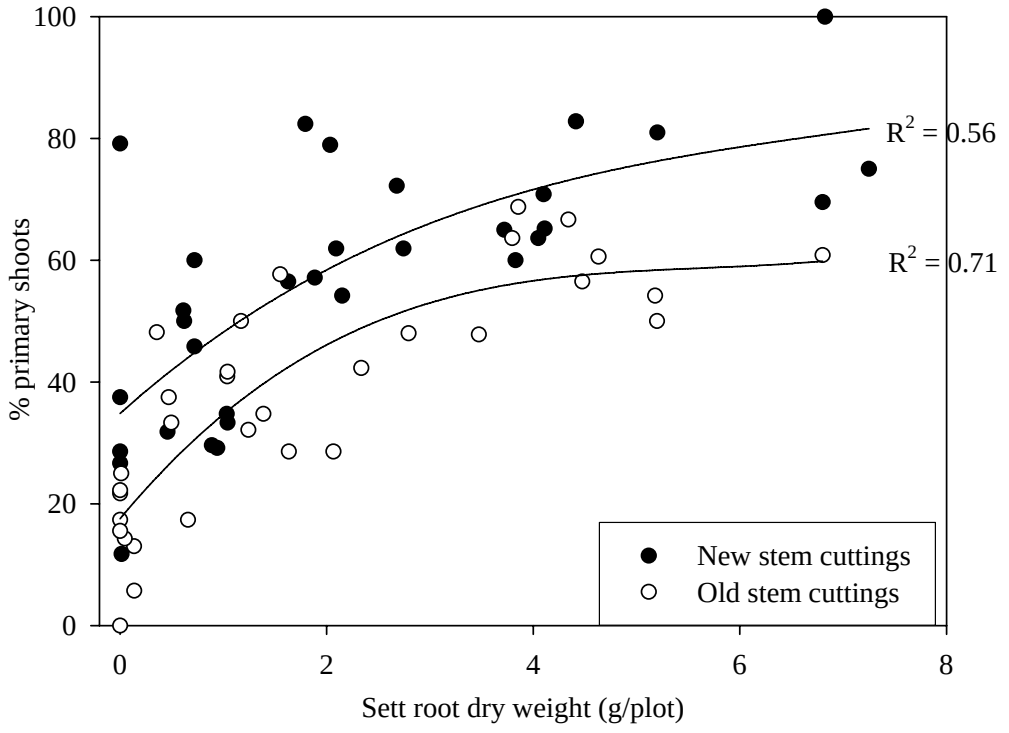


Figure 9.3.2.1: Percent of primary shoots establishing from buds on old and new stem cuttings, relating to sett root weight.

In untreated soil, removing 90% or more of sett roots significantly reduced primary shoot numbers. Thus, the associated primary and secondary shoot biomasses and associated shoot roots also weighed less. The same situation occurred in fumigated soil, but only 60% of sett roots had to be removed (Table 9.3.2.2). Soil fumigation increased shoot root weight and shoot biomass by over 100%. The nematicide increased the new buds that became primary shoots (42%), and shoot root weight per plot (48%), but there were no significant differences at the level of replication used (P<0.05, Tables 9.3.2.1 and 9.3.2.2). However, the primary and secondary shoot increases from the nematicide, yielded a significantly higher total shoot weight (59%). *Pratylenchus zeae* significantly reduced shoot root weight (48%) in reinfected fumigated plots. The lower weight of primary and secondary shoots was not significant (P<0.05). Primary and secondary shoot numbers were not reduced compared to the fumigated control. Plants reinoculated with *P. zeae* had a higher shoot/root ratio than plants from most other treatments (Table 9.3.2.2).

Table 9.3.2.2: Effect of soil treatment and root primordia shaving on shoot roots, shoot weights and shoot numbers per plot at 100 DAP.

Primordia intact (%)	Soil treatment	Shoot root wt. (g)	Primary shoot wt. (g)	Secondary shoot wt. (g)	Total shoot wt. (g)	Secondary shoot number	Shoot/ root ratio
0	Untreated	34 a	226 ab	136 a	362 ab	9 ab	8.13 a
10	Untreated	26 a	117 a	106 a	223 a	6 a	11.39 b
40	Untreated	70 bc	512 cd	282 ab	794 cd	16 cd	11.37 b
100	Untreated	52 ab	351 bc	213 ab	564 bc	13 bc	10.60ab
100	Nematicide	77 bc	573 de	325 b	898 d	16 cd	11.86 b
10	Fumigated	68 bc	427 cd	328 b	755 c	14 bc	11.22bc
40	Fumigated	68 bc	472 cd	343 b	815 cd	19 cd	12.38bc
100	Fumigated	108 d	801 f	530 c	1331 e	21 d	12.32 b
100	Fumigated + lesion nema.	73 bc	701 ef	377 bc	1078 de	20 d	14.96 c
	LSD (P=0.05)	27	166	177	323	6	2.58
Mean of treatments							
	New buds	71	548 a	303	851 a	13.4 a	11.47
	Old buds	57	380 b	284	664 b	16.5 b	11.56
	LSD (P=0.05)	ns	100	ns	182	2.9	ns

Values in the same column followed by the same letter are not significantly different at P=0.05
Industry standard

Different shoot root weights developed on established stools (individual primary shoots) in untreated, nematicide treated and fumigated soil, and correlated with shoot biomass (Table 9.3.2.3). The weight of sett roots on established stools correlated poorly with primary shoot weight, and was unrelated to the secondary shoot biomass per stool.

Table 9.3.2.3: Linear correlations (R^2) between shoot biomass per stool* versus root biomass per stool, using data from individual plots.

	Primary shoot weight/stool	Number of secondary shoots/stool	Secondary shoot weight/stool
Sett root weight/ stool	0.11	ns	ns
Shoot root weight/ stool	0.67	0.40	0.76
*Per established primary shoot			

Significant differences in the number of primary shoots emerging from the soil was evident by 30 DAP (Figure 9.3.2.2). When secondary shoots were emerging at 30-100 DAP, early differences were consistently maintained, but became less when shoot numbers declined from 100-150 DAP due to shoot competition. An exception was 10% sett roots remaining, in untreated plots, where shoot numbers did not decline. Untreated plots with 0% sett roots were not maintained beyond 100 DAP. In untreated soil, 90% of sett roots had to be removed for consistently fewer shoots to emerge during the experiment. In fumigated soil, only 60% of sett roots had to be removed for consistently fewer shoots to emerge during the experiment. Shoot emergence in all treatments is reported in Appendix 9.3.3.

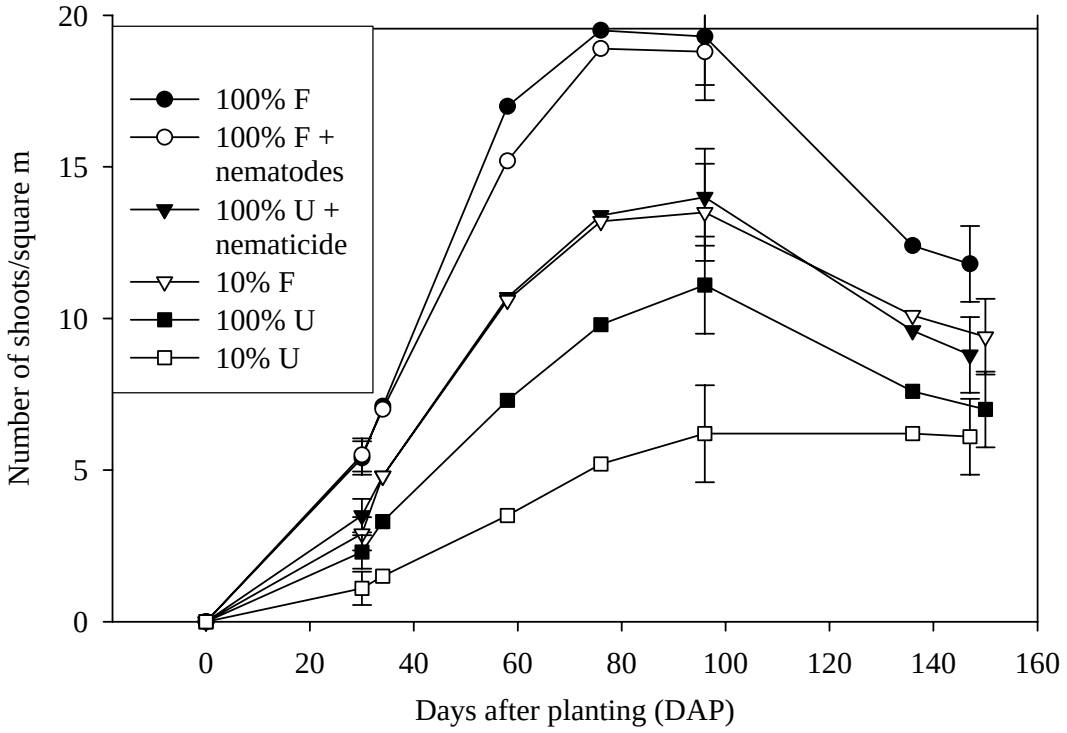


Figure 9.3.2.2: Effect of sett root pruning and soil treatment on number of shoots emerging from the soil (U = untreated, F = fumigated, LSD bars represent P=0.05).

Plant-parasitic nematodes at the site were lesion (*P. zeae*), spiral (*Helicotylenchus dihystera* Cobb) and ring (*Criconemella curvata* de Grisse and Loof). At 7 DAP, there were 305, 365 and 170 of these nematodes/200 mL of soil, respectively. The nematicide had significantly reduced lesion, spiral and ring nematodes by 49%, 48% and 88%, respectively. There was also control of lesion (68%) and ring nematodes (67%) at 50 DAP. However, nematode populations in the nematicide-treated rhizosphere were no different to those in untreated soil at harvest (100 DAP). Full details of soil populations are found in Appendix 9.3.1. There were 3000-5000 lesion nematodes/g of sett root in untreated soil at harvest, and populations were not significantly affected by root shaving or bud age (data not shown). In nematicide-treated plots, lesion nematode densities were not significantly lower inside sett roots at harvest (Table 9.3.2.4). There were 1500-3500 lesion nematodes/g of shoot root in untreated soil at harvest. Populations tended to be lower where the nematicide was used. Fumigation reduced all nematodes species to very low levels in soil and roots, and for the entirety of the experiment. At 7 DAP, fumigated soils reinoculated with lesion nematode had equivalent densities to those in untreated soil. By harvest, 7640

and 4271 lesion nematode/g of root were present in sett and shoot roots respectively in reinoculated plots (Table 9.3.2.4).

Table 9.3.2.4: (Lesion nematode + 0.5)$^{1/3}$ per g root, at harvest (100 DAP).

Primordia remaining (%)	Soil treatment	Sett roots	Shoot roots
0	Untreated	No sett roots	10.79 ab (1545)
10	Untreated	16.16 ab (4826)	12.94 a (2102)
40	Untreated	14.55 ab (3264)	11.55 ab (1907)
100	Untreated	16.20 ab (4525)	14.66 a (3325)
100	Nematicide	11.52 b (1957)	7.46 b (669)
10	Fumigated	3.77 c (45)	1.72 c (3)
40	Fumigated	1.28 c (5)	2.81 c (30)
100	Fumigated	5.51 c (211)	2.27 c (17)
100	Fumigated + lesion nematode	19.02 a (7640)	14.74 a (4271)
	LSD (P=0.05)	4.64	4.14

Values in the same column followed by the same letter are not significantly different at P=0.05
Values in parentheses are back-transformed means. Back-transformed means are the arithmetic means of the raw data
Industry standard

9.3.3 Experiment 2

Sett root weight was significantly reduced by shaving the sett root primordia in untreated, nematicide-treated and fumigated soil. Unshaved sett roots grew significantly better in fumigated soil or nematicide-treated soil than untreated soil ($P<0.05$, Table 9.3.3.1). The health of Q138 sett roots in untreated soil was significantly better than those of Q117. More Q138 buds became active than those of Q117. However, bud shooting was independent of the differing sett root weights induced by the treatments. About 8% of Q138 buds and 16% of Q117 buds that were activated, failed to develop into primary shoots. However, this death was also independent of sett root weight. Thus, the percent of primary shoots that established was independent of the differing sett root weights induced by the treatments (Table 9.3.3.1). Hence, differences in primary shoot emergence from 0-30 DAP, were also minor (Figures 9.3.3.1 and 9.3.3.2). Shoot emergence in all treatments is reported in Appendix 9.3.4.

Table 9.3.3.1: Effect of soil treatment and root primordia shaving on sett roots, buds activated and primary shoots established at 70 DAP.

Primordia remaining (%)	Soil treatment	Sett root wt./plot	Sett root health rating		% buds active	% primary shoots established	
		Mean	Q117	Q138	Mean	Q117	Q138
0	Untreated	0.00 a	No sett roots (nsr)		80	54	73
25	Untreated	0.85 bc	2.10 a	3.05 a	75	51	74
100	Untreated	1.52 d	2.17 a	3.25 abc	77	58	73
25	Nematicide	1.26 cd	3.22 b	3.3 abcd	78	55	80
100	Nematicide	2.79 e	3.50 bc	3.0 ab	71	52	72
25	Fumigated + nematodes	0.59 b	3.50 bc	3.57 cd	72	64	62
0	Fumigated	0.63 b	3.42 bc	nsr	79	59	69
25	Fumigated	0.91 bc	3.45 bc	3.67 d	76	54	77
100	Fumigated	2.35 e	3.63 c	3.62 cd	75	63	67
	LSD (P=0.05)	0.53	0.37	0.37	ns	ns	ns
Mean of treatments							
	Q117	1.03	3.08 a		71 a	57 a	
	Q138	1.25	3.32 b		80 b	72 b	
	LSD (P=0.05)	ns	0.16		4	5	

Values in the same column followed by the same letter are not significantly different at P=0.05

Industry standard

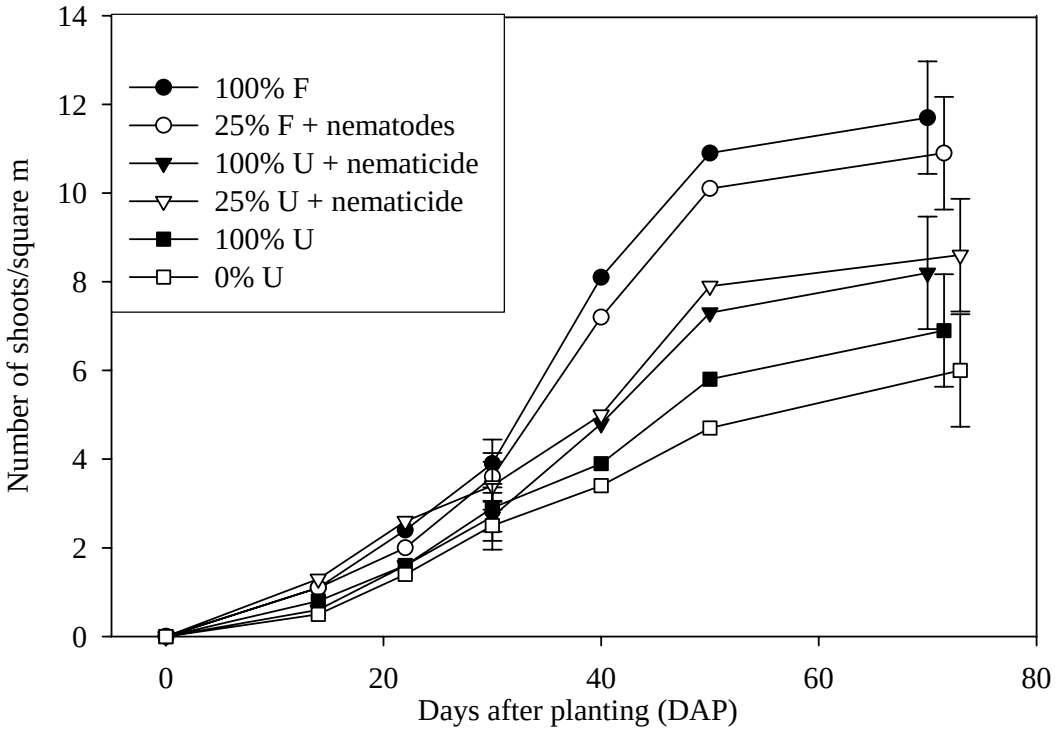


Figure 9.3.3.1: Effect of sett root pruning and soil treatment on number of Q117 shoots emerging from the soil in Experiment 2 (U = untreated, F = fumigated, LSD bars represent P=0.05).

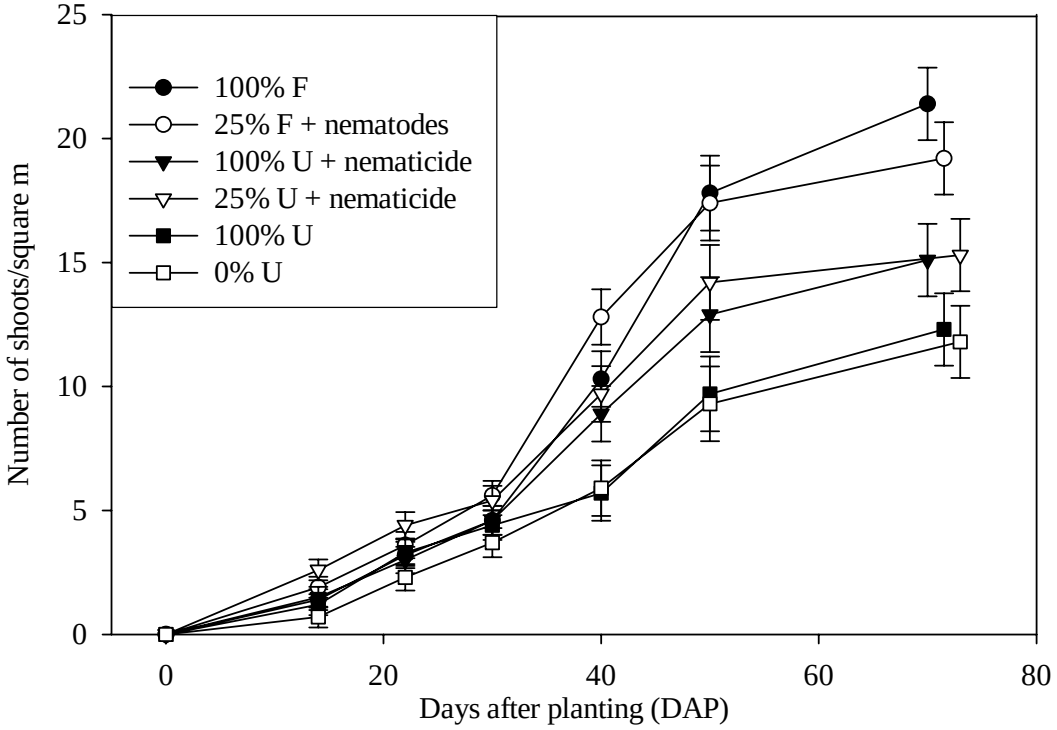


Figure 9.3.3.2: Effect of sett root pruning and soil treatment on number of Q138 shoots emerging from the soil in Experiment 2 (U = untreated, F = fumigated, LSD bars represent P=0.05).

In untreated soil, removing all the sett roots did not affect the percent of primary shoots established, but appeared to inhibit later growth. In particular, a low number and weight of secondary shoots were produced by Q117. In contrast, removing all the sett roots stimulated shoot root growth and subsequent shoot growth in fumigated soil. Thus, the secondary shoot weight of Q117 was significantly higher without sett roots than with 25% and 100% of sett roots in fumigated soil (Table 9.3.3.2).

In untreated soil, shoot root weight and health, and primary shoot weight was significantly less than in nematicide-treated or fumigated soil. Shaved sett roots (25% remaining) in nematicide-treated soil stimulated more shoot roots, so there was no significant difference to roots in fumigated soil ($P<0.05$, Table 9.3.3.2). Shoot root health was the same in nematicide-treated soil as in fumigated soil. In nematicide-treated soil, primary shoot weights were less, but not significantly, than shaved counterparts in fumigated soil ($P<0.05$). Nematicide-treated plots had more secondary shoots than untreated plots, but differences were usually not significant ($P<0.05$). Fumigated plots had significantly higher secondary shoot numbers than untreated or nematicide-treated plots. Secondary shoot weights reflected these trends. Thus, total shoot weights increased by 70% in nematicide-treated soil and by 164% in fumigated soil (Table 9.3.3.2). In fumigated soil reinoculated with lesion nematode, the lower sett root and shoot root weights (54% and 17% respectively) were not significant according to ANOVA comparisons. Nor was root health and shoot biomass significantly reduced ($P<0.05$, Table 9.3.3.2).

While Q138 established significantly more primary shoots (26%) than Q117, there was no difference in primary shoot weight per plot. Cultivar Q138 produced 125% more secondary shoots than Q117. Thus, secondary shoot weight and shoot root weight were significantly higher than Q117 ($P<0.05$, Table 9.3.3.2).

Plants in untreated and nematicide-treated soil tended to have lower shoot/root ratios than plants in fumigated soil. Plants in fumigated soil reinoculated with lesion nematode had a higher shoot/root ratio than plants in any other treatment ($P<0.05$, Table 9.3.3.2).

Table 9.3.3.2: Effect of soil treatment and root primordia shaving on shoot roots, shoot weight and secondary shoot numbers, per plot.

Primordia remaining (%)	Soil treatment	Shoot root wt. (g)	Shoot root health rating	Primary shoot wt. (g)	Secondary shoot wt. (g)		Total shoot wt. (g)	Secondary shoot number		Shoot/root ratio
		Mean	Mean	Mean	Q117	Q138	Mean	Q117	Q138	Mean
0	Untreated	28.5 a	2.68 a	249 a	58 a	174 a	365 a	11 a	31 a	12.96 ab
25	Untreated	35.6 a	2.67 a	300 a	110 ab	197 a	453 a	13 ab	32 ab	12.87 ab
100	Untreated	35.3 a	2.56 a	302 a	118 ab	202 a	462 a	14 ab	34 ab	13.26 ab
25	Nematicide	61.2 bcd	3.52 b	410 b	222 bc	382 a	712 b	22 bc	45 ab	11.69 a
100	Nematicide	54.6 b	3.25 b	395 b	286 cd	394 a	735 b	22 bc	47 b	14.16 bc
25	Fumigated + nematodes	57.0 bc	3.31 b	437 bc	458 e	821 b	1076 c	29 cd	69 c	18.86 e
0	Fumigated	71.0 d	3.55 b	505 c	594 f	697 b	1204 c	37 d	74 c	16.78 d
25	Fumigated	66.5 bcd	3.34 b	430 bc	402 de	937 b	1099 c	34 d	78 c	16.59 d
100	Fumigated	68.7 cd	3.53 b	441 bc	436 e	849 b	1083 c	34 d	76 c	16.01 cd
	LSD (P=0.05)	13.4	0.31	79	131	241	195	9	15	1.96
Mean of treatments										
	Q117	45.0 a	3.09 a	393	298 a		669 a		24 a	15.06
	Q138	61.3 b	3.22 b	390	517 b		928 b		54 b	14.53
	LSD (P=0.05)	6.3	ns	ns	60		92		5	ns

Values in the same column followed by the same letter are not significantly different at P=0.05

Industry standard

Plant-parasitic nematodes at the site were lesion (*P. zeae*), spiral (*H. dihystera*) and ring (*C. curvata*). At 7 DAP, there were 490, 54 and 4 of these nematodes/200 mL of soil, respectively. The nematicide significantly reduced numbers of lesion, and spiral nematodes by 82% and 56%, respectively. At harvest (70 DAP), numbers of these nematodes in the rhizosphere soil were 87% less in nematicide-treated plots. While the control of ring nematode was not as good, its density was low in untreated soil. Full details of soil populations are found in Appendix 9.3.2. There were 5400-5700 lesion nematodes/g of sett root in untreated soil at harvest, and numbers were over 90% less in nematicide-treated plots (Table 9.3.3.3). Numbers of lesion nematode in sett roots were not significantly affected by root shaving or cultivar ($P<0.05$). An exception was very high densities of lesion nematode inside Q117 sett roots, in reinoculated plots (9939 nematodes/g of root). These densities were significantly higher than indigenous populations in the sett roots of Q117 in untreated soils (data not shown).

There were 5800-9500 lesion nematodes/g of shoot root in untreated soil at harvest. Shaved sett root primordia increased lesion nematode in shoot roots, but cultivar had no significant effect on nematode densities ($P>0.05$). Numbers of lesion nematode in shoot roots were over 95% less in nematicide-treated plots.

Fumigation reduced all nematode species to very low levels in soil and roots, and for the entirety of the experiment. At 7 DAP, fumigated soils reinoculated with lesion nematode had equivalent densities to those in untreated soil. By harvest, 7454 and 13286 lesion nematode/g of sett and shoot root respectively, were present in reinoculated plots (Table 9.3.3.3).

Table 9.3.3.3: (Lesion nematode + 0.5)$^{1/3}$ per g of root, at harvest (70 DAP).

Primordia remaining (%)	Soil treatment	Sett roots	Shoot roots
0	Untreated	No sett roots	20.42 ab (9516)
25	Untreated	17.53 a (5702)	17.43 bc (6365)
100	Untreated	16.82 a (5423)	16.71 c (5829)
25	Nematicide	7.14 b (476)	5.91 d (260)
100	Nematicide	6.64 b (337)	5.51 d (219)
25	Fumigated + lesion nematode	17.82 a (7454)	22.76 a (13286)
0	Fumigated	No sett roots	0.91 e (1)
25	Fumigated	0.86 c (1)	1.01 e (2)
100	Fumigated	1.11 c (2)	1.32 e (3)
	LSD (P=0.05)	2.53	3.5

Values in the same column followed by the same letter are not significantly different at P=0.05

Values in parentheses are back-transformed means. Back-transformed means are the arithmetic means of the raw data

Industry standard

9.4 Discussion

At planting, good primary shoot establishment is an important precursor to a numerous and regular stand of shoots along the row. From the viewpoint of crop production, it leads to better light interception and more efficient capture of water and nutrients by the root system (Bull and Bull 1996).

A range of sett root weights were successfully induced in these experiments because shaving the sett root primordia reduced the number of sett roots initiated, and soil biocides increased sett root weight. This affected the number of buds activated and primary shoots establishing in Experiment 1, but the relationship was curvilinear. Specifically, sett roots had to be severely pruned to impact negatively on bud shooting, and greatly improved sett root weights in biocide-treated soil elicited only minor increases in the primary shoots establishing. In Experiment 2, sett roots and sett root health did not influence the numbers of primary shoots establishing.

These findings point to the parent stem cutting providing the developing shoot with nutrients interim to producing functional shoot roots. Bud shooting is increased when phosphorous and nitrogen are applied to the parent crop a month before planting-out

(King 1965; Croft 1998). When internodes are removed from stem cuttings, and nodes of 3-5 cm are planted, primary shoots are 50% smaller at 50 DAP, indicating some reliance upon internode resources (Croft 1998). In Experiment 1, the age of the stem cutting also influenced the importance of sett roots. Stem cuttings from the top of the stalk, with new buds, were more reliant upon sett roots to become primary shoots. Thus, there was a high mortality (33%) of buds that became active without sett roots, and biocides stimulated more primary shoots to establish by improving the volume of sett root. In contrast, old buds appeared to be sustained more by nutrients stored in the bottom of the stem, and so old buds were generally insensitive to the size of sett roots in association. However, compared to new buds more old buds were inherently dormant, and very inactive with 10% or less sett roots in untreated soil. Thus it appears that some sett roots were required to break bud dormancy.

During the period 10-50 DAP, the soil was much wetter in Experiment 2, and perhaps also diminished reliance upon sett roots in this experiment. While the harvest date (70 DAP) should have coincided with optimal sett root volumes in Experiment 2, sett root weights were over 50% less than comparable treatments in Experiment 1. The wetter conditions may have stimulated faster shoot root development, to the detriment of sett roots. In Experiment 2, severe shaving (0 sett roots) appeared to slow the growth of Q117 in untreated soil, so secondary shoots weighed 47% less than unshaved counterparts. The shoot roots of shaved stem cuttings had more lesion nematode at harvest, suggesting sett root removal stimulated earlier emergence of shoot roots. Lesion nematode therefore had a longer period to enter and multiply in those roots. In contrast to untreated soils, severe shaving stimulated 36% heavier secondary shoots in fumigated soil, perhaps by advancing shoot root emergence in a favourable soil environment. It must be noted that differences in plant biomass between treatments were sometimes large (eg. 47%) but not significantly different according to ANOVA comparisons. In future experiments, larger plot sizes or more replicates are recommended to reduce variability between treatment replicates.

It was found in these experiments that sett roots are not as important in establishment as reported previously (Ryker and Edgerton 1931; Cadet and Spaull 1985). However, about 35% of Q117 buds were inherently dormant, even in fumigated soil, and damage from mechanised planting in the sugar industry is likely to further reduce the

number of viable buds. Where nutrient deficient or aged planting material is used as well, it would be desirable to manage YD biota and remove poor sett root growth as a further constraint to viable buds becoming established. In field crops, increased sett root volume has been associated with strategies such as crop rotation, fallowing and soil fumigation, which all reduce populations of YD biota (Garside *et al.* 2002 a; Pankhurst *et al.* 2002). Routinely, greater numbers of primary shoots are established, with a greater number of mature stalks surviving at harvest.

The results of these experiments were not confounded by increased invasion of bacteria and fungi into the stem cutting due to shaving. Shaving caused external scarring, but when stem cuttings were split open at harvest, they showed no evidence of increased tissue rotting. Thus, growth effects from the shaving were attributed solely to reduced sett root volumes.

In Experiment 2, removing YD biota by soil fumigation doubled shoot root weight and improved shoot root health. Concurrently, many more secondary shoots emerged after 30 DAP. There were about 140% more and larger secondary shoots at harvest (70 DAP) and primary shoots were about 50% larger. While soil fumigation greatly increases nitrogen availability, prior studies have found no nitrogen effect on shoot development from 0-60 DAP (Garside *et al*. 1999). Thus, responses to fumigation were attributed to the control of biota associated with YD. The results from Experiment 2 show that even when many primary shoots establish in YD soil, a low number of secondary shoots can ensue due to pathogenic biota on shoot roots. Pathogenic effects on shoot roots were also evident in Experiment 1. Fumigation and nematicides increased the number and weight of secondary shoots per stool (per established primary shoot). This was correlated with improved shoot roots per stool, but not sett root weight.

The nematode species found at the two experimental sites were typical for YD soils in north Queensland (Chapter 3). In Experiment 2, the lesion nematode density at planting was high, and relatively high densities of nematodes developed in sett and shoot roots by harvest. According to the nematicide response, over 50% of the improved root weight and health from fumigation was due to nematode control. Similarly, 40-50% of the associated shoot weight response was due to nematodes.

In Experiment 1, responses to the nematicide were less, and coincided with lower efficacy of the nematicide and lower densities of lesion nematode in untreated soil and roots. At early establishment (0-100 DAP), fumigation responses in this sugarcane field have been consistent over time (this Chapter; Pankhurst *et al.* 2002, 2004 a), suggesting that the general suite of YD biota always have an impact. In contrast, the variable nematicide responses (0-50%) indicate large temporal changes in the impact of nematodes on early establishment of sugarcane. In another study in the same field, nematodes did not reduce early establishment unless soil fungi were also controlled (Pankhurst *et al.* 2002). A pathogenic synergism between lesion nematode and other unknown YD biota was therefore suggested from these experiments. In Experiment 2 for example, the nematicide response was related to the control of high densities of lesion nematode. However, when lesion nematode was the only pathogen (i.e. inoculated into fumigated plots), it had only minor effects on shoots despite multiplying to high densities in the root system. The higher shoot/root ratios of plants in fumigated soil indicated better performance of shoots relative to their root volume. Thus, the combination of root pathogens in YD soil perhaps inhibited root function as well as volume.

Compared to Q117, Q138 established more shoots. Fewer buds were inherently dormant, the mortality of active buds was lower, and secondary shoot tillering was more vigorous. Thus Q138 developed a more than adequate number of shoots in untreated soil. However, the biocide responses showed that the size of primary and secondary shoots was reduced by nematodes and other YD biota. Since soil fumigation can increase mature crop yield by 28% without increasing the number of stalks established (Garside *et al*. 1999), the yield of cultivars such as Q138 may still be limited by YD pathogens, despite vigorous shoot establishment.

To conclude, this study showed that poor primary shoot establishment (row gaps) at planting can occur from a combination of damaged buds, dormant buds, dry soil conditions and poor sett root growth in YD soil. Prudent selection of planting material can lower the reliance of buds upon sett roots, and the risk associated with poor sett root growth in YD soil. However, practices that reduce nematodes and other YD biota will improve sett roots and provide a buffer against situations that may result in poor primary shoot establishment. In turn, these practices will also reduce

biotic constraints on shoot roots, and this is likely to increase the number and size of secondary shoots established.

CHAPTER 10

THE ROLE OF PLANT-PARASITIC NEMATODES IN REDUCING SUGARCANE YIELD AND YIELD COMPONENTS ON FERTILE SOILS OF THE SOUTH AND CENTRAL QUEENSLAND COAST

In the Brisbane region, G R Stirling and P J L Whittle implemented, monitored and harvested four of the field experiments reported in this thesis Chapter (Sites 1-4). The raw data was communicated to the author, and was analysed and incorporated as a sub-set of similar field experiments from the south and central Queensland coast (sites 5-14).

10.1 Introduction

Soil fumigation or pasteurisation, long term fallowing, and rotation crops improve the root health and volume of the following sugarcane crop, resulting in yield responses of 30% or more (Lawrence 1984; Garside *et al*. 1999). Most studies have pursued the role of soil fungi as the causal agent, especially after the root rotting fungus (*Pachymetra chaunorhiza*) was discovered, and root health was improved by applying fungicides to the soil. However, identifying and demonstrating the pathology of other fungal pathogens to sugarcane has proved difficult (Magarey 1996).

In Australia, the importance of nematodes as pests of sugarcane is largely unknown. However, recent surveys of Queensland's sugarcane fields have shown that plant-parasitic nematodes are ubiquitous. Five species are widespread (Blair *et al*. 1999 a, b) and all are known sugarcane pathogens in other parts of the world (Spaull and Cadet 1990). Pathogenicity to sugarcane cultivars grown widely in Australia has been demonstrated in the glasshouse and in field miniplots (Chapters 6, 7, 8 and 9). However, these experiments only examine plant crop events from 0-100 days after planting (DAP), whereas field sugarcane grows for over 300 DAP, and about 75% of the crop is ratoon.

The poor establishment of sugarcane due to nematode attack in sandy soils is well documented around the world (Spaull and Cadet 1990). These soils are termed 'class

A' soils for convenience in this report. However, in Australia as in many other countries, these soils are minor in area compared to the sugarcane grown on the more fertile sandy loam to clay soils (class B). Estimates of yield losses in these soils are very speculative and unsupported by field experiments (Sasser and Freckman 1987).

It was the central aim of these field experiments to quantify the role of plant-parasitic nematodes in contributing to the 'yield decline' (YD) of sugarcane in Queensland. To this end, non-volatile nematicides were used as a research tool to selectively control nematodes for the entire growing season. This approach departs from grower uses of nematicides on sugarcane, to protect the plant from the serious nematode problems that occur at planting and at early tillering of the ratoon, in 'class A' soils (Bull 1979, 1981; Spaull and Cadet 1990). The experiments reported in this Chapter were all done on sandy loam to clay soils, where chronic nematode problems have not been identified.

Relationships between yield and nematode densities were explored as a general regional trend by combining data from all of the sites into linear correlations. Firstly, these correlations aimed to identify the relative importance of particular nematode species/groups impacting on tillers emerged, stalks established, stalk length and final yield. Secondly, significant correlations would support the contention that nematicide responses were primarily due to nematode control.

10.2 Materials and Methods

10.2.1 Field details

The experimental sites were located in sugarcane fields along the Queensland coast between Rocky Point (just south of Brisbane) and Mackay, a distance of about 1200 km. Locations of the sites across Queensland are appended (Appendix Plates 10.2.1-10.2.3).Sugarcane fields were selected in different districts and on different soil types to represent broad areas currently under sugarcane cropping (Table 10.2.1). Fields had grown sugarcane for at least 20 years, and growers practised widely adopted methods of production. Growers perceived nematodes to be an insignificant production issue at the sites and had not implemented farming strategies specifically to manage nematodes. Experimental sites were located away from districts where

acute nematode problems were recognised, such as in coarse sandy soils where nematicides were routinely in use by sugarcane growers. In most of the experiments, the sugarcane cultivar planted was Q124. Exceptions were CP51-21 at Coolum (Site 2), and Q138 at Maryborough (Site 5). Experiments in south Queensland were conducted from 1995 to 1998, whilst experiments in central Queensland were conducted in 1998 and 1999.

Table 10.2.1: Details and location of nematicide experiments.

Site number and location (district) [A]	Soil texture[B]	Soil description	Duration of experiment[C]	Irrigation status
South Queensland				
1. Rocky Point	16: 32: 18: 34	Clay loam	P, 1R	Dryland
2. Coolum	10: 15: 25: 50	Clay	P, 1R	Dryland
3. Maroochydore	12: 16: 22: 50	Clay	P, 1R	Dryland
4. Yandina	5: 13: 16: 66	Clay	P, 1R	Dryland
5. Maryborough	15: 46: 23: 16	Silty sand loam	P, 1R, 2R	Irrigated
6. Childers	27: 56: 6: 11	Fine sandy loam	P, 1R, 2R	Irrigated
7a. Elliot Heads	44: 40: 8: 8	Loamy sand	P, 1R	Irrigated
7b. Elliot Heads	44: 40: 8: 8	Loamy sand	P, 1R	Irrigated
8. Fairymead	40: 36: 9: 15	Fine sandy loam	P, 1R	Irrigated
9. Fairymead	17: 51: 18: 13	Fine sandy loam	P, 1R	Irrigated
10. Bingera	28: 49: 7: 16	Fine sandy loam	P, 1R, 2R	Irrigated
Central Queensland				
11. Plane Creek	19: 43: 25: 13	Silty sand loam	1R	Irrigated
12. Racecourse	42: 32: 13: 13	Sandy loam	1R	Irrigated
13. Mirani	44: 29: 9: 18	Sandy clay loam	P	Dryland
14a. Farleigh	51: 32: 2: 15	Coarse sand loam	P	Dryland
14b. Farleigh	36: 40: 13: 11	Sandy loam	P	Dryland

[A]Sites 1-4, 7-10 and 11-14 are located near Brisbane, Bundaberg and Mackay respectively

[B]% coarse sand: fine sand: silt: clay

[C]P = plant, IR = 1st ratoon, 2R = 2nd ratoon

10.2.2 Experimental design

Experiments were a paired comparison of +/- nematicide, with six replicates of each treatment usually allocated in a randomised complete block design. However, paired plot designs were sometimes used. Individual plots were 12 m long × 6 rows wide, and situated in one area of the field in the case of randomised blocks. At Bundaberg (Site 8), paired plots were scattered over a 6 ha field. At Elliot Heads (Site 7), three pairs of untreated and nematicide-treated plots were located on opposite sides of a 5 ha field. At this site, nematode populations, crop management (irrigation), and subsequent sugarcane growth varied markedly on each side of the field, so each

group of plots was analysed as a separate experiment of three replicates (Sites 7a, 7b). The Farleigh site also constituted two experiments of six replicates on different soil types at opposite ends of the same 4 ha field (Sites 14a, 14b).

10.2.3 Nematicide program

The nematicides used were aldicarb (Temik 15G®) and fenamiphos (Nemacur 10G®), which were alternated to minimize the development of enhanced biodegradation problems (Smelt and Leistra 1992; Stirling *et al.* 1992 b). However, at three central Queensland sites (13, 14a, 14b) the experiments were done only on the plant crop, and fenamiphos was used three times in succession for prolonged nematode control. Because most of the nematicidal activity of aldicarb and fenamiphos dissipates in 30-60 days (Hough *et al.* 1975; Stirling and Dullahide 1987), applications were repeated to control nematodes for the entire crop cycle.

Stem cuttings (billets) with 2-3 nodal buds were planted end-to-end into the bottom of a planting furrow of 20 cm depth, and in rows 1.5 m apart. After tillers emerged, the sugarcane row was progressively profiled into a raised bed by transferring soil from the inter-row according to established industry practice. Thus, to maintain the control of nematodes in the row, where possible, granules were applied prior to hilling and weed scarifying operations to better mix granules with soil being introduced around the developing plant. If irrigation was available, granules were applied immediately before the field was watered. The nematicide applications occurred (a) at, or soon after planting in August/September, (b) at the 3-5 leaf stage in November and/or (c) in December when plants measured about 1 m to the top of the canopy. The nematicide was applied in a 0.3 m wide band over the row at 10 kg a.i./treated ha, which meant that Temik and Nemacur applications were 2 g and 3 g of product/m of row, respectively.

After the row mound had been established in December, and to cater for more expansive root growth, nematicide applications were extended to a 1 m wide band over the row. Thus, Temik and Nemacur applications were 6.67 g and 10 g of product/m of row, respectively. Those wider bands were usually applied in January (120 DAP) and March (190 DAP). Similarly, ratoon crops were treated with 1 m

wide bands of nematicide across the row, with the first application soon after harvest of the plant crop, and 2-3 further applications during the growing season. Where nematode control was deemed to be satisfactory according to nematode counts, nematicides were applied less frequently. Full details of the nematicide regime at the 16 sites are appended (Appendix 10.2.3).

In plant crops, a hand rake was used to incorporate the nematicide granules into the soil, or the applications coincided with cultivation by the grower. In ratoon crops, trash blankets were maintained at some sites, and this dictated the method of nematicide applications (Appendix 10.2.3).

10.2.4 Nematode and crop sampling

Samples for nematodes were collected regularly from each site in both plant and ratoon crops. To avoid damaging roots of sugarcane in the middle two rows from which yields were collected, all samples were taken from the outer rows in each plot. Holes approximately 20 × 20 × 20 cm were dug near the stool and a handful of roots and soil was placed in a bucket. Material from 10 holes in each plot was then mixed and sub-samples of soil (200 mL) and roots (50-100 g) were retained for analysis. Nematodes were extracted by placing soil on a Baermann tray for 96 hours (Whitehead and Hemming 1965), and by misting roots for 96 hours (Seinhorst 1950). After processing, roots were dried and weighed. Nematodes were concentrated by sieving twice through a 38 μm sieve. Nematodes were identified to species, except for the vermiform stage (J2) of root-knot nematode (*Meloidogyne* spp.), and the ring nematodes (Criconematidae).

At some sites, roots collected in March and April for nematode analysis were rated for root health before nematodes were extracted. Ten root pieces (10-15 cm long) were randomly selected from each root sample and rated individually using the scale in Table 10.2.4. A diagrammatic representation of the ratings is appended (Appendix Plate 10.2.4). The 20 ratings from each sample (plot) were then averaged to obtain a mean health rating.

Table 10.2.4: Root health ratings for primary and secondary roots, and tertiary roots.

Primary/Secondary Roots	
1. Severe disease	Almost all (>95%) of primary roots dark and necrotic, with sparse and stunted secondary roots. Lesions apparent on new primary roots.
2. High disease	Most (>66%) of primary roots diseased. Secondaries tend to be stunted and thickened.
3. Moderate disease	Similar proportions of diseased and healthy primary and secondary roots.
4. Low disease	<33% of primary roots diseased. Most roots healthy, with functional secondaries.
5. Healthy	Few (<5%) of primary roots diseased. Almost all roots white and healthy, with many long white secondary roots.
Tertiary Roots – Based on ratios of tertiary root length (TRL):primary root length (PRL)	
1. Severe disease	No tertiary roots.
2. High disease	Tertiary roots erratically distributed and generally diseased (TRL:PRL<2:1).
3. Moderate disease	Moderate tertiary root system (TRL:PRL = 2-10:1).
4. Low disease	Large numbers of tertiary roots (TRL:PRL = 10-20:1).
5. Healthy	Extensive, uniform mass of healthy tertiary roots, contributing a major proportion of the total root length (TRL:PRL>20:1).

At early establishment of the plant crops, the primary and secondary shoots emerging from the soil were collectively termed ‘tillers’ in these experiments. The tillers that survived competition and continued growing after 150 DAP were then termed ‘stalks’.

At most sites, the number of tillers (SN_1) and number of established stalks (SN_2) per 20 m of row were counted at about 120 and 200 days after planting (DAP) respectively. In each plot, 20 tillers/stalks were randomly tagged, and their length (SL) was measured from ground level to the highest leaf collar. At some sites, the diameter (SD) of those 20 stalks was also measured. In ratoon crops, the number and

length of stalks were measured only at about 210 days after ratooning (DAR). At harvest, yield (T/ha) was estimated by weighing a sample of stalks (with leaves removed) from the two middle rows of each plot. At some sites, the commercial cane sucrose (CCS) recoverable from the stalk juice was estimated from a sample of six stalks.

10.2.5 Regional trend between nematode densities and yield

To relate yield to nematode levels as a general regional trend, data was combined from all sites into linear correlations. Untreated yields varied greatly from site to site (70-164 T/ha), no doubt due to differences in the environment (temperature, light, rainfall, other pathogens and pests, etc.) and level of crop management (fertiliser inputs, irrigation frequency, skills of the grower, etc.) that were the primary determinants of crop growth. These factors overshadowed the effects of more subtle influences on yield, such as nematodes. For instance, untreated plots yielded more than 150 T/ha at some sites, whereas the nematicide-treated plots yielded less than 100 T/ha at other sites. A plant response to the nematicide was therefore calculated for each site (biomass in treated plots – biomass in untreated plots), and used in correlations.

When yield data were examined, there was no response to nematicides in the highest yielding crops (>150 T/ha). Since the final yield at any site ultimately reflects the sum of the environmental and management influences that impinge on the crop during the year, the yield of nematicide-treated plots was used to formulate a rating for the adversity or suitability of the environment (EM) at each site in a given year (Table 10.2.5). Nematicide-treated yield was used to exclude a nematode bias from the rating. This rating was co-factored with nematode populations in correlations with biomass responses to the nematicides.

Nematode control varied from site to site, so the nematicide response was correlated with the number of nematodes controlled by the nematicide (nematode levels in untreated plots – nematode levels in treated plots). During the first 90 DAP, nematicide-treated soil was periodically covered by untreated soil in row filling operations, which appears to have contaminated the soil samples collected. In this situation, the nematode levels in untreated soil at planting (P_i) were correlated with

nematicide responses on the assumption of nematode control in nematicide-treated plots in the underlying root zone.

Table 10.2.5: Crop yields used to generate an environment/management (EM) rating for each site.

Yield of nematicide-treated sugarcane (T/ha)	Environment/management (EM) rating
<80	0
80-89	1
90-99	2
100-109	3
110-119	4
120-129	5
130-139	6
140-149	7
150-159	8
160-169	9
>170	10

10.2.6 Statistical analyses and correlations

Depending on the experimental design, analysis of variance (ANOVA) or a paired T-test was used to compare sugarcane yield and yield components in treated and untreated plots at individual sites. Where these tests revealed significant differences at the 5% level, means were compared using least significant difference (LSD) at P=0.05. GENSTAT Version 6, Rothamstead Experiment Station, U. K. (statistical program) was used for all analyses.

Multiple linear regression was used to correlate trends between nematode density and the influence of EM with yield responses across all sites. Stalk length/m^2 of plot SL/m^2) was also used in these correlations, and was calculated from $SN_2 \times SL$.

10.3 Results

10.3.1 Nematodes on plant crops

Lesion nematode (*Pratylenchus zeae* Graham) occurred at all sites, usually at higher population densities than any other nematode species. Numbers of lesion nematode ranged from 16-359 nematodes/200 mL of untreated soil at planting, increasing to 408-2747 nematodes/200 mL of soil by mid-season, usually followed by a decline toward harvest (Figures 10.3.1.1-10.3.1.5). Mid-season populations in roots ranged

from 175-14851 nematodes/g of root (Table 10.3.1.1) and also declined towards harvest. Root-knot nematode (*Meloidogyne* spp.) was present at most sites, but population densities were usually lower than for lesion nematode. At planting and during the season, root-knot nematode juveniles were detected at between 70-75% of sites (Table 10.3.1.2) and mid-season populations declined greatly by harvest (Figures 10.3.1.1-10.3.1.5). Species of ectoparasite nematodes were also present, particularly spiral nematode (*Helicotylenchus dihystera* Cobb) at the rain-fed sites with a high clay content, and stunt nematode (*Tylenchorhynchus annulatus* Cassidy) at loam and sandy loam soils in south Queensland (Table 10.3.1.3).

Soil samples collected between 0-100 DAP indicated about 50% control of all nematode species in nematicide-treated plots. In nematicide-treated plots after 100 DAP, populations of lesion nematode in soil and roots were reduced by >90% at all irrigated sites and by >66% at rain-fed sites (Table 10.3.1.1). After 100 DAP the control of root-knot nematode in soil and roots was more variable, but was usually >50% (Table 10.3.1.2). The control of ectoparasitic nematodes was highly variable (data not presented).

10.3.2 Nematodes on ratoon crops

Population densities of all species were often higher at the start of the ratoons than at planting (P_i). But later in the ratoons, they usually did not reach levels achieved in the plant crops (Tables 10.3.1.1 and 10.3.1.2, Figures 10.3.1-10.3.5). This was particularly the case for root-knot nematode, which rarely exceeded mid-season population densities of 150 nematodes/200 mL of soil. Early in the ratoon (0-100 DAR), root-knot nematode juveniles were detected at 72% of sites compared to 62% of sites by mid-season (Table 10.3.1.2).

In nematicide-treated plots, the level of control of lesion nematode in the ratoon crops did not rival that of plant crops, but was usually >66% (Table 10.3.1.1). The control of root-knot nematode was very variable, with more nematodes found in nematicide-treated plots than untreated plots by mid-season at two sites (Table 10.3.1.2, Figure 10.3.1.5). The control of ectoparasites in ratoons was usually around 50%.

Table 10.3.1.1: Densities of lesion nematodes (*Pratylenchus zeae*) in untreated soil and roots, and level of control in nematicide-treated plots, at each site.

Site	Nematodes/ 200 mL of soil at planting (P_i)	Maximum nematode density[A] in untreated plots, 200 mL of soil	g of root	% control due to nematicides[B], in soil	in roots
Plant crops					
1. Rocky Point	205	2747	2571	80	84
2. Coolum	56	408	175	66	82
3. Maroochydore	89	947	467	81	91
4. Yandina	16	1621	2870	96	88
5. Maryborough	63	1035	4811	94	38
6. Childers	178	1368	10916	96	98
7a. Elliot Heads	390	2313	10734	98	96
7b. Elliot Heads	359	1133	14851	97	97
8. Fairymead	77	672	3180	94	97
9. Fairymead	169	647	1811	95	86
10. Bingera	50	1010	3301	94	96
11. Plane Creek	135	245	175	94	91
12. Racecourse	174	492	833	94	86
13. Mirani	113	617	1254	91	92
14a. Farleigh	130	1103	2816	46	71
14b. Farleigh	224	680	2662	97	98
	Ratoon, early season				
1st ratoon crops					
1. Rocky Point	859	1216	2240	32	84
2. Coolum	178	278	181	68	81
3. Maroochydore	183	301	46	68	28
4. Yandina	254	1105	380	96	84
5. Maryborough	263	208	621	60	83
6. Childers	432	700	3353	29	29
7a. Elliot Heads	657	507	3178	95	99
7b. Elliot Heads	323	993	2863	99	97
8. Fairymead	355	337	1353	75	91
9. Fairymead	664	1776	1986	91	84
10. Bingera	970	303	1334	75	73
11. Plane Creek	825	527	154	99	99
12. Racecourse	507	453	110	98	97
2nd ratoon crops					
5. Maryborough	no data	633	160	81	79
6. Childers	no data	247	325	100	99
10. Bingera	430	317	290	97	93

[A]Maximum nematode density in soil and roots of untreated plots in samples taken between February and April (mid-season)

[B]Percent of nematodes controlled by the nematicide in samples taken between February and April (mid-season)

Table 10.3.1.2: Densities of root-knot nematodes (*Meloidogyne* spp.) in untreated soil and roots, and level of control in nematicide-treated plots, at each site.

Site	Nematodes/ 200 mL of soil at planting (P_i)	Maximum nematode density[A] in untreated plots, 200 mL of soil	g of root	% control due to nematicides[B], in soil	in roots
Plant crops					
1. Rocky Point	2	124	59	76	92
2. Coolum	3	167	9	66	78
3. Maroochydore	4	217	97	74	70
4. Yandina	4	319	1529	85	44
5. Maryborough	0	0	0		
6. Childers	3	270	4747	49	92
7a. Elliot Heads	0	730	6133	80	52
7b. Elliot Heads	0	113	475	98	69
8. Fairymead	23	12	321	50	83
9. Fairymead	9	0	7		-100
10. Bingera	13	838	5387	89	93
11. Plane Creek	0	0	0		
12. Racecourse	0	430	661	97	97
13. Mirani	5	47	0		
14a. Farleigh	34	150	1682	100	75
14b. Farleigh	12	0	0		
	Ratoon, early season				
1st ratoon crops					
1. Rocky Point	0	0	0		
2. Coolum	4	6	0	50	
3. Maroochydore	5	58	4	31	0
4. Yandina	21	403	371	91	92
5. Maryborough	0	0	0		
6. Childers	302	1032	1786	-50	-95
7a. Elliot Heads	13	23	109	26	83
7b. Elliot Heads	23	0	15		80
8. Fairymead	0	0	0		
9. Fairymead	8	0	0		
10. Bingera	142	143	980	-78	-15
11. Plane Creek	0	0	0		
12. Racecourse	67	8	12	13	100
2nd ratoon crops					
5. Maryborough	no data	0	0		
6. Childers	no data	80	1830	89	86
10. Bingera	100	143	773	85	89

[A]Maximum nematode density in soil and roots of untreated plots in samples taken between February and April (mid-season)

[B]Percent of nematodes controlled by the nematicide in samples taken between February and April (mid-season)

Table 10.3.1.3: Maximum mid-season densities of ectoparasitic nematodes/200 mL of soil at each field site.

Site	Stunt[A]	Spiral[B]	Stubby-root[C]	Ring[D]	Dagger[E]
Plant crops					
1. Rocky Point	46	362	311	23	0
2. Coolum	226	538	41	2	0
3. Maroochydore	121	671	92	12	8
4. Yandina	0	239	0	32	0
5. Maryborough	2140	792	22	0	0
6. Childers	1043	8	382	0	5
7a. Elliot Heads	203	100	137	0	70
7b. Elliot Heads	620	105	155	0	0
8. Fairymead	1012	137	107	13	3
9. Fairymead	1956	90	80	0	6
10. Bingera	0	83	136	0	0
11. Plane Creek	730	131	3	2	2
12. Racecourse	382	167	82	0	0
13. Mirani	47	0	15	2	5
14a. Farleigh	22	14	25	30	0
14b. Farleigh	18	36	57	10	0
1st ratoon crops					
1. Rocky Point	24	146	91	45	0
2. Coolum	111	486	65	1	0
3. Maroochydore	24	327	51	4	24
4. Yandina	5	405	2	35	0
5. Maryborough	901	293	50	0	0
6. Childers	14	0	46	0	0
7a. Elliot Heads	417	40	258	0	117
7b.Elliot Heads	640	148	250	0	0
8. Fairymead	590	200	95	12	40
9. Fairymead	226	102	93	0	0
10. Bingera	0	23	25	0	7
11. Plane Creek	75	170	60	0	0
12. Racecourse	8	42	22	0	0
2nd ratoon crops					
5. Maryborough	649	257	26	0	0
6. Childers	126	13	321	1	1
10. Bingera	0	50	93	0	0

[A]*Tylenchorhynchus annulatus*, [B]*Helicotylenchus dihystera*, [C]*Paratrichodorus minor* Colbran, [D]Criconematidae and [E]*Xiphinema elongatum* Schuurmans, Stekhoven and Teunissen

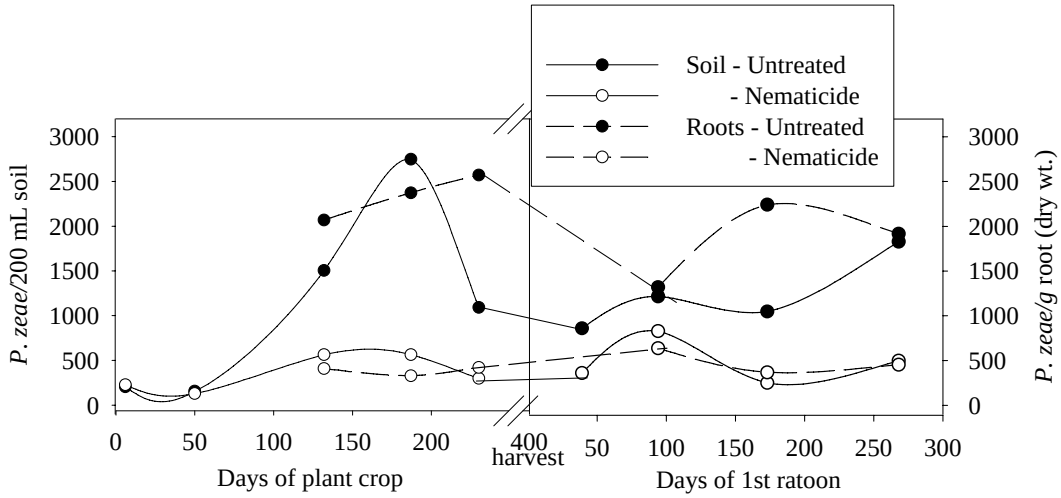


Figure 10.3.1.1: Plant crop and 1st ratoon densities of *Pratylenchus zeae* in soil and roots in untreated and nematicide-treated sugarcane, at a rain-fed site (1) in south Queensland.

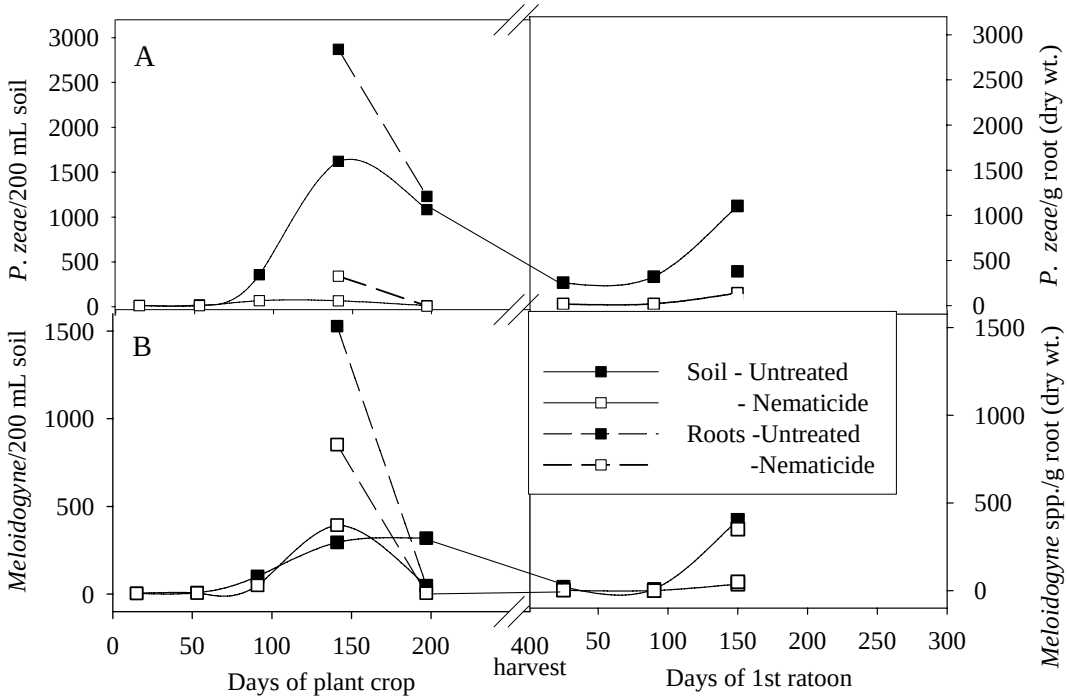


Figure 10.3.1.2: Plant crop and 1st ratoon densities of (A) *Pratylenchus zeae* and (B) *Meloidogyne* spp. in soil and roots in untreated and nematicide-treated sugarcane, at a rain-fed site (4) in south Queensland.

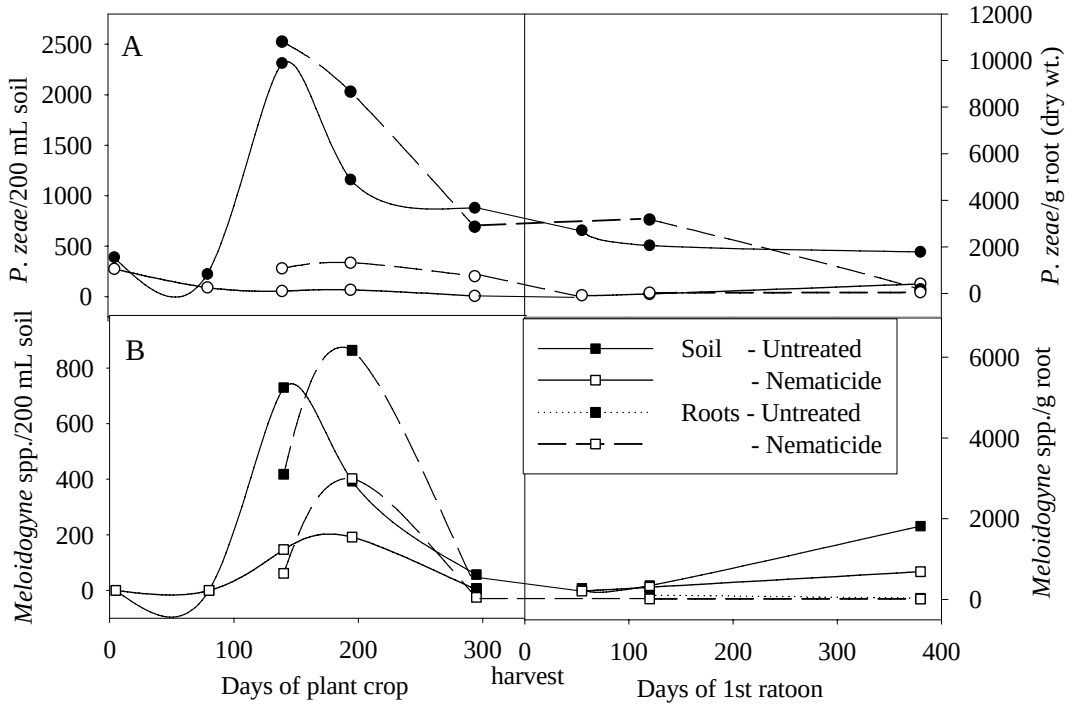


Figure 10.3.1.3: Plant crop and 1st ratoon densities of (A) *Pratylenchus zeae* and (B) *Meloidogyne* spp. in soil and roots in untreated and nematicide-treated sugarcane, at Elliot Heads (Site 7a) in south Queensland.

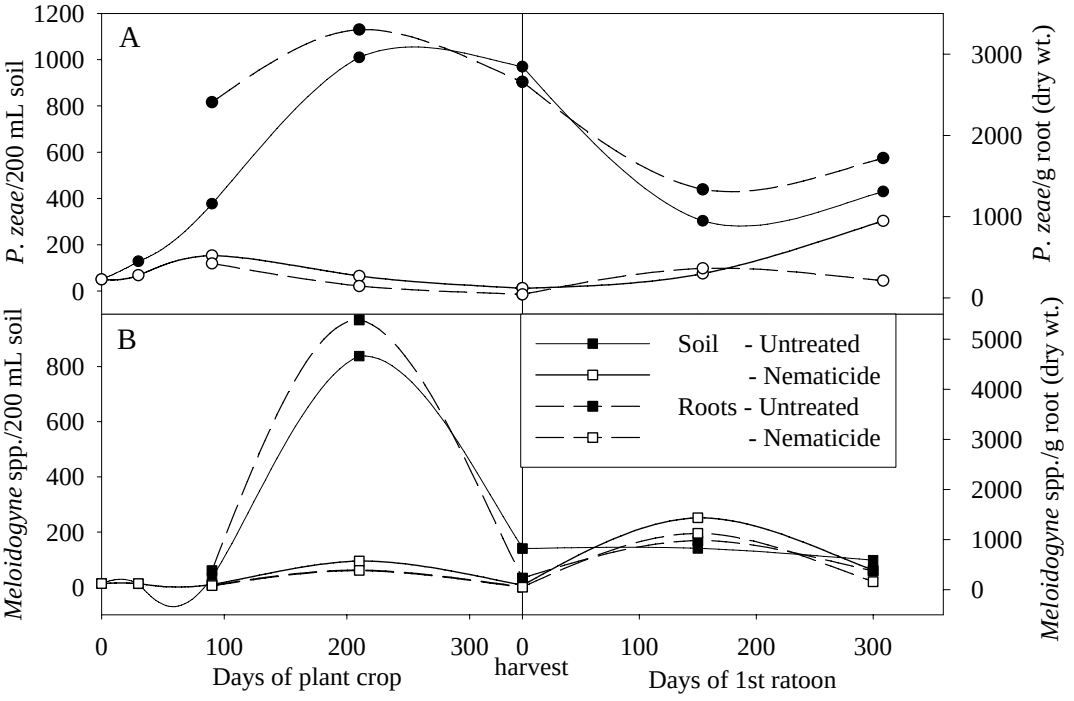


Figure 10.3.1.4: Plant crop and 1st ratoon densities of (A) *Pratylenchus zeae* and (B) *Meloidogyne* spp. in soil and roots in untreated and nematicide-treated sugarcane, at Bundaberg (Site 9) in south Queensland.

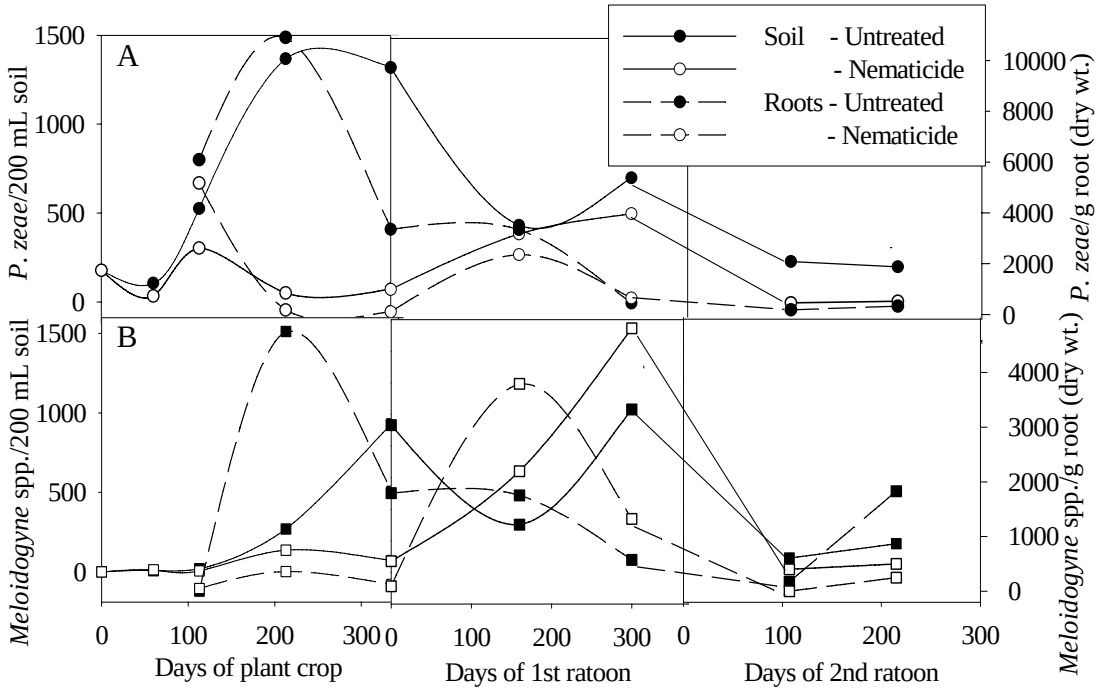


Figure 10.3.1.5: Plant crop and ratoon densities of (A) *Pratylenchus zeae* and (B) *Meloidogyne* spp. in soil and roots in untreated and nematicide-treated sugarcane, at Childers (Site 6) in south Queensland.

10.3.3 Plant crop yields

Between 6 and 12 tillers/m^2 had emerged in untreated plots by 100-150 DAP (Table 10.3.3.1). Although up to 22% more tillers emerged in treated plots at some sites, this was never significant ($P>0.05$). Tiller numbers were then either maintained or declined to become 6-10 established stalks/m^2. By about 200 DAP, numbers of stalks were increased by 10% or more in nematicide-treated plots only at three sites, and significantly ($P<0.05$) only in one field (Site 7a, 7b). These responses developed after 100 DAP (Table 10.3.3.1). Where nematicides increased tiller numbers prior to 100 DAP, the responses tended to disappear as the tillers developed into established stalks (Figure 10.3.3).

Visual responses to nematicides were often apparent by 100 DAP due to differences in tiller length. Tiller length was significantly increased by the nematicides at four of the seven sites where these measurements were taken at 100-150 DAP (Table 10.3.3.1). By about 200 DAP, nematicides had significantly increased stalk length at

10 of the 14 sites where these measurements were taken. The mean diameter of established stalks was never more than 4% larger in treated plots at the six sites where these measurements were taken. This effect was statistically significant only at one site (Table 10.3.3.1).

The untreated plant crops yielded between 71-155 T/ha and typically were above average for plant crops in their district. Yields in nematicide-treated plots were routinely larger, usually by 10-20%, and significantly ($P<0.05$) at six sites (Table 10.3.3.2). One exception was the highest yielding site (Site 5), where the nematicides had no effect on yield.

Table 10.3.3.1: Percent increases in tiller number (SN_1), stalk number (SN_2), tiller/stalk length (SL) and stalk diameter (SD) due to the nematicides at sites where these measurements were taken.

Site	Plant crop 120 DAP		Plant crop 200 DAP			1st Ratoon 210 DAP		2nd Ratoon 210 DAP	
Region or mill area –	SN_1	SL	SN_2	SL	SD	SN_2	SL	SN_2	SL
South Queensland									
1. Rocky Point	22	33*	4	9*	2				
2. Coolum	-3	3	10	6*	-2	-4	6*		
3. Maroochydore	2	5	4	5*	1	9*	6*		
4. Yandina	2	9	2	9					
5. Maryborough			1	5*		1	0		
6. Childers			6	15*		-2	-2	14*	4
7a. Elliot Heads	9	31*	13*	22*	4	17*	22*		
7b. Elliot Heads	-2	19*	12*	12*	3*	-10	13*		
8. Fairymead	11	17*	2	13*	1	2	7*		
9. Fairymead			3	3		5	8*		
10. Bingera			6	10*		0	0	5	8*
Central Queensland									
11. Plane Creek						6	6		
12. Racecourse									
13. Marian			3	6*					
14a. Farleigh			0	1					
14b. Farleigh			8	3					

*significant at P=0.05

Table 10.3.3.2: Final yields in untreated plots, comparison to the district average, and yield improvements when nematodes were selectively controlled.

Region or mill area –	Sugarcane yield in untreated plots (T/ha)			% yield increase in treated plots			Untreated plot yield versus the 'district average' (%)[A]		
	Plant	1R	2R	Plant	1R	2R	Plant	1R	2R
South Queensland									
1. Rocky Point	85	121		13*	2		-8	7	
2. Coolum	81	131		19*	2		0	28	
3. Maroochydore	86	117		14	11		6	15	
4. Yandina	104	136		12	5		23	31	
5. Maryborough	155		84	-1		8	104		-2
6. Childers	113	123	107	23*	3	26*	11	26	9
7a. Elliot Heads	71	129		51*	37*		-24	17	
7b. Elliot Heads	103	164		16*	0		17	49	
8. Fairymead	117	130		13*	11*		13	10	
9. Fairymead	93	108		8	20*		-19	-13	
10. Bingera	112	94	104	10	8	14*	10	15	13
Central Queensland									
11. Plane Creek		113			20*			27	
12. Racecourse		110			7			17	
13. Marian	89			11			7		
14a. Farleigh	88			16			1		
14b. Farleigh	105			10			21		

*significant at P=0.05
[A](Yield in untreated plots) – (District average yield for the same year and crop class)
1R = 1st ratoon crop, 2R = 2nd ratoon crop

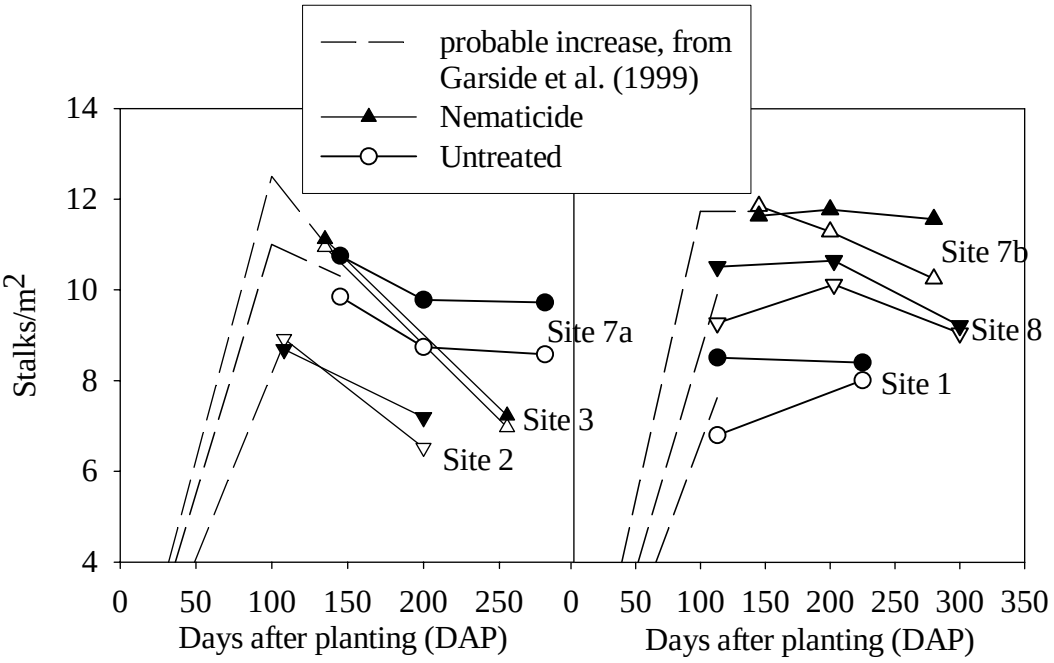


Figure 10.3.3: Number of tillers emerged and developing into mature stalks at some sites in south Queensland.

10.3.4 Ratoon crop yields

At about 210 DAR, between 7-11 stalks/m^2 had been established in untreated plots, and nematode control had induced significant increases ($P<0.05$) at three of 12 sites where these measurements were taken (Table 10.3.3.1). Stalks were significantly longer ($P<0.05$) in nematicide-treated plots at seven of 12 sites (Table 10.3.3.1).

The untreated crops yielded 84-164 T/ha. These yields were usually larger than district averages of crops of the same age. In nematicide-treated plots the ratoon yields were usually larger, with responses >10% observed at about half of the sites. Responses in 1st ratoon crops were more variable than those of the preceding plant crop (Table 10.3.3.2).

10.3.5 Root health

Roots in nematicide-treated plots were often visibly different to those in untreated plots (Plate 10.3.5). Roots from untreated plots were generally poor, consisting largely of black primary roots with few secondary or tertiary (fine feeder) roots. Where fine roots were present they were generally dark in colour. Nematicide-treated root systems had more fine-feeder roots and they tended to be either white or golden brown in colour. Even though root-knot nematode juveniles were routinely extracted from untreated roots, those roots showed no obvious swellings or gross abnormalities. At the 12 sites where root health was rated, it was generally improved where nematicides were applied. Improvements were significant ($P<0.05$) at about half the sites irrespective of the crop being in south or central Queensland (Tables 10.3.5.1 and 10.3.5.2), or plant or ratoon.

1 

2 

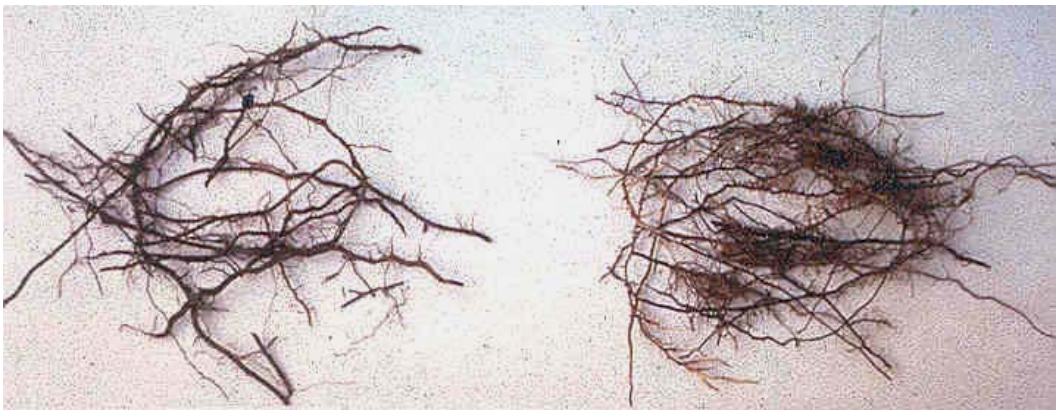
3 

Plate 10.3.5: Visual differences in roots from untreated (left) and nematicide-treated (right) plots at Sites 1, 2 and 3 in south Queensland. Courtesy of G Stirling.

Table 10.3.5.1: Root health ratings for nematicide-treated and untreated sugarcane where root samples were analysed in south Queensland between March and June.

	1. Rocky Point		2. Coolum		3. Maroochydore		4. Yandina	6. Childers	7a. Elliot Heads	7b. Elliot Heads	8. Fairymead	10. Bingera
	Plant	1st R	Plant	1st R	Plant	1st R	1st R	2nd R	Plant	Plant	Plant	Plant
Nematicide	2.78	2.55	1.95	3.00	2.47	2.37	3.35	2.72	2.86	2.60	3.08	3.28
Untreated	2.08	2.16	1.96	2.07	1.65	1.63	2.88	2.24	2.60	2.50	2.57	2.93
LSD (P=0.05)	0.55	ns	ns	0.31	0.27	0.45	ns	0.14	ns	ns	0.23	0.34

Plant = Plant crop, 1st R = 1st ratoon crop, 2nd R = 2nd ratoon crop.

Table 10.3.5.2: Root health ratings for nematicide-treated and untreated sugarcane where root samples were analysed in central Queensland between March and April.

	11. Plane Creek 1st Ratoon crop	13. Mirani Plant crop	14a. Farleigh Plant crop	14b. Farleigh Plant crop
Nematicide	3.72	3.80	3.22	3.38
Untreated	3.53	3.05	2.63	3.05
LSD (P=0.05)	ns	0.20	0.41	ns

10.3.6 Commercial cane sucrose (CCS)

At the sites where CCS was measured, differences due to the nematicides were always minor and significant at only two sites (Table 10.3.6). The site where CCS was significantly lowered by the nematicides was also the site with the largest nematicide response (51%).

Table 10.3.6: Commercial cane sucrose (CCS) from stalks in untreated and nematicide-treated plots at harvest.

Site	Untreated CCS	Nematicide CCS	LSD	% change with nematicide
Plant crops				
1. Rocky Point	15.64	15.96	ns	2
2. Coolum	14.22	14.17	ns	0
3. Maroochydore	14.32	14.27	ns	0
4. Yandina	14.18	14.43	ns	2
5. Maryborough	11.2	10.96	ns	-2
6. Childers	13.28	13.64	ns	3
7a. Elliot Heads	16.81	16.21	0.47	-4
7b. Elliot Heads	16.66	16.63	ns	0
8. Fairymead	16.32	16.34	ns	0
10. Bingera	15.58	15.68	ns	1
Ratoon crops				
1. Rocky Point	15.22	15.79	ns	4
2. Coolum	14.27	14.13	ns	-1
3. Maroochydore	14.89	15.21	0.32	2
4. Yandina	14.79	14.93	ns	1
7a. Elliot Heads	14.83	15.11	ns	2
7b. Elliot Heads	14.57	14.81	ns	2
8. Fairymead	15.68	15.5	ns	0
10. Bingera	13.47	13.74	ns	2

10.3.7 Relationships between nematode density and plant crop response

The total population of all nematode species in the soil at planting (P_i) was not correlated with biomass increases at 100 DAP in nematicide-treated plots (data not shown). However, P_i was related to established stalk ($R^2 = 0.44$) and stalk length ($R^2 = 0.42$) responses at 200 DAP. An influence of EM was found with the SN_2 response ($R^2 = 0.68$, Figures 10.3.7.1 and 10.3.7.2). When mid-season yield was expressed as stalk length/m^2 of plot, the increase with nematicides was correlated with P_i ($R^2 =$

0.70). As a cofactor, EM did not improve this correlation (Figure 10.3.7.3). The response curve showed that a P_i of between 25 and 500 nematodes/200 mL of soil correlated with an increase of between 0 and 7 m of stalk/m^2 of treated plot. Contrary to this trend, P_i did not correlate with harvest yield.

While the nematicides increased SN_2 significantly at some sites, this increase was poorly related to the populations of nematodes controlled inside roots. *Pratylenchus zeae* and *Meloidogyne* spp. densities in roots were correlated ($P<0.05$), but poorly ($R^2 = 0.32, 0.43$) and only with EM as a cofactor (Figures 10.3.7.4 and 10.3.7.5).

Mid-season increases in SL were significantly correlated with the number of lesion nematodes controlled in roots prior to 150 DAP ($R^2 = 0.62$) with little influence of EM ($R^2 = 0.66$). The control of between 16 and 8000 lesion nematodes/g of root correlated with a SL increase of between 0 and 30 cm (Figure 10.3.7.6). This control was also significantly related to increased final yield ($P<0.05$), but was poorly correlated ($R^2 = 0.28$) unless the influence of EM was introduced ($R^2 = 0.62$, Figure 10.3.7.7). Control of root-knot nematode inside roots prior to 150 DAP was never significantly related to any plant responses.

Increased final yields were significantly related ($P<0.05$) to the population densities of *P. zeae* + *Meloidogyne* spp. controlled inside sugarcane roots at 150-200 DAP (mid-season). However, the correlation was poor ($R^2 = 0.32$) until the influence of environment and/or management (EM) was introduced, whereby R^2 increased to 0.69 (Figure 10.3.7.8). A reduction of between 100 and 8000 endoparasites/g of root correlated with a yield increase of between 5 and 20 T/ha, mindful that EM influenced this relationship. Numbers of endoparasites in the soil at 150-200 DAP also correlated with improved final yield ($R^2 = 0.39$), reflecting the trend inside the roots. Inclusion of EM improved this relationship ($R^2 = 0.52$) but not to the extent of root populations (Figure 10.3.7.9). Even though the EM rating was generated using final yields, EM was never singularly correlated ($P>0.05$) with any biomass responses due to the nematicides. The mid-season control of ectoparasites was not correlated ($P>0.05$) with yield increases.

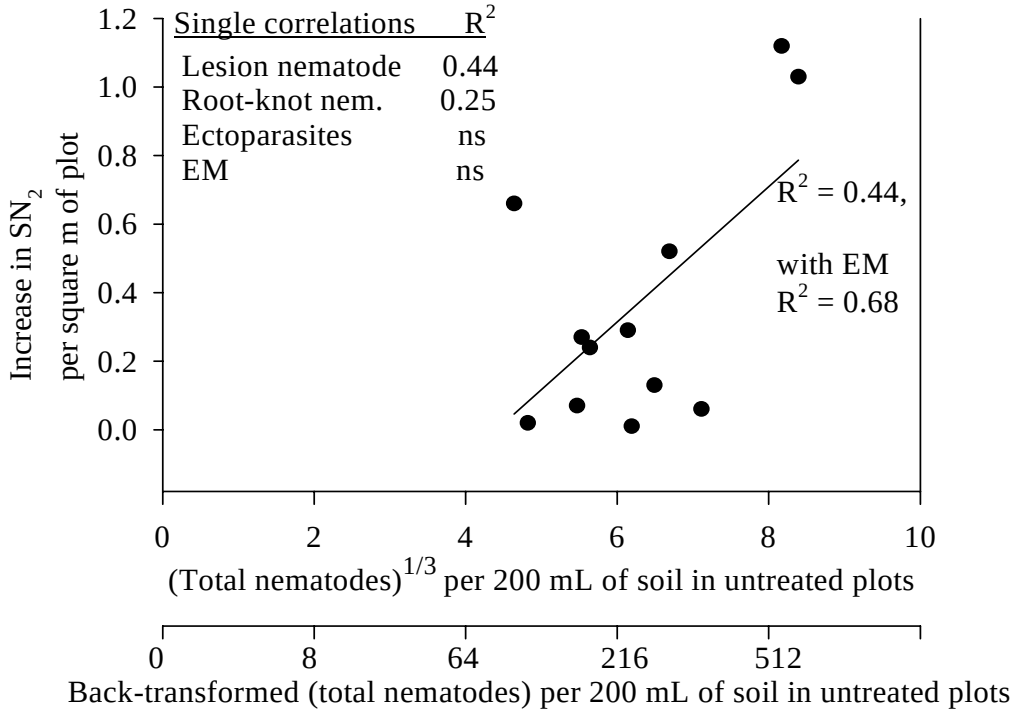


Figure 10.3.7.1: Plant crop increases in established stalks (SN_2) at 200 DAP due to the nematicide, relating to the density of total nematodes (endoparasites + ectoparasites) at planting (Pi), and EM.

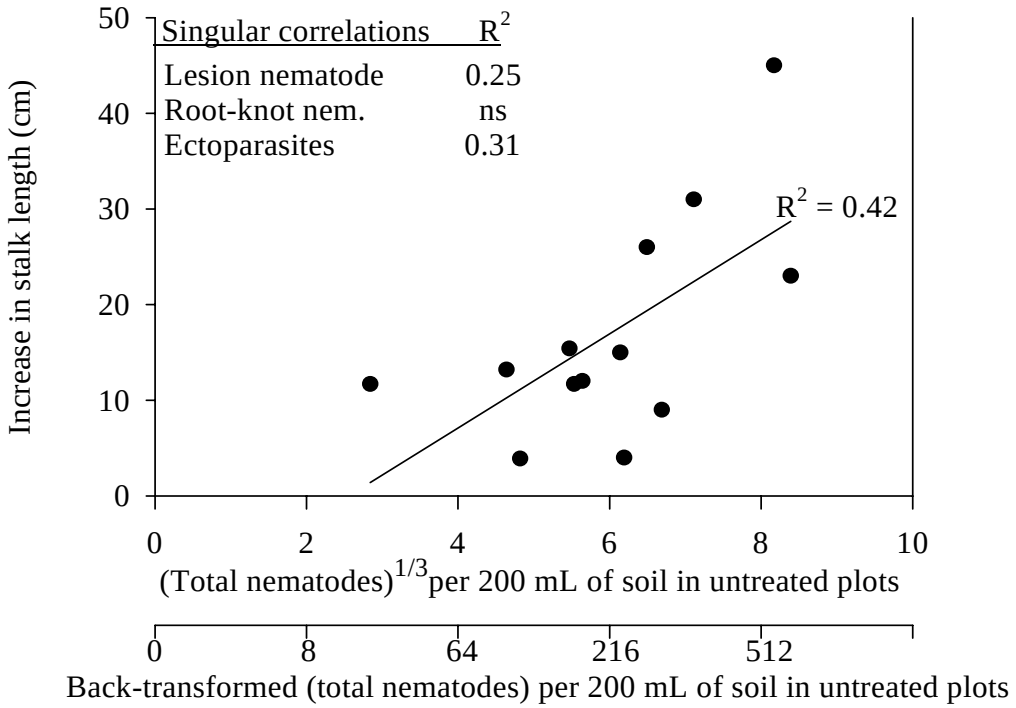


Figure 10.3.7.2: Plant crop increases in stalk length at 200 DAP due to the nematicide, relating to the density of total nematodes (endoparasites + ectoparasites) at planting (Pi).

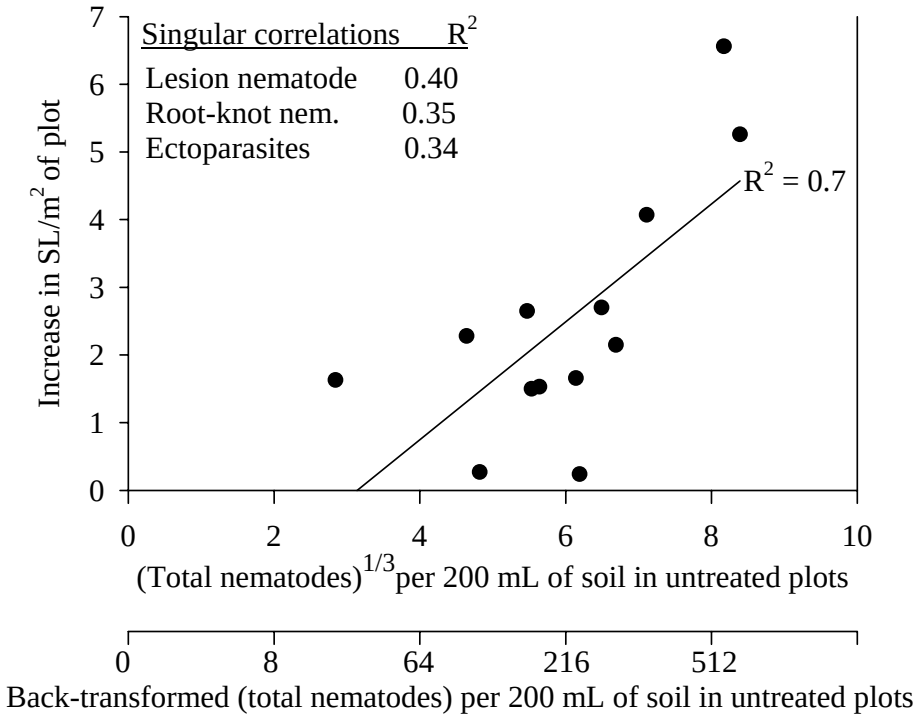


Figure 10.3.7.3: Plant crop increases in stalk length/m^2 of treated plot at 200 DAP, relating to the density of total nematodes (endoparasites + ectoparasites) at planting (Pi).

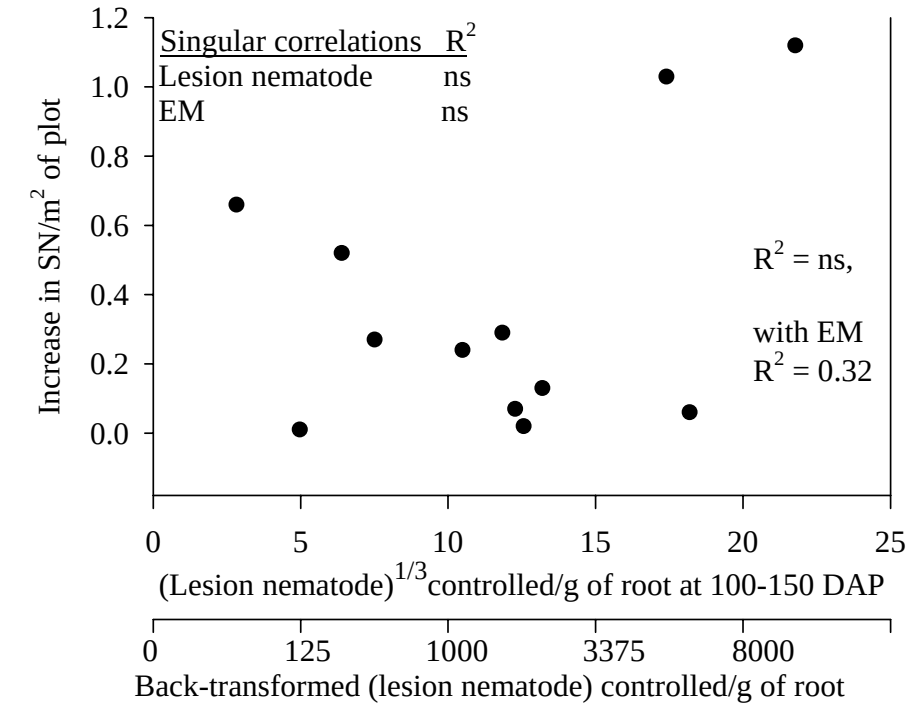


Figure 10.3.7.4: Plant crop increases in established stalks (SN) due to the nematicide, relating to the density of lesion nematode controlled inside roots at 100-150 DAP, and EM.

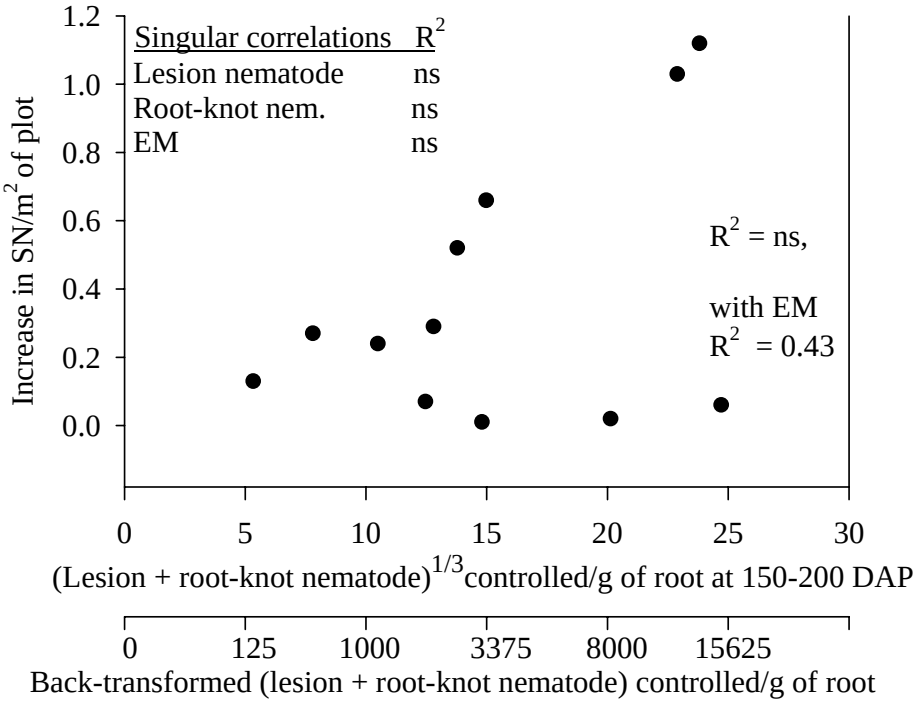


Figure 10.3.7.5: Plant crop increases in established stalks (SN) due to the nematicide, relating to the density of endoparasites controlled inside roots at 150-200 DAP, and EM.

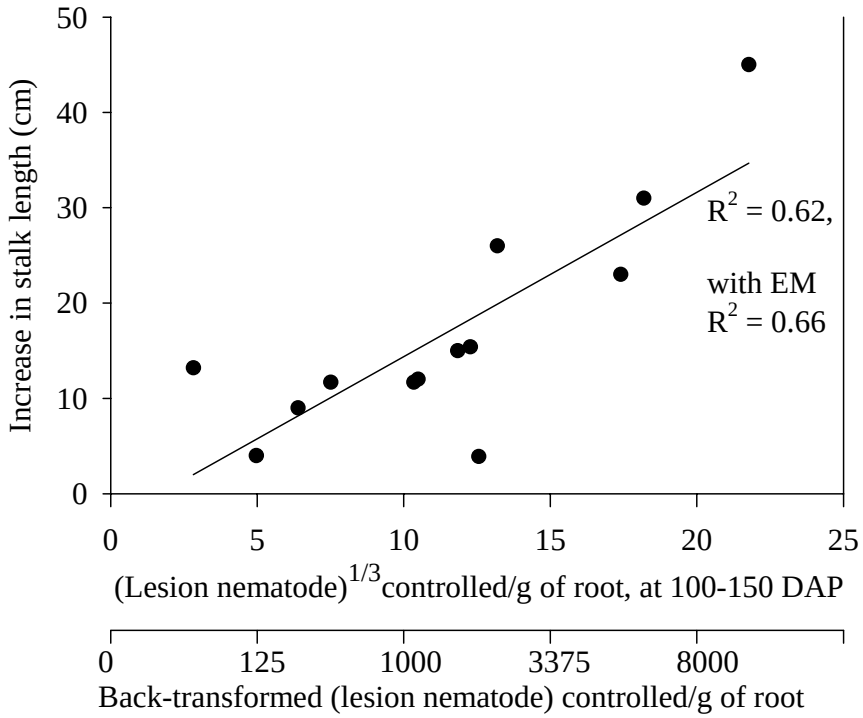


Figure 10.3.7.6: Plant crop increases in stalk length at 200 DAP due to the nematicide, related to the density of lesion nematode controlled inside roots at 100-150 DAP, and EM.

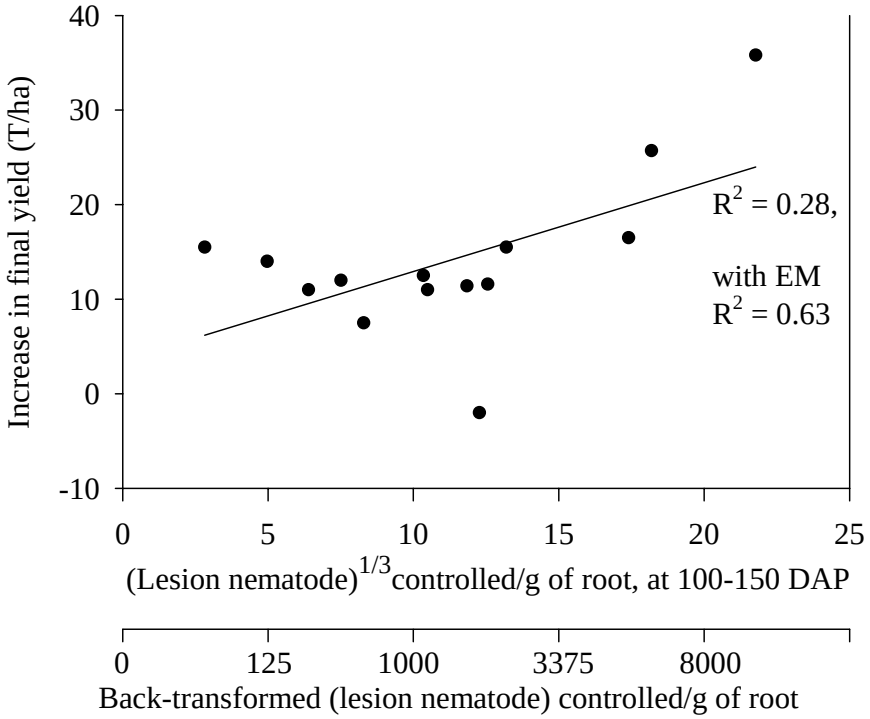


Figure 10.3.7.7: Plant crop increases in final yield due to the nematicide, related to the density of lesion nematode controlled inside roots at 100-150 DAP, and EM.

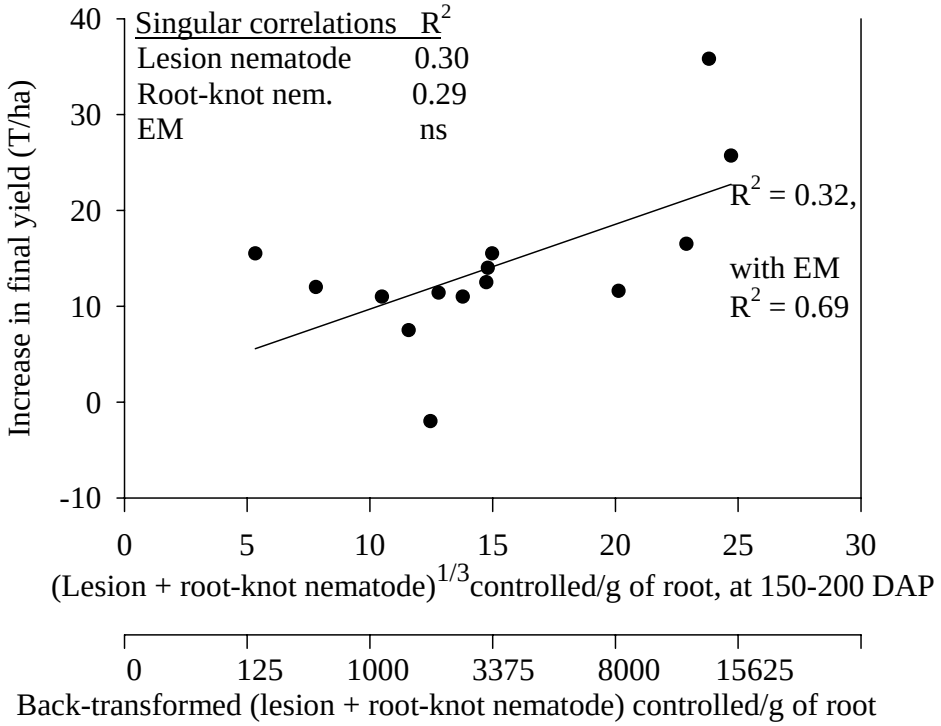


Figure 10.3.7.8: Plant crop increases in final yield due to the nematicide, related to the density of endoparasites controlled inside roots at 150-200 DAP, and EM.

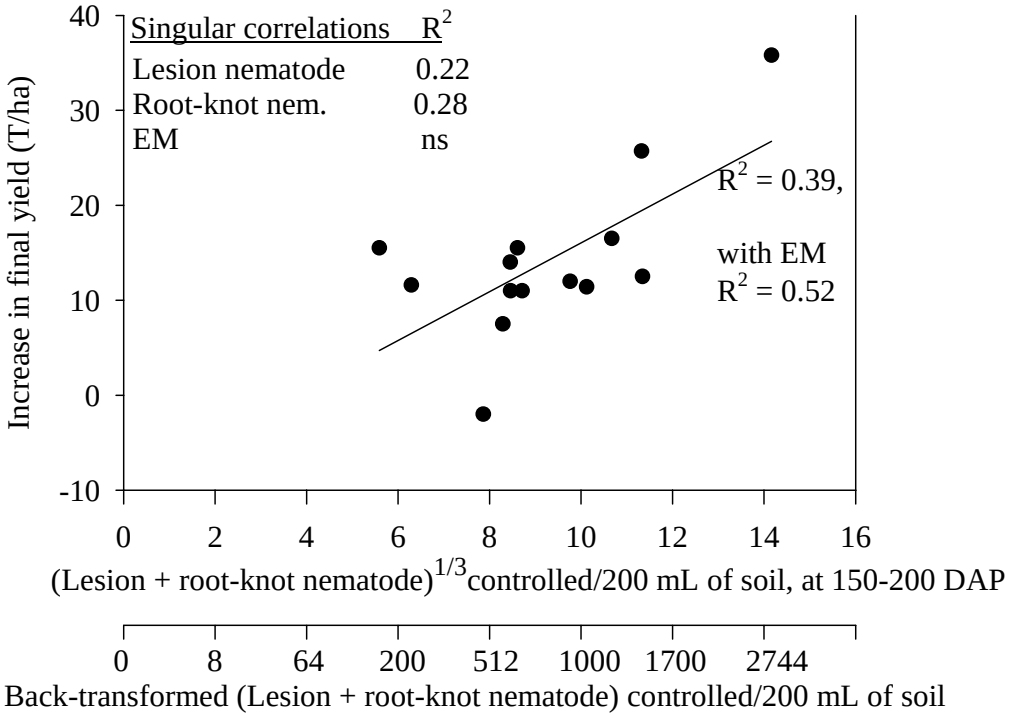


Figure 10.3.7.9: Plant crop increases in final yield due to the nematicide, related to the density of endoparasites controlled in soil at 150-200 DAP, and EM.

10.3.8 Relationship between nematode density and ratoon crop responses

In ratoons, SL was measured at around 210 DAR, and nematicide responses were significantly related ($P<0.05$) to the populations densities of ectoparasites ($R^2 = 0.39$) and endoparasites (*P. zeae* + *Meloidogyne* spp.) controlled in soil ($R^2 = 0.79$) and roots ($R^2 = 0.39$) (Figures 10.3.8.1-10.3.8.3). The control of nematodes was not related to stalk number responses at around 210 DAR, unless stalk length was also considered. Hence, increased SL/m^2 of treated plot was significantly related to the total number of nematodes (endoparasites + ectoparasites) controlled in the soil ($R^2 = 0.7$, Figure 10.3.8.4). The control of lesion nematode provided the best singular correlation with SL responses, while the control of root-knot nematode provided the best singular correlation with SL/m^2 of plot.

In ratoon crops, the numbers of endoparasites (*P. zeae* + *Meloidogyne* spp.) controlled in soil and inside sugarcane roots was significantly related to increases in final yield ($P<0.05$). However, this relationship was poorly correlated ($R^2 = 0.36$,

0.23), and not improved when the rating for EM was introduced. The control of between 200 and 1000 endoparasites/200 mL of soil, or 100 and 2700 endoparasites/g of root, correlated with a yield increase of between 7 and 22 T/ha (Figures 10.3.8.5 and 10.3.8.6). The control of ectoparasites (*T. annulatus*, *H. dihystera*, and *P. minor*) was also significantly ($P<0.05$) but rather poorly related ($R^2 = 0.32$) to increased final yield, and was independent of endoparasites in the soil. The control of between 0 and 512 ectoparasites/200 mL of soil correlated with a yield increase of between 0 and 20 T/ha (Figure 10.3.8.7). Unlike harvest yields in the plant crops, adding EM as a factor did not improve correlations between the density of nematodes controlled and biomass responses in the ratoons.

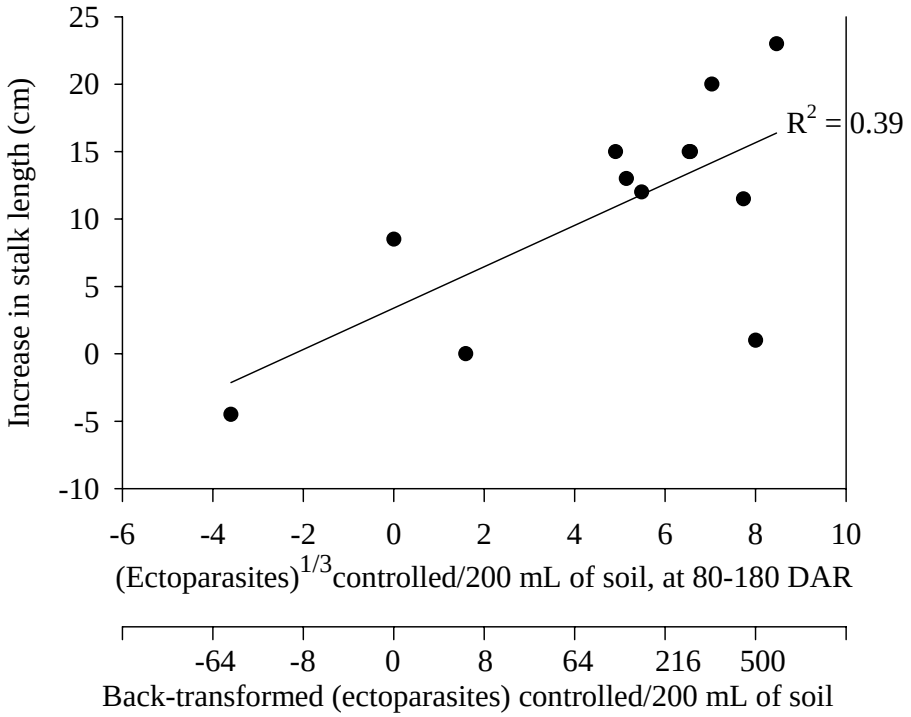


Figure 10.3.8.1: Ratoon crop increases in stalk length around 200 DAR due to the nematicide, related to the density of ectoparasites controlled in soil at 80-180 DAR.

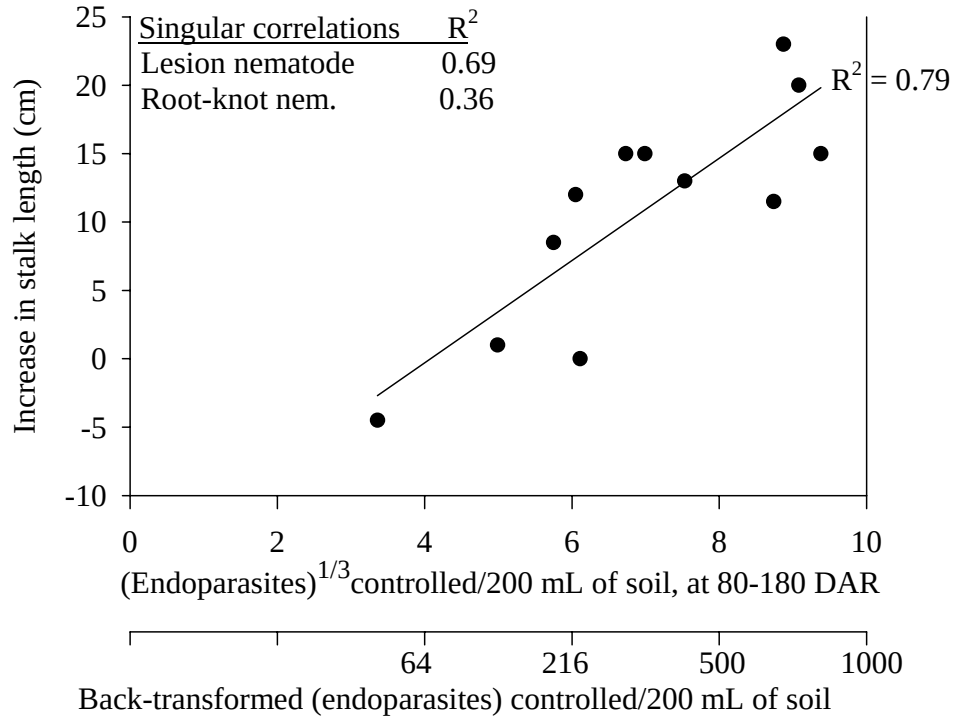


Figure 10.3.8.2: Ratoon crop increases in stalk length around 200 DAR due to the nematicide, related to the density of endoparasites controlled in soil at 80-180 DAR.

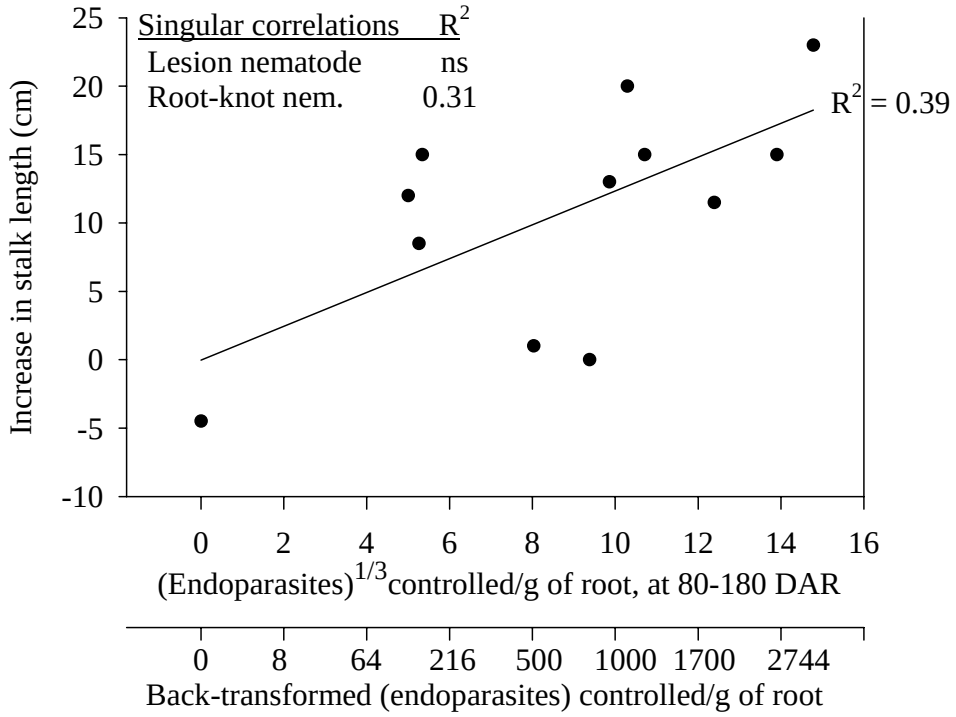


Figure 10.3.8.3: Ratoon crop increases in stalk length around 200 DAR due to the nematicide, related to the density of endoparasites controlled inside roots at 150-200 DAR.

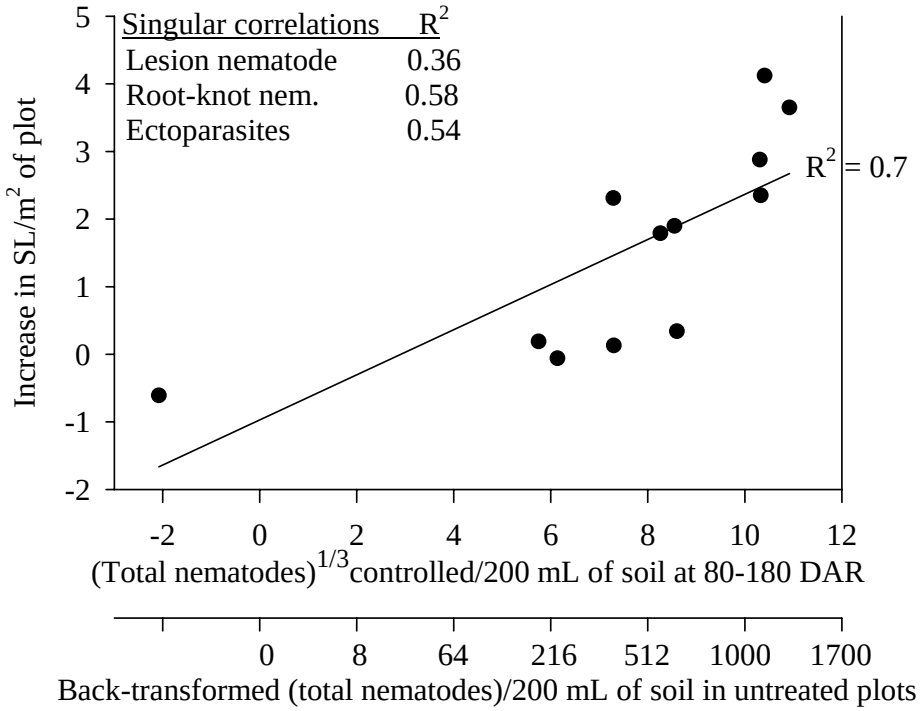


Figure 10.3.8.4: Ratoon crop increases in stalk length/m^2 of treated plot at around 200 DAR, relating to the density of total nematodes (endoparasites + ectoparasites) controlled in soil at 80-180 DAR.

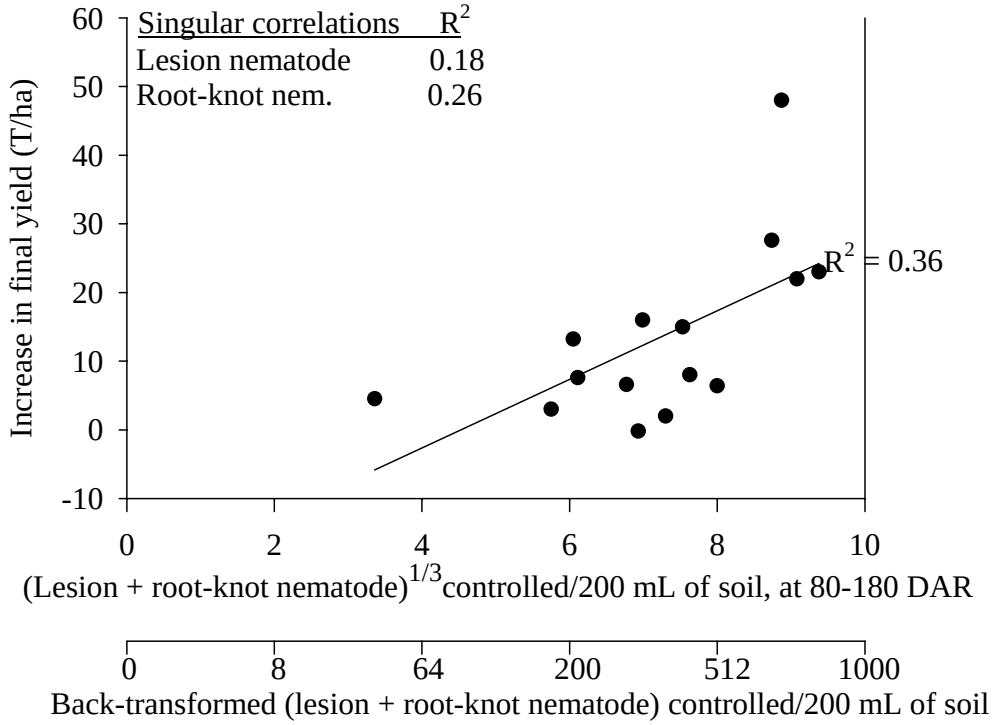


Figure 10.3.8.5: Ratoon crop increases in final yield due to the nematicide, related to the density of endoparasites controlled in soil at 80-180 DAR.

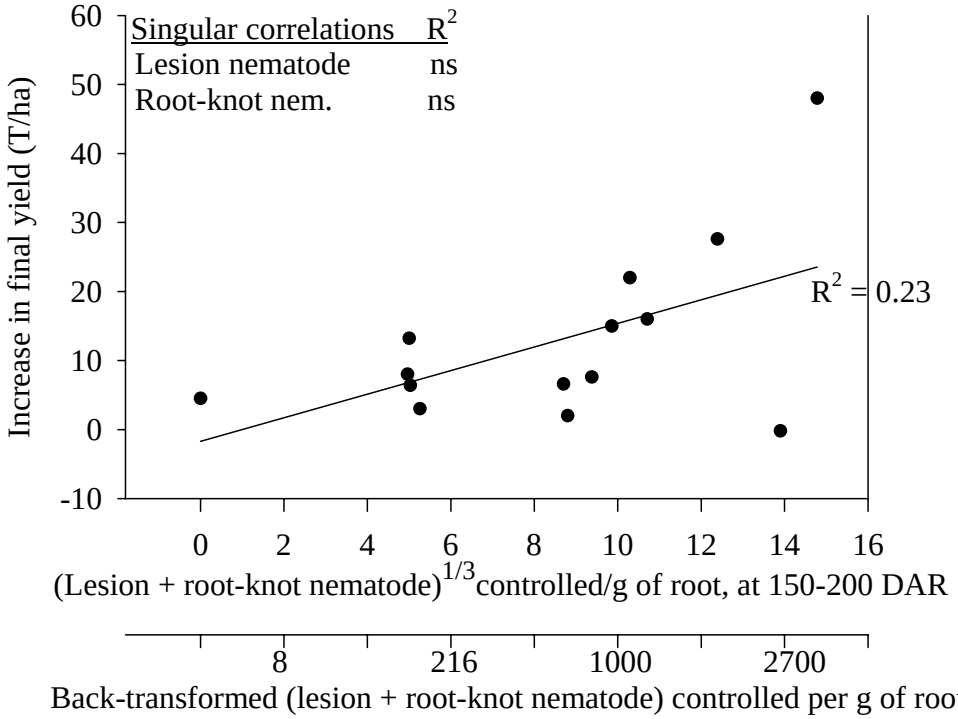


Figure 10.3.8.6: Ratoon crop increases in final yield due to the nematicide, related to the density of endoparasites controlled inside roots at 150-200 DAR.

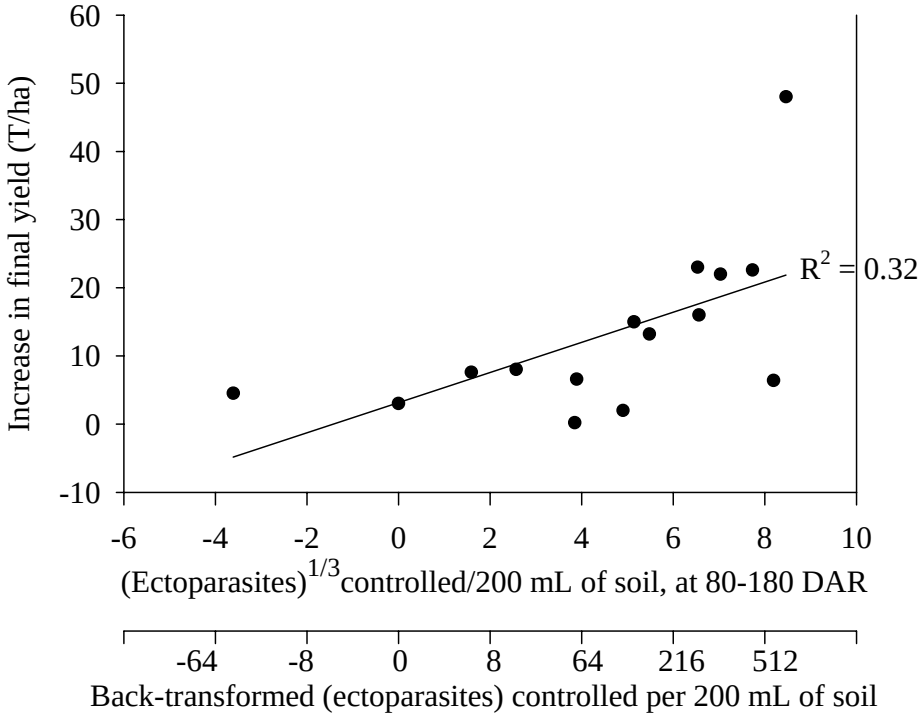


Figure 10.3.8.7: Ratoon crop increases in final yield due to the nematicide, related to the density of ectoparasites controlled in soil at 80-180 DAR.

10.4 Discussion

These experiments represent the first attempt in Australia to assess nematode damage in the sandy loam, clay loam and clay soils (class B soils) on which the bulk of Australia's sugarcane is grown. The approach used in this work also departs from traditional uses of nematicides on sugarcane worldwide to temporarily control nematodes during crop establishment in coarse sandy soils (class A soils). This data demonstrate the difficulties involved in controlling nematodes on sugarcane with non-volatile nematicides. Despite using aldicarb and fenamiphos 4-6 times in two years, good nematode control was obtained only in irrigated situations on sandy loam soils. Although the level of nematode control on non-irrigated clay loam and clay soils was not as good as hoped, it was sufficient to demonstrate that nematodes are causing economic damage in those soils.

10.4.1 Plant crop establishment

Prior findings have shown great benefits in controlling nematodes in the first 100 DAP (Spaull and Cadet 1990). Also, rotation crops and soil fumigation improve the number of tillers emerging in the sugarcane planting that follows, implicating deleterious soil biota (Garside *et al*. 1999). Thus, this early period of crop growth is given particular attention in this discussion. Counts of nematodes found only partial control from 0-100 DAP in the soil zone sampled. However, cultivation practices probably confounded the perceived level of nematode control. During tillering, the row furrow was progressively filled with untreated inter-row soil in between nematicide applications. Deeper in the row profile where sett roots were growing and shoot roots were initiating, nematode control was expected from the prior nematicide treatments. Significant shoot length responses by 120 DAP also suggested nematodes were controlled. In retrospect, freshly deposited surface soil should have been excluded from the samples collected.

Despite this expected control, SN_1 responses were variable and not significant, and did not necessarily coincide with high numbers of nematodes. Lesion nematode predominated at planting, with populations >100 nematodes/200 mL of soil at 10

sites. Other studies in ‘class B’ soils have shown that in such situations, 250-2100 nematodes/g of sett root develop by 60 DAP in glasshouse pots (Pankhurst *et al*. 2001) and the field (Garside *et al.* 1999; Garside *et al.* 2002 a; Blair unpub.). At those sites, lesion nematode was controlled by fallowing, crop rotation and soil fumigation, and increased tiller numbers were routinely established when planted to sugarcane. However, the lack of response in my study indicates that lesion nematode is not a primary cause of poor tiller numbers in sandy loam to clay soils (>10% clay). Pankhurst *et al*. (2001) also found the nematicide response was significantly lower than that from fumigation, in a short-term pot experiment.

At the 16 sites, root-knot nematode counts at planting were also usually low (<13 nematodes/200 mL of soil). While this density can cause root galls and significant yield loss in highly susceptible vegetable crops (Netscher and Sikora 1990), poor tillering of sugarcane is associated with higher nematode numbers (Bull 1981). Prior to 150 DAP in my study, nematicide responses were unrelated to the number of root-knot nematodes controlled inside shoot roots.

In terms of early establishment, findings at the 16 sites depart significantly from those around the world in ‘class A’ soils. Attack by root-knot and lesion nematode on sett roots in infertile soils of 2-3% clay was attributed to failed emergence of the primary shoot (–26 and –34%) and greatly reduced populations of secondary shoots (–82 and –43%) in West and South Africa (Cadet and Spaull 1985). Similarly, in sandy soils in Australia (<10% clay), nematicides applied at the 3-5 leaf stage improved harvest yields between 13-64%. Most nematode damage was observed in the least fertile soils where yields were <60 T/ha (Bull 1979, 1981). The timing of the nematicide application and observed root galls in untreated plots suggested attack by root-knot nematode on newly emerging shoot roots as the cause of the low shoot numbers in those experiments. However, the bulk of those responses occurred in infertile ‘class A’ soils (<10% clay) where nutrient deficiencies and lack of moisture probably exacerbated the impact of nematodes. In the ‘class B’ soils in Queensland where crop rotation and soil fumigation have improved tiller numbers in the following sugarcane crop, factors additional to nematodes appear to be involved.

In the first 120 DAP, nematodes had a greater effect on tiller length, with significant responses at many of the sites. Those responses were maintained through to mid-

season and related to P_i densities of nematodes (R^2 = 0.42) and lesion nematode controlled in shoot roots (R^2 = 0.62). Overall, the responses indicated that nematodes decreased SL at more sites and more significantly than SN_2 in 'class B' soils. Stalk diameter was also decreased less than SL.

10.4.2 Final yield

A loss of tillered shoots that became harvestable stalks was observed during crop growth, as is common for sugarcane (Garside *et al*. 1999). This is attributed to competition for light, water and nutrients at canopy closure. Thus, when high tiller numbers (37/m^2) are established with soil fumigation and high density planting, relatively few (10/m^2) become mature stalks, depending on environmental conditions (Garside *et al.* 2002 b). This dynamic appeared to influence the effects of nematode parasitism reported in this paper. Early tiller number responses to the nematicide were sometimes lost by harvest. At other sites, the control of mid-season populations of endoparasites appeared to reduce competition between maturing stalks, so SN_2 differences emerged by harvest. Increased SN_2 due to the nematicides were related to nematode control only when EM was factored in, suggesting major environmental influences from mid to late season. The 6-10 stalks/m^2 established in untreated plots is standard for the sugar industry and final responses were less than 0.4 stalks/m^2 at 66% of sites. Also, the control of over 3000 endoparasites/g of root had no effect on the number of stalks establishing in some environments.

Mid to late season effects of the environment and/or level of management (EM) on the nematicide responses probably also involved SL. Thus, lesion nematode control was poorly related to final yield responses (R^2 = 0.28) unless EM was considered (R^2 = 0.63). Similarly, when populations of lesion and root-knot nematode reached a maximum between 150-200 DAP, their control inside roots was correlated with responses in harvest yield (R^2 = 0.32), particularly when EM was considered (R^2 = 0.69). More specifically, when environmental conditions and management were favourable for producing a large crop (>130 T/ha), nematodes had no impact on final yield despite the fact that high populations were present. In contrast, nematodes tended to have a more severe impact on final yield in crops growing in a harsh

environment or poorly managed, where untreated yields were <90 T/ha. The reduced impact of nematodes in situations where water and nutrients are abundant has been documented in many crops (McSorley and Phillips 1993). In sugarcane, nematicide responses in South Africa were higher in situations of higher water stress (Donaldson 1985; Donaldson and Turner 1988), while in Australia, crops grown with high inputs of fertiliser and water in favourable environments were less responsive to soil fumigation (Muchow *et al*. 1994 b; Garside *et al*. 2000). In the latter situation, a glasshouse bioassay confirmed that pathogen levels were no different than in other sugarcane soils (Bell *et al*. 2000).

In plant crops of sugarcane much emphasis has been given to biotic factors causing poor early establishment (Garside *et al.* 2002 a; Pankhurst *et al.* 2002), but mid-season growth and development is also important. Stem biomass is mainly accumulated after 125 DAP in spring-planted crops (Muchow *et al*. 1993) and the importance of pathogens such as nematodes should not be ignored. Improved stalk length is a significant component of crop rotation responses and the nematicide responses reported in this paper. Similarly, soil fumigation can increase yield by 28% without increasing the stalk numbers established (Garside *et al*. 1999).

10.4.3 Ratooning

In the ratoon crops, stalk numbers were measured only at around 210 DAR, with significant responses at 25% of sites. The density of nematodes controlled was not solely correlated with stalk number responses. However, when nematicide responses were analysed combining stalk number with stalk length (SL/m^2 of plot), the control of root-knot nematode in soil was prominent in correlations. This suggests a greater effect of root-knot nematode on stalk number than stalk length.

The nematicides had greater effects on stalk length by 210 DAR, and were related to lesion and root-knot nematode controlled in soil (R^2 = 0.79) and roots (R^2 = 0.39) earlier in the season. This finding further implicates lesion and root-knot nematode as the most important nematode pests of sugarcane, especially by reducing stalk length in ‘class B’ soils. In the ratoons, the control of ectoparasites (*T. annulatus, H. dihystera* and *P. minor*) was also related to increases in stalk length and SL/m^2 of

plot. The impacts of these species may be less due to low densities (<500 nematodes/200 mL of soil) and mild pathogenicity on sugarcane (Harris 1974; Spaull and Cadet 1990). Nonetheless, as the crop ages, the whole community may need to be considered when assessing the impact of nematodes.

The decline in vigour of ratoon crops is not attributed to poor stalk numbers, provided that account is made of row gaps due to stool damage (Chapman *et al*. 1993). Thus, the inability of the root system to promote shoot elongation has been implicated in ratoon decline, and attributed to soil compaction and a build-up of soil pathogens. The significant nematicide responses and correlations between stalk length and nematode densities indicate nematodes are contributing to ratoon decline. Anomalies in the correlation between nematodes and harvest yield in plant crops were attributed to the influence of EM. However, a similar situation in ratoon crops could not be related to any single factor, such as EM. In ratoons, stalk number responses were also unrelated to the density of nematodes controlled. An explanation for these observations, in part, may be carry-over responses from the preceding plant crops. Thus, improved ratoon yields are often obtained despite the return of pathogens that were controlled early in the plant crop (Spaull and Cadet 1990; Stirling *et al.* 2001). This response is attributed to the treated crop developing a larger stool, which then confers an advantage in the following ratoon. Thus, the control of nematodes in the ratoon may account for biomass responses only in part, with protection from nematodes in the previous plant crop also contributing. Also, by mid-season in the ratoons, nematodes did not attain the high densities observed in the plant crops. Nevertheless, ratoon roots exhibited the same symptoms of nematode damage as observed in plant crops, and high correlations between the nematode populations controlled and biomass responses, were found mid-season.

Nematode densities on the ratoons from 0-80 DAR were poorly related to mid-season yield responses in general (data not shown). When axillary buds are ratooning, they may source reserves from the stool and be less vulnerable to root pathogens. Nematicides can be delayed up to 60 DAR without reducing yield responses in nematode infested ratoons in South Africa (Rostron 1976; Spaull and Donaldson 1983), suggesting that bud formation is insensitive to nematodes even in acute situations.

10.4.4 Regional crop losses

In plant crops, yield increases of 10-20 T/ha were observed at most sites in response to nematode control. Yield increases ranged from 0-20 T/ha in ratoon crops. Because responses were usually less than 20 T/ha in crops yielding more than 100 T/ha, they were not obvious on visual inspection. This subtlety of damage differs from visibly reduced growth and yield collapses caused by localised attacks of insect pests (Logan 1997; Ward 1997; Allsopp 2001) or acute nematode problems in 'class A' soils (Bull 1979).

The following evidence points to the growth responses in this study being largely due to the control of plant-parasitic nematodes:

(a) Root health improved when nematicides were applied. This improvement was less than is observed with soil fumigation (Chandler 1984; Pankhurst *et al.* 2002), but was consistent at all sites where root health was assessed. The health of roots in untreated plots was indicative of lesion nematode damage (i.e. darkened primary and secondary roots and pruned tertiary roots). Similar symptoms were observed in field microplots inoculated solely with lesion nematode (Chapter 8; Stirling *et al*. 1999 a). This suggests that control of lesion nematode is a major cause of the responses observed in this series of experiments, and is at least partly responsible for the poor health of sugarcane roots in Queensland sugarcane fields. Nematicide responses in the absence of root-knot nematode further implicates root lesion nematode as the major pathogen. Nevertheless, root-knot nematode seems to have contributed to the poor health of roots at some sites despite the fact that root galling was not readily apparent.

(b) Growth processes within the sugarcane plant are not stimulated by aldicarb or fenamiphos independently of nematodes (Chapter 6; Spaull 1995; Stirling *et al*. 1999 b) despite findings to the contrary with aldicarb in some other crops (Barker and Powell 1988).

(c) Stalk number, stalk length and final yield responses were significantly correlated with the number of nematodes controlled in the soil and inside

sugarcane roots. In particular, lesion and root-knot nematodes were correlated with reduced final yields in plant crops, and lesion nematode was correlated with reduced stalk length throughout the crop cycle. These species are demonstrated pathogens of sugarcane (Spaull and Cadet 1990).

(d) There was no evidence of the nematicides controlling organisms other than nematodes. Because aldicarb is systemic, it has the potential to impact on foliar sucking insects such as aphids, scale insects (*Aulacaspis madiunensis*), mealybugs (*Saccharicoccus sacchari*), planthoppers (*Perkinsiella saccharicida*) and froghoppers (*Eoscarta carnifex*). However, these pests do not normally cause crop losses (Agnew 1997) and were not usually detected in untreated plots. When soil and roots were being sampled, root-feeding symphylans (*Hanseniella* spp.) and insect larvae such as canegrubs (*Lepidiota* and *Antitrogus* spp.), wireworm (*Heteroderes* spp.) or ground pearls (*Eumargarodes laingi* and *Promargarodes australis*) were not observed, and damage to roots or shoot bases was not apparent (Agnew 1997). Also, chlorpyrifos had been applied across most experiments to provide protection against canegrubs and wireworm.

(e) Aldicarb reduced populations of soil fungi and bacteria in the sugarcane rhizosphere in the glasshouse, but this action was either temporary or affected organisms considered beneficial for plant growth, such as mycorrhizal fungi and *Pseudomonas* spp. (Pankhurst *et al*. 2001). Fenamiphos does not alter total numbers of bacteria, fungi or actinomycetes on sugarcane in the glasshouse or wheat grown in the field (Thompson *et al*. 1980; Magarey and Bull 1996). In wheat fields, Thompson *et al.* (1980) also found no effect of aldicarb on soil biota other than nematodes. Fungal propagules were not significantly reduced by aldicarb or fenamiphos in a plant and ratoon crop of sugarcane in south Queensland (Stirling *et al.* 1999 b).

The following evidence indicates the 16 sites used in this study represent the wider industry, and that similar responses to nematicides are likely to have been obtained elsewhere:

(a) Yields in untreated plots were comparable to, or greater than average yields in the regions that surrounded them. This demonstrates that the sites were managed by competent growers, and that the environment for growth was not sub-standard. Since high yielding crops tend to have reduced nematode impacts (Figures 10.3.7.1-10.3.7.9), the level of nematode damage within the industry may actually be higher than observed at the 16 experimental sites.

(b) While the 16 sites were situated in south and central Queensland, similar levels of crop loss could be expected in the northern half of the industry as the system of sugarcane farming is similar throughout Queensland. Strategic interventions such as long fallow, rotation crops and soil fumigation increase the growth of sugarcane in north Queensland (the Burdekin, Ingham and Tully) (Garside *et al.* 1999, 2000), implicating the presence of pathogens such as nematodes. More specifically, where lesion and root-knot nematodes were controlled in north Queensland using nematicides under optimal conditions, responses of 15-111% were observed (Chandler 1980).

(c) The environment for crop growth appears to be no better in far-north Queensland than elsewhere, as regional averages range from 63-90 T/ha. (Morgan 2003). In the experiments reported in this paper, nematode damage was minimal in untreated crops yielding more than 130 T/ha. Yield averages of this magnitude are only obtained after very favourable years in plant crops of the Burdekin catchment.

(d) Nematode counts and species composition in untreated soil and roots at the 16 experimental sites were similar to those observed in surveys of 711 sugarcane fields throughout south, central and north Queensland (Blair *et al*. 1999 a, b).

To accurately extrapolate the findings of this study to crop losses throughout the Queensland sugar industry requires details of soil-types, proportions of crop classes and expected yields across the regions. Results from this study indicate average losses of 10 T/ha, thus an estimate of lost productivity from nematodes may exceed 5 million T of sugarcane per year, or a monetary loss of more than A$ 100 million annually ($ 20/T sugarcane). This estimate significantly departs from historical perceptions of nematode damage (Bull 1981). Prior estimates appear to be based on

obvious nematode damage in the small areas of ‘class A’ soils in south Queensland, with no expectation of nematode damage in ‘class B’ soils. Most of Queensland’s sugarcane is grown in sandy loam, clay loam and clay soils and these results show that nematodes are subtle but important pests in those soils.

10.4.5 Other comments

While the EM factor was generated using final yields, it is important to note that EM was never singularly correlated with any yield responses due to the nematicides. Where nematode counts were related to nematicide responses, EM explained major departures from the correlations observed at some sites, and never was the basis of correlations.

Higher yielding crops sometimes have lower CCS levels because active growth delays sugar storage in the stem (Cadet *et al.* 2004). However, nematode control did not affect CCS at the majority of sites. A significant negative effect was observed at Site 7a only, where there was a very large difference (51%) in yield between the nematicide-treated and untreated crop. Thus, the increased crop tonnage achieved by controlling nematodes will usually translate to increased sugar yield as well.

CHAPTER 11

GENERAL DISCUSSION

In Queensland, sugarcane cropping has been based on a monoculture for 80 years or more in most districts. In the last 30 years, plough-out and replant (no fallow) has increased, as has a reliance upon inorganic fertilisers, and intensive tillage to remove soil compaction. While this system of cropping has delivered profitable yields, it is linked to physical, chemical and biological factors in the soil being degraded. The productive capacity of the soil to grow sugarcane has been reduced, a problem that has been termed 'yield decline' (YD). Prior studies indicate that soil pathogens are contributing to YD. For instance, yields are better in virgin soils, and when sugarcane soils are fumigated, the subsequent health and volume of sugarcane roots is improved and routinely increases yield by about 30% (Magarey and Croft 1995). Similarly, lower pathogen levels are associated with fallowing and rotation crops that also increase subsequent sugarcane growth (Garside *et al*. 2001; Meyer and Van Antwerpen 2001). The monoculture provides a continual food source for sugarcane specific pests and pathogens, while the low organic matter levels in the soil and intensive tillage have also reduced microbial diversity and associated mechanisms that naturally suppress pathogens such as nematodes (Stirling *et al*. 2003). Predatory mesofauna have probably also declined with cultivation, as found under wheat crops (Gupta 1994). However, in Australia, there have been few studies to examine the incidence of plant-parasitic nematodes on sugarcane, nor their role in YD.

In Queensland, sugarcane is cultivated in the wet and dry tropics and sub-tropics, and on coastal plains and foothills with soil types that range from coastal sand dunes, through to alluvial loams, cracking clays and kraznozems. Nematode distribution was examined across these climates and soil types by surveying fields of maturing crops. Soil surveys in far-north Queensland (Chapter 3) revealed a high incidence of plant-parasitic nematodes on sugarcane crops. A total of 35 species were detected in this and other surveys (Blair *et al*. 1999 a, b). Collectively, these surveys suggest every sugarcane field in Queensland is populated with lesion nematode (*Pratylenchus zeae*). Root-knot nematode (mainly *Meloidogyne javanica*) is also widespread in sugarcane fields, as are three ectoparasitic species also found in a high proportion of fields (>66%), namely stubby root nematode (*Paratrichodorus minor*),

stunt nematode (*Tylenchorhynchus annulatus*) and spiral nematode (*Helicotylenchus dihystera*).

Geographic location, soil type and soil origin had varying effects on the distribution of each nematode species. In far-north Queensland, lesion nematode densities were significantly lower in poorly drained clays of the Tully River delta, but not in kraznozems (>60% clay) formed *in situ* on upland slopes. In other regions, densities of lesion nematode tended to be lower in soils with more than 35% clay, but this nematode was never absent (Blair *et al*. 1999 a, b). Populations of root-knot nematode were higher in sandy soils. Thus in far-north Queensland, this nematode was detected in 76% of sandy loams in the Mulgrave River delta compared to 19% of fields with more than 30% clay in adjacent districts. In other regions, the densities of root-knot nematode also tended to be higher in sandy soils (<20% clay). Stubby root nematode (*P. minor*) was detected in fewer fields in far-north Queensland than to the south, while the incidence of ring nematode (*Criconemella curvata*) and burrowing nematode (*Achlysiella williamsi*) was higher in the north (Chapter 3; Blair *et al*. 1999 a, b). Historically, the sugar industry has perceived nematode problems to be confined to very sandy soils in south Queensland. However, plant-parasitic nematodes occur in all soils, suggesting a greater role in YD than previously suspected.

There are few published studies (Allsopp 1990) of nematode dispersion within sugarcane fields in Australia, and its implication for field sampling and minimising sampling error. Nematodes were distributed in clumped patterns (aggregations) across the field when point samples were collected a standard distance (20 cm) from the sugarcane stool (Chapter 4). Lesion nematode densities differed up to five-fold in samples as little as 1.4 metres apart. These aggregated dispersions were attributed to localised gradients in root density, and the nematode's limited dispersive ability (<50 cm). Compared to lesion nematode, ring and spiral nematode were even more aggregated, perhaps due to more sedentary feeding habits and greater sensitivity to edaphic gradients (eg. soil texture and moisture) across the field at the macro-distributional level (Hussey *et al*. 1991; Delaville *et al*. 1996).

The implications for field sampling were examined using the 'negative binomial model' to describe nematode aggregation. From this model, the accuracy of estimates

of the mean nematode density in the field was correlated with the number of sub-samples that were taken and combined. In a 30 m^2 plot, 10 and 25 sub-samples estimated the mean densities of lesion and spiral nematodes, respectively, with good precision. However, in large field experiments (>3 ha), gradients in nematode macro-distribution are likely to further vary mean nematode densities between replicated plots across the field. The densities of nematodes on sugarcane require transformation for plot counts to comply with normality in ANOVA comparisons. A transformation of $x^{1/3}$ was found adequate for soil populations of ring and spiral nematodes and lesion nematode in the soil and roots.

In this thesis, studies to describe horizontal dispersion were confined to the top 30 cm of soil. Prior studies in South Africa have found that about 90% of sugarcane roots grow in this zone (Ryker and Edgerton 1931), and Queensland cultivars exhibit a similar growth habit (Reghenzani 1993). Mean nematode densities across the row, near row, and inter-row were very similar during a plant and first ratoon crop in far-north Queensland (Chapter 5). This was attributed to the extensive shallow and fibrous root network established by sugarcane, and the ability of the nematodes present (*P. zeae* and *H. dihystera*) to parasitise those fine-roots. In contrast, Magarey (1999) reported that *Pachymetra* spore loads were 20 times higher in the row, largely because they were associated with attack upon emerging primary roots. Because high densities of *P. zeae* and *H. dihystera* were regularly recovered from near the row (20-30 cm from the stool), this area is recommended as a sampling zone.

In general, nematode densities varied during the crop cycle depending on the volume of the root system and its growth rate, as influenced by season. Because sugarcane develops a new root system annually, nematode densities increased and then declined annually, interrupted only by fallowing between crop cycles. This trend was similar for far-north Queensland (Chapter 5), and central and south Queensland (Chapter 10). General population levels were as follows. At planting, up to 400 lesion nematode and up to 100 spiral nematode/200 mL of soil were present, whereas the densities of other species were usually lower (<50 nematodes/200 mL of soil). Shortly after planting, sett roots emerging from the stem cuttings were colonised by lesion nematode, and between 250 and 5700 lesion nematode/g of sett root can be extracted from the roots of sugarcane in north Queensland (Chapter 9; Garside *et al.*

1999, 2002 a; Blair unpub.). However, static nematode levels were detected in the soil from 0-100 days after planting (DAP) probably because the practice of row filling from 30-100 DAP contaminated samples with inter-row soil containing fewer nematodes. Also, the root system was not yet extensive and this period is associated with dry seasonal conditions, which inhibit crop growth. Low soil moisture probably also inhibited the ability of nematodes to infect emerging roots by inhibiting nematode movement. In contrast, under wet and humid conditions in far-north Queensland, sugarcane planted in late-spring developed very high populations of lesion nematode inside roots and in rhizosphere soil by 70 DAP (Chapter 9).

Rapid increases in nematode populations on plant crops after 100 DAP were associated with large increases in root volume providing an abundant nutrient source. A decline in nematode populations toward harvest was linked to the root system losing vigour and becoming suberised, as well as low soil moisture. In particular, *Meloidogyne* spp. declined markedly nearing crop harvest. Most nematode species increased to higher densities on plant crops than ratoons by mid-season. This was evident by the first ratoon in south Queensland (Chapter 10) and was detected in third ratoon or older crops in survey samples (Blair *et al.* 1999 a). A generally lower impact of nematodes on ratoon yields was found from nematicide experiments (Chapter 10). The reduced vigour and sparser root systems of ratoons may have contributed to lower nematode populations (Glover 1970), or alternatively, the sugarcane cropping system may not favour natural predators of nematodes until late in the crop cycle. Lesion nematode generally attained higher densities inside and around sugarcane roots than other species and, although declining at harvest, prevailed into the next ratoon at higher densities than other species.

Fallowing reduced nematode densities in the soil. Thus, sugarcane planted after a fallow had fewer lesion nematodes than ploughed-out/replanted sugarcane, at least in far-north Queensland (Chapter 3). In this region, grass fallows of weeds tended to support more nematodes than well managed legume fallows, probably because legumes are poor hosts of most sugarcane nematodes. A 240 day bare fallow induced with herbicide (untilled) was as effective as a tilled bare-fallow, reducing lesion nematode numbers by about 80%. Spiral nematode was not affected greatly by tillage and fallowing operations, which could account for densities at planting sometimes rivalling those of lesion nematode (Chapters 5 and 10).

To summarise, nematode populations and dynamics were found to be related to root habit, root distribution across the row to inter-row profile and root vigour during the crop cycle.

As a consequence of descriptive studies in Chapters 3, 4 and 5 of this thesis, preliminary experiments were conducted to assess nematode pathogenicity (Chapter 6), and particularly that of *P. zeae* (Chapter 7). The relative effects of nematodes were examined in two field soils, by comparing sugarcane growth in pasteurised and nematicide-treated soil in glasshouse pots for 60 DAP (Chapter 6). Soil pasteurisation (70 °C, 90 minutes) improved sugarcane root and shoot growth by about 100%, and the number of shoots were increased by 100-200%, illustrating that typical YD soils were used in these experiments (Magarey *et al*. 1995). In fenamiphos-treated soil, the root and shoot growth was stimulated less, and more variably. Nonetheless, indigenous populations of *P. zeae* and *P. minor* were suppressed and responses were usually significant, suggesting part of the response to pasteurisation was due to the control of nematodes. The growth of plants was increased more by nematicides in these experiments than has been found previously (Magarey and Bull 1996). In the past, soils from the Tully River delta were used, which have low nematode densities (Chapter 3). Also, the watering system used, and rate of fenamiphos used (40 mg/L of soil), may have inhibited the multiplication of nematodes and had a slight phytotoxic effect respectively, contributing to lower nematicide responses. In the pot experiments reported in this thesis (Chapter 6), nematicides did not stimulate sugarcane growth in the absence of nematodes (using pasteurisation), supporting findings by others (Spaull 1995; Stirling *et al*. 1999 b). Thus, yield responses from nematicides are suitable as a research tool to quantify nematode damage to sugarcane crops (Chapter 10).

The pathogenicity of *P. zeae* to a cultivar (Q 114) of Australian sugarcane was demonstrated in 1.5 L pots, as root weight was reduced. Lesions were visible on new shoot roots, and roots were darker in colour than the healthy roots in sterile soil. Inoculated plants developed fewer secondary shoots and shorter primary shoots, but only when high nematode densities were added directly to the root system. Pathogenicity was less pronounced when nematodes were mixed with the soil beforehand, perhaps because this process killed many of the nematodes. Even when cultured nematodes were inoculated by ‘watering-in’, less than 20% were active 1-2

weeks later according to recovery methods based on nematode movement (Chapters 8 and 9).

In the glasshouse experiments (Chapters 6 and 7) primary shoots grew quite variably from stem nodes despite efforts to select plants of even size. Thus, the mean weight of control plants sometimes differed by up to 50% from plants inoculated with nematodes or treated with nematicide, but were not significantly different according to ANOVA comparisons. Future experiments of this nature require greater replication if ANOVA comparisons are used to determine significant effects. Similarly, larger miniplots (Chapter 9) are needed to reduce yield variability in same-treatment plots and/or more replicates are needed, to show subtle treatment effects as significant according to ANOVA comparisons.

The relationship between nematodes and sugarcane is certain to be more complex in the field than found in pots because the crop is a relatively large perennial that develops an extensive root system. To simulate these conditions, *P. zeae* was established in 50 L field pots, and sugarcane (cultivar Q124) was grown for 140 days (Chapter 8). Nematodes were established at densities (P_i) that are typical in sugarcane fields prior to planting. Although root weight was not significantly reduced, the root systems in infected pots had an overall darkened appearance. Tertiary (fine) root length was reduced by over 50%, which is an important observation, as fine root growth is poor in YD soils (Croft and Magarey 1984). Shoot biomass was not markedly affected, perhaps because plants were grown with an excess of water and fertiliser and lesion nematode was the sole pathogen present.

Buds and associated sett roots were activated in sterile soil in the above pot experiments (Chapters 6, 7 and 8) to select for evenly sized test plants. Past researchers have also used this system (Magarey and Grace 1998). Thus, the role of YD biota and nematodes on bud shooting, and its association with sett roots, has not been researched in Australia. Shoot establishment and root production on sugarcane is an involved process. The stem cutting produces sett roots in concert with shooting buds, which become primary shoots. The growth and decline of sett roots overlaps the emergence of secondary shoots after 30 DAP, and the growth of shoot roots, which eventually become dominant. Thus, in two field experiments (Chapter 9) the

dynamics of early plant establishment was examined by physically pruning sett roots and manipulating detrimental soil biota with soil biocides and nematode inoculants.

Only severe sett root pruning inhibited the number of buds activated, and primary shoot establishing in one experiment, with no effect in the other experiment. Thus, sett roots were less important than found previously overseas, and suggested from field studies in Queensland (Garside *et al.* 2002 a; Pankhurst *et al.* 2002). It was concluded that in the period before the developing primary shoot produces functional shoot roots, germinating buds rely upon sett roots in some situations, whereas they are supported entirely by the stem cutting in other situations. Old stem cuttings from the bottom of the stem had many dormant buds, and more than 40% of the sett root system had to be intact to prevent very poor shooting of these buds, perhaps by breaking dormancy. Plant crops are vulnerable to poor primary shoot establishment (row gaps) from a combination of damaged buds, dormant buds and poor sett root growth in YD soils. It is desirable to control deleterious soil biota to increase sett root health and volume, especially where poorly nurtured or aged planting material is used.

Deleterious soil biota and nematodes also reduced the volume and health of shoot roots, which reduced tillering of the secondary shoots. Primary and secondary shoots were smaller, suggesting that even when vigorous cultivars (eg. Q138) establish an over-abundance of shoots in YD soils, final yield may still be reduced by pathogens. The sugarcane field used for these experiments had a consistent history of fumigation responses (>80%), whereas the variable nematicide responses (0-50%) indicate large temporal changes in the impact of nematodes on early establishment (Chapter 9; Pankhurst *et al.* 2002, 2004 b).

To further explore nematode impacts on crop yield, a series of field experiments were conducted using nematicides. The vast majority of these types of experiments on sugarcane crops throughout the world have focused on testing the economics of nematicide use. A review by Spaull and Cadet (1990) identified nematicides as economic only in sandy soils (<10% clay), where they protect against chronic nematode damage at planting, or during tillering of the ratoon. At these sites, root-knot nematode was usually the most prevalent nematode pest (Bull 1979, 1981). Also, untreated crops yielded less than 80 T/ha, suggesting that sub-optimal growing

environments perhaps exacerbated nematode damage. Nematicide experiments reported in this thesis (Chapter 10) aimed to control nematodes more thoroughly to assess their role in YD. To that end, economics were ignored, and nematicides were used as a research tool to control nematodes for the entire crop cycle. Secondly, very sandy soils were avoided, and the 16 sites chosen represented a range of environments in the more fertile sandy loam to clay soils on which 95% of Australia's sugarcane is grown. Throughout the world, the extent of losses from nematodes in these soils has been based on speculation (Sasser and Freckmann 1987).

When nematodes were controlled, stalk length was increased more consistently and significantly than stalk number at 120 DAP across the sites. Low densities of root-knot nematode at planting, and better soil fertility, may have contributed to lower stalk number responses than observed in sandy soils in Queensland and around the world (Bull 1981; Spaull and Cadet 1990). These findings, and those previously (Chapter 9), indicate varied impacts of nematodes on shoot numbers soon after planting (0-100 DAP). In some environments, the control of over 3000 endoparasites/g of root had no effect on the numbers of stalks established. By 200 DAP, there were significant stalk length responses at most sites. Correlations suggested that lesion, root-knot and ectoparasitic nematodes in the soil at planting (P_i) all contributed to stalk length and stalk number losses. However, P_i was not correlated with final yield losses. In contrast, the control of lesion nematode inside roots was strongly correlated with stalk length responses at 200 DAP and final yield increases. Untreated roots usually had 1000-15000 lesion nematode/g (dry weight) after 150 DAP both in plant and ratoon crops. In field microplots (Chapter 8), the incidence of lesion nematode was more consistently correlated with shorter primary shoots than other biomass measurement, above ground.

More than 1000 root-knot nematode/g of shoot root developed at five sites after 150 DAP, and the control of this nematode also appeared to contribute to nematicide responses (Chapter 10). However, the level of crop management and/or environmental factors (EM) determined whether nematode parasitism translated to significant final yield losses. Thus, nematodes had a more severe impact on plant crops that were growing in harsh environments or were poorly managed (i.e.

untreated yields were <90 T/ha). This factor seemed to influence the nematicide response mainly after 200 DAP according to correlations between nematodes, yield and EM. Garside *et al.* (1999) also reported that crop rotation, fallowing and soil fumigation effects on the subsequent sugarcane crop changed between 240 DAP and harvest.

In ratoon crops, reduced populations of nematodes were also linked to increased stalk length rather than stalk number by 210 days (DAR). The control of root-knot nematode was correlated more strongly with stalk length and stalk number increases in ratoons, than on plant crops. Similarly, the control of ectoparasites correlated more strongly with yield responses in ratoons, than in plant crops. This suggests as the crop ages, the whole community may need to be considered when assessing the impact of nematodes. Nematode densities on ratoons correlated poorly with stalk number and final yield, perhaps due to persisting and confounding responses from the preceding plant crops. Improved ratoon yields are often obtained despite the return of pathogens that were controlled early in the plant crop (Spaull and Cadet 1990, Stirling *et al.* 2001). Thus, the nematodes controlled on the ratoons may account for biomass responses only in part, with improved root stooling from the previously treated plant crop also contributing.

In plant crops, yield increases of 10-20 T/ha were observed at most sites in response to nematode control. In ratoons, yield increases ranged from 0-20 T/ha (Chapter 10). Similar responses could be expected across other fields in the sugar industry, given the large number of experimental sites and the range of environments they represented. Also, yields in untreated plots were average or above for the districts that surrounded them. In addition, nematode densities and species composition at the 16 sites were comparable to those in surveys of 711 sugarcane fields throughout south, central and north Queensland (Chapter 3, Blair *et al.* 1999 a, b).

The following observations indicate that nematicide responses were attributable solely to nematode control. Nematicides can potentially control insects, but foliar sucking insects were not detected in untreated plots, or were regarded as insignificant pests (Agnew 1997). When soil and roots were being sampled, root-feeding symphylans (*Hanseniella* spp.) and insect larvae such as canegrubs (*Lepidiota* and *Antitrogus* spp.), wireworm (*Heteroderes* spp.) or ground pearls (*Eumargarodes*

laingi and *Promargarodes australis*) were not observed, and damage to roots or shoot bases was not apparent. In untreated soil, the poor growth of fine roots and darkened primary roots were not symptomatic of insect pests, but were similar to roots inoculated with lesion nematode (Chapters 7 and 8). Total numbers of soil biota, such as bacteria, actinomycetes and fungi, are not reduced by aldicarb or fenamiphos in field situations (Thompson *et al.* 1980; Stirling *et al.* 1999 b). In the absence of nematodes, aldicarb and fenamiphos did not significantly stimulate sugarcane growth in glasshouse pots (Chapter 6; Spaull 1995; Stirling *et al*. 1999 b).

Presuming these results can be applied across the major sugarcane growing regions of Queensland, lost productivity from nematodes is conservatively estimated at over 5 million tonnes of sugarcane per year, or a monetary loss of more than A$ 100 million annually. This estimate is much greater than earlier perceptions of nematode damage (Bull 1981). Prior estimates appear to be based on minor areas in south Queensland where nematode damage is obvious in sandy soils. There was no expectation of nematode damage in more fertile soils. Since most of Queensland's sugarcane is grown in sandy loam to clay soils, these results show that nematodes are subtle but important pests throughout the sugar industry.

Findings from experiments in this thesis point to synergistic effects between nematodes and other root pathogens, such as fungi (Magarey *et al.* 1997 a), in reducing the shoot biomass of sugarcane. When nematodes were selectively removed from YD soil using nematicides, the root and shoot biomass of sugarcane was improved in proportion (Chapters 6, 9 and 10), so shoot/root ratios remained the same. In contrast, plants in fumigated soil tended to develop higher shoot/root ratios, particularly in the field environment (Chapter 9), pointing to root function as well as root biomass being improved when all root pathogens are controlled. When inoculated as a single pathogen, lesion nematode caused a general blackening of roots, reduced fine root length (Chapter 8) and reduced the weight of sett and shoot roots (Chapters 7 and 9). However, in response to this root damage, a comparable loss of shoot biomass was generally not found, so this treatment had the highest shoot/root ratios (Chapters 7, 8 and 9). Thus, during biomass accumulation by the plant, there is some insensitivity to the root damage caused by *P. zeae* when present as an isolated parasite of the root system.

Nonetheless, the pathogenicity of *P. zeae* to sugarcane roots was demonstrated. *Pratylenchus zeae* generally persisted at higher densities than other pest species on plant and ratoon crops, and was present in all sugarcane fields. Yield responses to nematicides were consistently related to the populations of *P. zeae* that were controlled, as was the increased root health and fine root growth in nematicide-treated plots (Chapter 10), indicative of *P. zeae* control. These findings indicate that lesion nematode is the most important nematode pest on sugarcane in Queensland, and is contributing significantly to YD.

A limited number of cultivars were used in the experiments reported here, and sugarcane cv. Q124 was the major cultivar planted in field experiments to assess crop losses from nematodes (Chapter 10). However, it is expected that findings from these experiments generally represent the nematode relationship with sugarcane in Queensland. Stirling *et al.* (1999 c) found few differences in the multiplication of *P. zeae* on nine cultivars grown widely in Queensland. A similar result was found with *Meloidogyne javanica* on six cultivars. These cultivars reflect genetic diversity from 16 different parents with breeding origins in South Africa, India, southern USA, Hawaii and Queensland. From nematode surveys of sugarcane fields in south Queensland, no difference was found in densities of the most common nematodes (*P. zeae*, *Meloidogyne* spp., *Tylenchorhynchus annulatus* and *Paratrichodorus* spp.) on different Q cultivars (Blair *et al.* 1999 a). To iterate, findings in this thesis were expected to apply equally to the range of Q cultivars available to growers.

Resistance of sugarcane to *P. zeae* and *M. javanica* has been reported in other countries (Spaull and Cadet 1990; Dinardo-Miranda *et al.* 1996), and the sourcing of overseas germplasm is possible. However, a committed breeding and testing program would be required to develop nematode resistance in Queensland-bred clones. Imported clones with resistance to the major nematode pests (*P. zeae* and *M. javanica*) are likely to possess other traits unsuited to the agricultural environment in Queensland. These traits need to be selected against, while retaining nematode resistance and the local traits that have established Q cultivars as economic in the Queensland agricultural environment.

CHAPTER 12

COLLABORATED RESEARCH RELATING TO NEMATODES

The ‘sugarcane yield decline joint venture’ (YDJV) was formed to unite expertise in soil physics, chemistry, microbiology and sugarcane agronomy. Observations and experiments were implemented to (a) identify sub-optimalities associated with continuous sugarcane cropping, and (b) examine strategies to improve the profitability of sugarcane farming systems. The thesis author collaborated in this research and findings relating to nematodes are summarised below.

12.1 General physical, chemical and biological sub-optimalities associated with yield decline (YD)

Soil properties associated with YD were examined by comparing fields under long-term sugarcane cropping with adjacent virgin plots that had never grown sugarcane and were usually grass pastures. Because sugarcane yields are greater in virgin soils, it suggests the capacity of YD soils to grow sugarcane has degraded over time. Compared to virgin soils, the physical and chemical descriptions of YD soils were generally (a) higher bulk density, (b) less available water, (c) less organic carbon in surface horizons, (d) lower pH, (e) lower CEC, (f) more available Al and Mn, and (g) less available Cu and Zn (Garside *et al*. 1997 b). Compared to virgin soils, YD soils had (a) lower microbial biomass, (b) lower numbers of actinomycete fungi, (c) lower numbers of *Pseudomonas* spp., (d) higher numbers of fungi, and (e) higher numbers of *Pachymetra chaunorhiza* spores. Populations of lesion nematode (*Pratylenchus zeae*) inside roots were generally no different.

Soil fumigation increased sugarcane growth more in YD soils, suggesting larger numbers of root pathogens. However, there was some response to fumigation when sugarcane was grown in virgin soils, suggesting some pathogens, such as lesion nematode, were being hosted by the grass pastures (Magarey *et al.* 1997 b).

The pathogenic aspect of YD and root infection was examined by exposing node cuttings to YD soils for 6, 10, 14 and 21 DAP, whereupon plants and sett roots were washed and transplanted into fumigated sand for further grown and expression of

YD. By 14 DAP, enough sett roots had been infected to subsequently infect shoot roots and reduce plant growth (Pankhurst *et al*. 2004 a).

12.2 Effect of chemical biocides and breaks from the sugarcane monoculture on soil biota and sugarcane yield

Chemical biocides and breaks from the sugarcane monoculture were used experimentally in YD soils to manipulate specific soil components and reveal their importance in YD. These studies showed that nematodes (particularly *P. zeae*) contribute to YD, but also showed that in some fields, nematode parasitism is minor compared to other YD biota. At a field site in far-north Queensland, soil fumigation significantly increased sett and shoot root weight, secondary shoot numbers and shoot biomass by 64 DAP (Pankhurst *et al*. 2004 a). Nematodes were controlled in both fumigated and nematicide-treated soil, but nematicide responses were quite poor in comparison. In contrast, a soil fungicide significantly improved root and shoot growth, indicating a major part of the fumigation response was due to the control of pathogenic fungi. *Pachymetra chaunorhiza* was not important in this experiment because a moderately resistant cultivar (Q117) of sugarcane was grown. Dematiaceous (dark sterile) hypomycetes have been implicated in the poor root health associated with YD (Magarey and Bull 2003), and may have been controlled in this case.

The relative responses to soil fumigation, fungicide and nematicide at 64 DAP were maintained to harvest at 365 DAP (Garside *et al*. 2002 a). Detrimental fungi and nematodes in association, appeared to be responsible for the poor root growth in continuous sugarcane soil because the yield response in ‘fungicide + nematicide’ plots was equivalent to soil fumigation. At this site, bare fallow, alternate crops and legume/grass pasture were implemented for 54 months, thereby introducing different plant species, levels of tillage and organic matter retention. The different crop breaks, soil fumigation and ‘fungicide + nematicide’ increased yield by similar amounts (30-48%) compared to untreated sugarcane grown continuously. However, physical, chemical and biotic components in the soil were affected differently. This suggested complex dynamics between soil fertility, soil biota and yield accumulation (Pankhurst *et al*. 2002). Populations of fungi and nematodes were reduced by rotation crops and pasture, but did not account fully for subsequent sugarcane

responses, indicating that abiotic factors in the soil were improved, such as soil nutrition.

Breaks in the monoculture (bare fallow, crop and legume/grass pasture) of 30-42 months duration, significantly increased yields in the subsequent sugarcane crop at four other sites throughout Queensland (Garside *et al*. 1999). In general, compared to continuous sugarcane, bare fallow reduced populations of both pathogenic and beneficial biota, and diminished the capacity of the soil to utilise carbon substrates. Crop and pasture reduced numbers of lesion nematode and *Pachymetra chaunorhiza* spores, and increased numbers of free-living nematodes, culturable fungi, mycorrhizal fungi and *Pseudomonas* spp. (Pankhurst *et al*. 1999, 2000). Pasture increased the capacity of the soil to utilise carbon substrates. Fumigation responses (14-38%) in pasture and crop soils suggested some detrimental soil biota were still being maintained in these systems (Pankhurst *et al*. 2004 b).

Ratoon yields were also improved following the break crops (Garside *et al*. 2001), probably because the plant crop developed a larger and more vigorous stool. The control of plant-parasitic nematodes, and increased numbers of free-living nematodes, *Pseudomonas* spp. and fungi, did not persist on ratoon crops (Pankhurst *et al*. 2004 b). However, there was no ratoon response in plots of continuous sugarcane soil that had been fumigated, suggesting crop and pasture breaks may induce changes in abiotic factors that endure into ratoons.

Compared to continuous sugarcane, short rotations with non-host legume crops (soybean, peanut) were beneficial in the short-term by reducing populations of plant-parasitic nematodes. Lesion nematode was reduced in number by 44-88% at planting at six field sites (Stirling *et al*. 2002). In some cases, a long-term crop (30 months) did not significantly increase sugarcane yield any more than a short-term crop (nine months) (Garside *et al*. 1999). Thus, even short breaks in the sugarcane monoculture can impact on YD.

12.3 Effect of crop history and organic matter on the suppression of YD biota

The sugarcane monoculture and associated farming system has reduced organic carbon levels and soil biodiversity. In turn there has been a decline in biotic mechanisms that naturally suppress soil pathogens such as nematodes (Pankhurst *et*

al. 2003 b). In sugarcane soils, pest species of nematodes are abundant, with fewer groups of saprophytic (free-living) nematodes that are indicative of a diverse and balanced microbial community (Stirling *et al*. 2001). The effect of different cropping histories and organic amendments on the suppression of YD biota, was examined.

Sand was infected with YD biota by adding chopped sugarcane roots (2% by weight) sourced from a field under long-term sugarcane monoculture. Sugarcane seedlings grown in the sand subsequently developed symptoms of YD. The ability of test soils (10% by weight added to the sand) to block the transfer of YD symptoms, was a measure of suppressive capability (Pankhurst *et al*. 2003 a). A sugarcane soil that had been under long-term pasture (seven years) was highly suppressive to YD biota. This was associated with accumulated and conserved organic matter (no tillage) stimulating high biodiversity and increasing microbial biomass by 50% compared to continuous sugarcane soil (Pankhurst *et al*. 2005). In contrast, the soil from tilled soybean fallows of short duration (nine months) did not become more suppressive than continuous sugarcane soil. The microbial biomass and suppression stimulated by pasture was very specific to the YD biota associated with sugarcane. Thus, the suppression of YD biota was low in a crop and rainforest soil that had no history of sugarcane production, and this was despite the rainforest soil having a high microbial biomass.

When 10-20 T/ha of organic matter from legume, grass, timber and animal sources were added to sugarcane soil and left to decompose, suppression developed at one and seven months, but had dissipated by 12 months. However, this suppression was significant only at seven months in soils amended with poultry manure and chitin. These experiments suggested a more proactive conservation of organic matter is needed to stimulate suppression in sugarcane soils. These amendments stimulated microbial biomass by only 10%, compared to over 50% in suppressive pasture soil. Throughout much of the sugar industry, sugarcane trash is now retained and not burned. However, the intensive tillage between crop cycles is probably negating any potential to build-up organic carbon and soil biodiversity.

While 10-20 T/ha of organic matter was poorly to moderately suppressive to the general suite of YD biota (Pankhurst *et al*. 2005), greater suppression toward lesion and root-knot nematode was observed at seven months (Stirling *et al*. 2003). The

level of lesion nematode control (61-96%) and duration was greater than expected of non-volatile nematicides used commercially on sugarcane. Amendments with low nitrogen contents were recommended, which stimulated fungi rather than bacteria, and increased numbers of omnivorous nematodes.

In summary, the sugarcane monoculture and associated tillage has reduced microbial biomass and diversity, and favoured pathogens of sugarcane roots. A number of abiotic components of the soil have also declined, such as bulk density, CEC and pH, but their relation to sugarcane pathogens is unknown. Sugarcane responses to soil biocides and breaks in the monoculture strongly implicate soil pathogens in YD. Specifically, the pathogenicity of nematodes (particularly *P. zeae* and *Meloidogyne* spp.) and fungi (*Pachymetra chaunhoriza* and *Pythium* spp.) to sugarcane has been demonstrated, but biocide responses suggest other unidentified pathogens are also involved. Dematiaceous (dark sterile) hypomycetes have also been implicated in YD. Long-term pasture and crop breaks also appear to improve soil fertility by changing abiotic components in the soil.

Because sugarcane is a low value per hectare crop, chemical biocides are uneconomic in controlling sugarcane pathogens, as are long-term rotations impractical in sugarcane based farming systems. A holistic approach to managing YD is currently being investigated and proposed with prototype farming systems (Bell *et al*. 2003). Short rotations with non-host crops are proposed to reduce detrimental biota, add organic matter and improve soil fertility. A proactive approach to add and conserve organic matter and reduce soil disturbance is required to encourage greater soil biodiversity and naturally suppress YD biota. Permanent traffic and cropping lanes are proposed to void the need for whole-field and wholesale tillage between sugarcane crop cycles. The planting of sugarcane with minimal soil disturbance (direct drill) is being investigated.

12.4 Collaborated (minor author) papers and text related to nematodes, and participation by B Blair

Paper or text	Participation (%)
Garside, A. L., Berthelsen, J. E., Pankhurst, C. E., Blair, B. L., Magarey, R. C., D'Amato, C. D. and Bull, J. I. (2002 a) Effects of breaks from sugarcane monoculture and biocides on the growth and yield of a subsequent sugarcane crop. *Proceedings of the Australian Society of Sugarcane Technologists* **24**: 12.	10
Magarey, R. C., Bull, J. I., Blair, B. L. and Johnson, E. J. (1997) Biological studies of soils in paired old and new land sites growing sugarcane. *Australian Journal of Experimental Agriculture* **37**: 451-457.	10
Pankhurst, C. E., Blair, B. L., D'Amato, C. D., Bull, J. I. and Magarey, R. C. (2001) Quantification of the effects of fumigation and nematicide treatments on the early shoot and root growth of sugarcane in a yield decline soil. *Proceedings of the Australian Society of Sugarcane Technologists* **23**: 260-267.	35
Pankhurst, C. E., Blair, B. L., Magarey, R. C., D'Amato, C. D., Bull, J. I. and Garside, A. L. (2002) Use of biocides to determine the impact of detrimental fungi and nematodes on establishment and early growth of sugarcane following rotation breaks. *Proceedings of the Australian Society of Sugarcane Technologists* **24**: 11.	30
Pankhurst, C.E., Blair, B. L., Magarey, R. C., Stirling, G. R., D'Amato, C., Bull, J. I. and Garside, A. L. (2003 a) Testing a plant bioassay to assess the capacity of soils to suppress the activity of soil organisms associated with yield decline of sugarcane. *Proceedings of the Australian Society of Sugarcane Technologists* **25**: 10.	15
Pankhurst, C. E., Magarey, R. C., Stirling, G. R., Blair, B. L., Bell, M. J. and Garside, A. L. (2003 b) Management practices to improve soil health and reduce the effects of detrimental soil biota associated with yield decline of sugarcane in Queensland, Australia. *Soil and Tillage Research* **72**: 125-137.	10
Pankhurst, C. E., Blair, B. L., Magarey, R. C., Stirling, G. R. and Garside, A. L. (2004 a) Quantification of the effects of biocides and rotation breaks on the early growth of sugarcane and soil organisms associated with yield decline. *Plant and Soil*: in press.	40
Pankhurst, C. E., Stirling, G. R., Magarey, R. C., Blair, B. L., Holt, J. A., Bell, M. J. and Garside, A. L. (2004 b) Quantification of the effects of rotation breaks on soil biological properties and their impact on yield decline in sugarcane. *Soil Biology and Biochemistry*: in press.	10
Pankhurst, C. E., Blair, B. L., Magarey, R. C., Stirling, G. R., Bell, M. J. and Garside, A. L. (2005) Effect of rotation breaks and organic matter amendments on the capacity of soils to develop biological suppression towards soil organisms associated with yield decline of sugarcane. *Applied Soil Ecology* **28**: 271-282.	15

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APPENDICES

Appendix 9.3.1: Nematodes in 200 mL of soil at 7 and 50 DAP, and rhizosphere soil at 100 DAP.

Primordia remaining	Soil treatment	$(Nematodes + 0.5)^{1/3}$ in soil at 7 DAP			$(Nematodes + 0.5)^{1/3}$ in soil at 50 DAP			$(Nematodes + 0.5)^{1/3}$ in soil at 100 DAP		
		Lesion	Spiral	Ring	Lesion	Spiral	Ring	Lesion	Spiral	Ring
0	Untreated							8.8 b (765)	7.3 a (474)	4.1 a (95)
10 %	Untreated				6.8 b (349)	5.0 a (147)	3.2 ab (34)	8.4 b (595)	6.6 ab (306)	4.3 a (119)
40 %	Untreated							7.0 b (361)	5.7 ab (207)	4.3 a (84)
100 %	Untreated	6.7 a (305)	7.2 a (365)	5.5 a (170)	7.0 ab (364)	4.9 a (127)	3.5 a (54)	6.8 b (350)	4.9 b (122)	4.0 a (102)
100 %	Nematicide	5.4 b (155)	5.7 b (190)	2.7 b (20)	4.8 c (118)	4.3 a (90)	2.6 b (18)	8.9 b (780)	7.2 a (434)	4.3 a (119)
10 %	Fumigated							1.8 c (8)	0.8 c (0)	0.8 b (0)
40 %	Fumigated							1.6 c (17)	2.1 c (20)	0.8 b (0)
100 %	Fumigated + lesion nemas.	6.2 ab (233)	0.8 c (0)	0.8 c (0)	8.1 a (538)	1.8 b (8)	1.4 c (5)	14.6 a (3453)	0.8 c (0)	1.8 b (22)
100 %	Fumigated	1.7 c (5)	0.8 c (0)	1.8 bc (6)	1.1 d (2)	0.8 b (0)	0.8 c (0)	2.5 c (30)	1.1 c (2)	1.0 b (1)
	LSD (P=0.05)	1.14	1.34	1.42	1.10	1.05	0.76	2.59	2.01	2.11

Values in the same column followed by the same letter are not significantly different at P=0.05

Values in parentheses are back-transformed means. Back-transformed means are the arithmetic means of the raw data

Industry standard

Appendix 9.3.2: Nematodes in 200 mL of soil at 7 DAP, and rhizosphere soil at 70 DAP.

Primordia remaining	Soil treatment	(Nematodes + 0.5)$^{1/3}$ in soil at 7 DAP			(Nematodes + 0.5)$^{1/3}$ in soil at 70 DAP		
		Lesion	Spiral	Ring	Lesion	Spiral	Ring
0	Untreated				11.67 b (2032)	6.79 a (326)	3.05 ab (30)
25 %	Untreated				11.58 b (1721)	6.51 a (102)	4.20 a (102))
100 %	Untreated	7.87 a (490)	3.76 a (54)	1.32 (4)	13.04 ab (2382)	6.38 a (272)	2.29 bc (17)
25 %	Nematicide				5.12 c (164)	2.10 b (12)	1.63 cd (5)
100 %	Nematicide	4.34 b (89)	2.49 b (24)	1.26 (3)	3.96 cd (79)	2.09 b (20)	1.28 cd (5)
25 %	Fumigated + lesion nematode	6.44 a (317)	0.79 c (0)	0.79 (0)	15.46 a (4080)	0.79 b (0)	0.79 d (0)
0 %	Fumigated				1.76 de (14)	1.14 b (2)	0.93 d (1)
25 %	Fumigated				0.79 e (0)	1.04 b (1)	1.19 cd (2)
100 %	Fumigated	0.79 c (0)	0.79 c (0)	0.79 (0)	1.39 de (4)	1.46 b (5)	0.79 d (0)
	LSD (P=0.05)	1.83	1.12	ns	3.04	2.04	1.27

Values in the same column followed by the same letter are not significantly different at P=0.05

Values in parentheses are back-transformed means. Back-transformed means are the arithmetic means of the raw data

Industry standard

Appendix 9.3.3: Sequential stalk emergence in Experiment 1 (see Appendix 9.3.3a and 9.3.3b below).

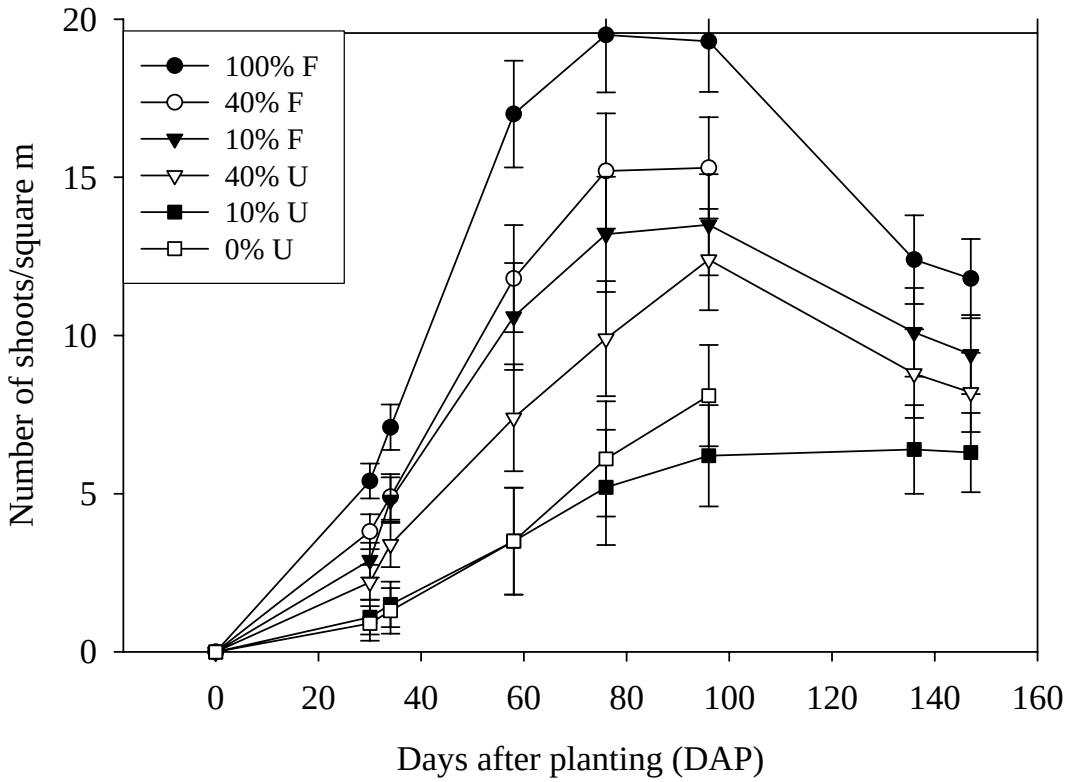
Primordia remaining	Soil treatment	30 DAP	34 DAP	57 DAP	75 DAP	96 DAP	136 DAP	147 DAP
0	Untreated	0.9 a	1.3 a	3.5 a	6.1 a	8.1 ab		
10 %	Untreated	1.1 a	1.5 a	3.5 a	5.2 a	6.2 a	6.4 a	6.3 a
40 %	Untreated	2.2 b	3.4 bc	7.4 bc	9.9 b	12.4 cd	8.8 ab	8.0 ab
100 %	Untreated	2.3 b	3.3 b	7.3 b	9.8 b	11.1 bc	7.6 ab	6.9 ab
100 %	Nematicide	3.5 c	4.8 cd	10.7 cd	13.4 bc	14.0 cd	9.6 bc	8.8 ab
10 %	Fumigated	2.9 bc	4.8 cd	10.6 cd	13.2 bc	13.5 cd	10.1 bc	9.4 bc
40 %	Fumigated	3.8 c	4.9 d	11.8 d	15.2 cd	15.3 d		
100 %	Fumigated + lesion nematode	5.5 d	7.0 e	15.2 e	18.9 de	18.8 e		
100 %	Fumigated	5.4 d	7.1 e	17.0 e	19.5 e	19.3 e	12.4 c	11.8 c
	LSD (P=0.05)	1.11	1.44	3.38	3.64	3.21	2.8	2.51

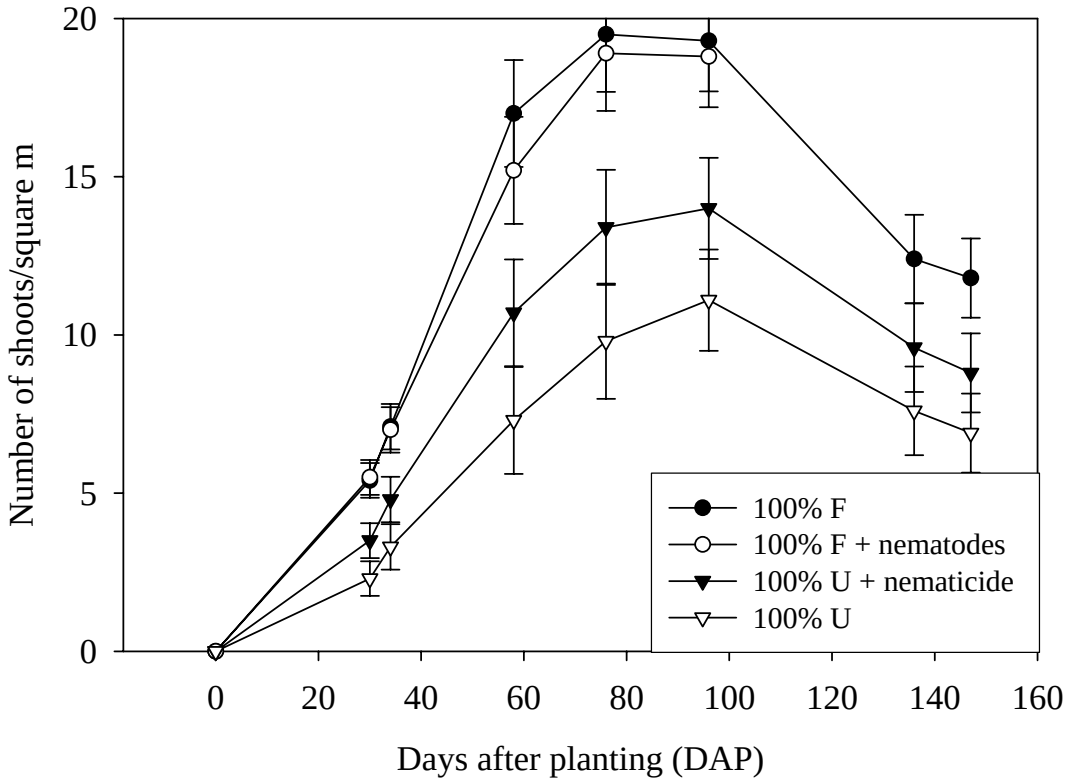
Values in the same column followed by the same letter are not significantly different at P=0.05

Appendix 9.3.4: Sequential stalk emergence in Experiment 2 (see Appendix 9.3.4a-9.3.4d below).

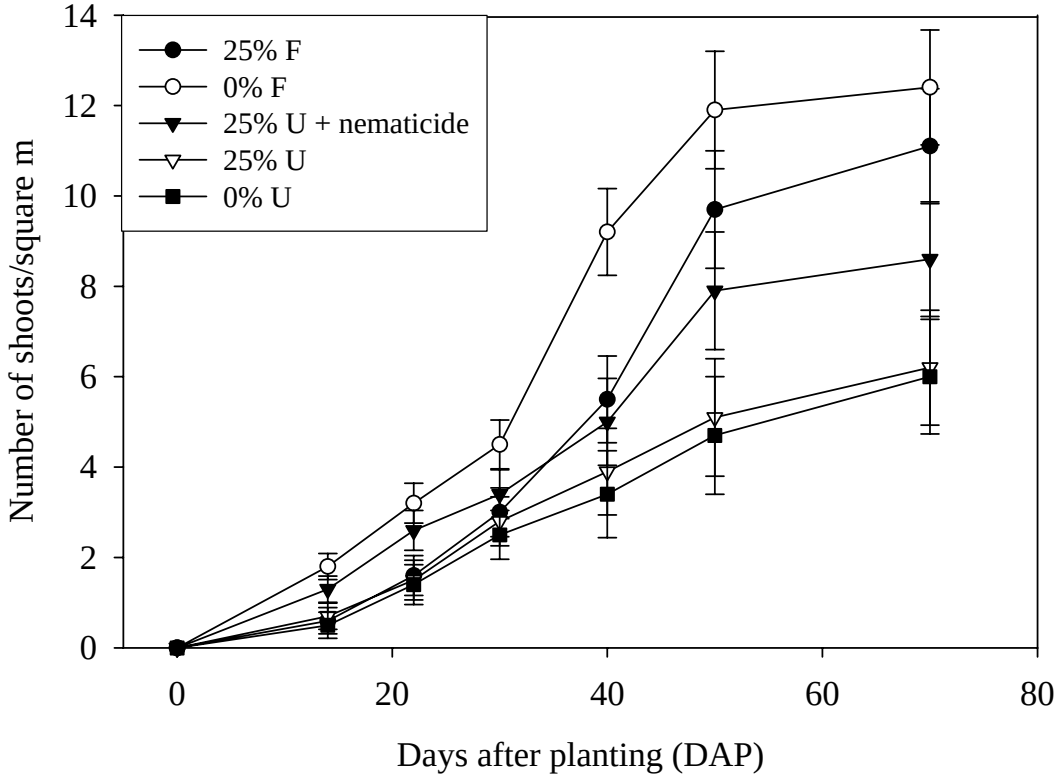
		Q117, days after planting (DAP)						Q138, days after planting (DAP)					
Primordia remaining	Soil treatment	14	22	30	40	50	70	14	22	30	40	50	70
0	Untreated	0.5 a	1.4 a	2.5 a	3.4 a	4.7 a	6.0 a	0.7 a	2.3 a	3.7 a	5.9 a	9.3 a	11.8 a
25 %	Untreated	0.7 abc	1.5 a	2.8 ab	3.9 ab	5.1 a	6.2 ab	1.5 ab	3.4 bc	4.2 ab	6.2 a	9.4 a	12.2 a
100 %	Untreated	0.8 abc	1.6 ab	2.9 abc	3.9 ab	5.8 ab	6.9 ab	1.2 ab	3.3 abc	4.4 ab	5.7 a	9.7 a	12.3 ab
25 %	Nematicide	1.3 cd	2.6 cd	3.4 abc	5.0 ab	7.9 bcd	8.6 bc	2.6 c	4.4 c	5.4 bc	9.7 b	14.2 bc	15.3 c
100 %	Nematicide	0.6 ab	1.6 ab	2.7 ab	4.8 ab	7.3 abc	8.2 ab	1.5 ab	3.0 ab	4.6 abc	8.9 b	12.9 b	15.1 bc
25 %	Fumigated + lesion nematode	1.1 bc	2.0 abc	3.6 bcd	7.2 cd	10.1 de	10.9 cd	1.9 bc	3.6 bc	5.6 c	12.8 d	17.4 d	19.2 d
0 %	Fumigated	1.8 d	3.2 d	4.5 d	9.2 e	11.9 e	12.4 d	0.9 a	2.8 ab	4.7 abc	9.6 b	16.5 cd	20.2 d
25 %	Fumigated	0.6 ab	1.6 ab	3.0 abc	5.5 bc	9.7 cde	11.1 cd	2.1 bc	3.8 bc	5.7 c	12.5 cd	18.8 d	21.6 d
100 %	Fumigated	1.1 bc	2.4 bcd	3.9 cd	8.1 de	10.9 e	11.7 d	1.4 ab	3.2 ab	4.6 abc	10.3 bc	17.8 d	21.4 d
	LSD (P=0.05)	0.58	0.89	1.09	1.92	2.6	2.54	0.85	1.06	1.19	2.24	3.02	2.93

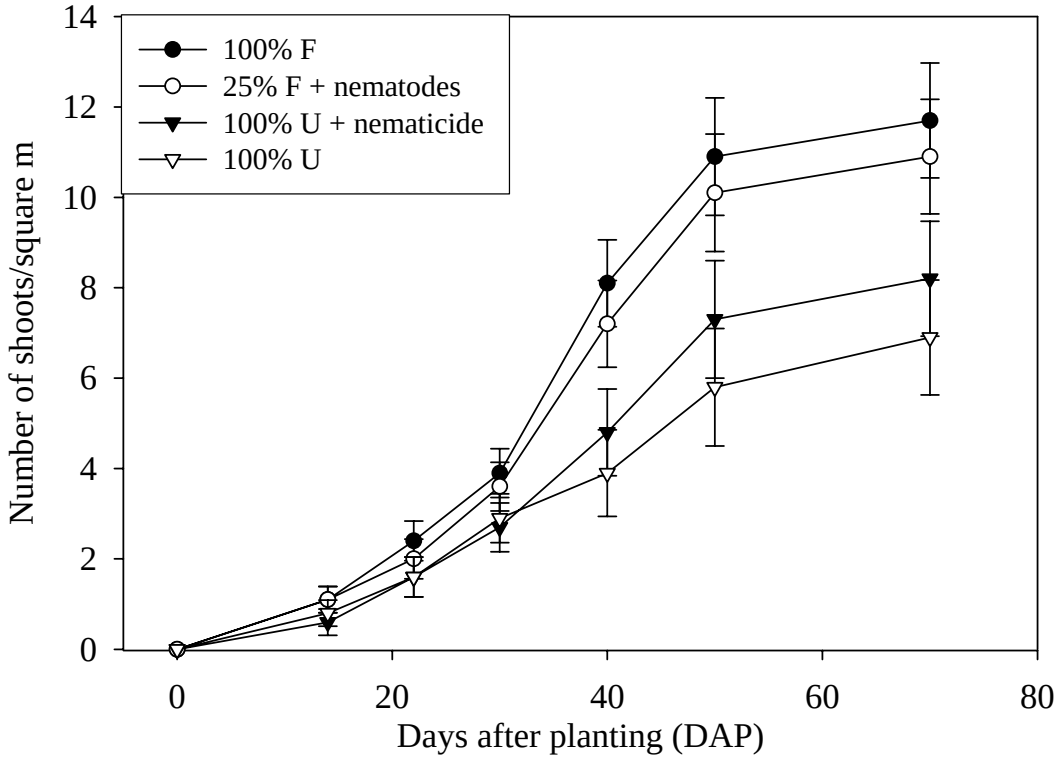
Values in the same column followed by the same letter are not significantly different at P=0.05



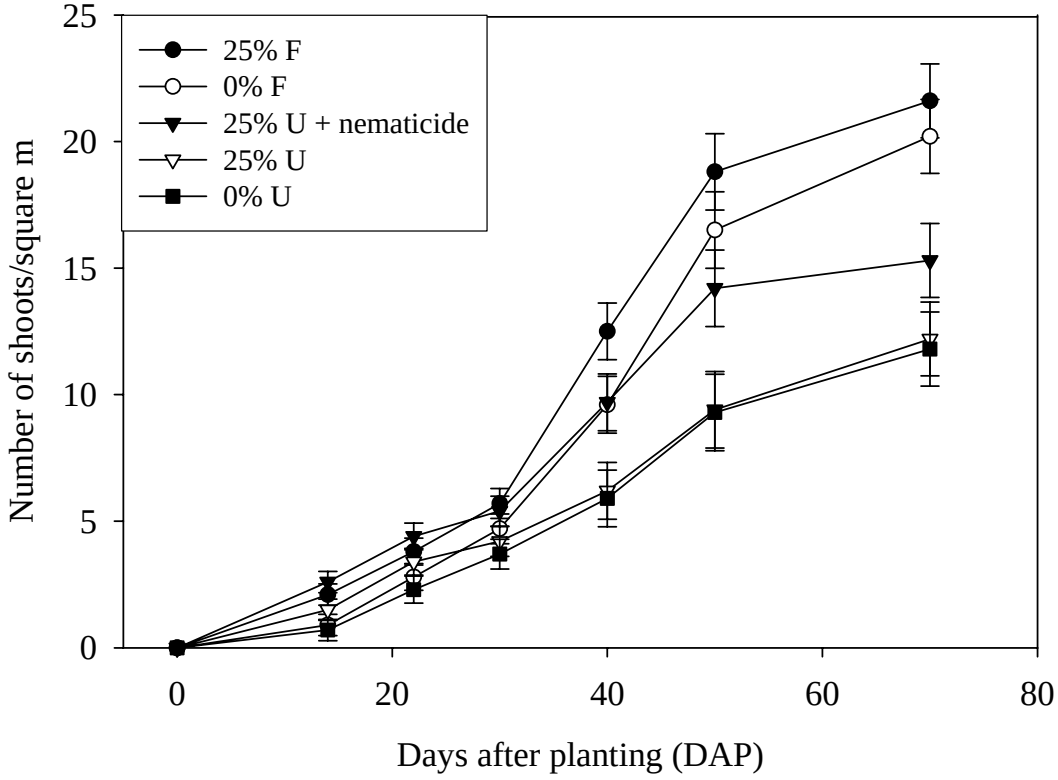


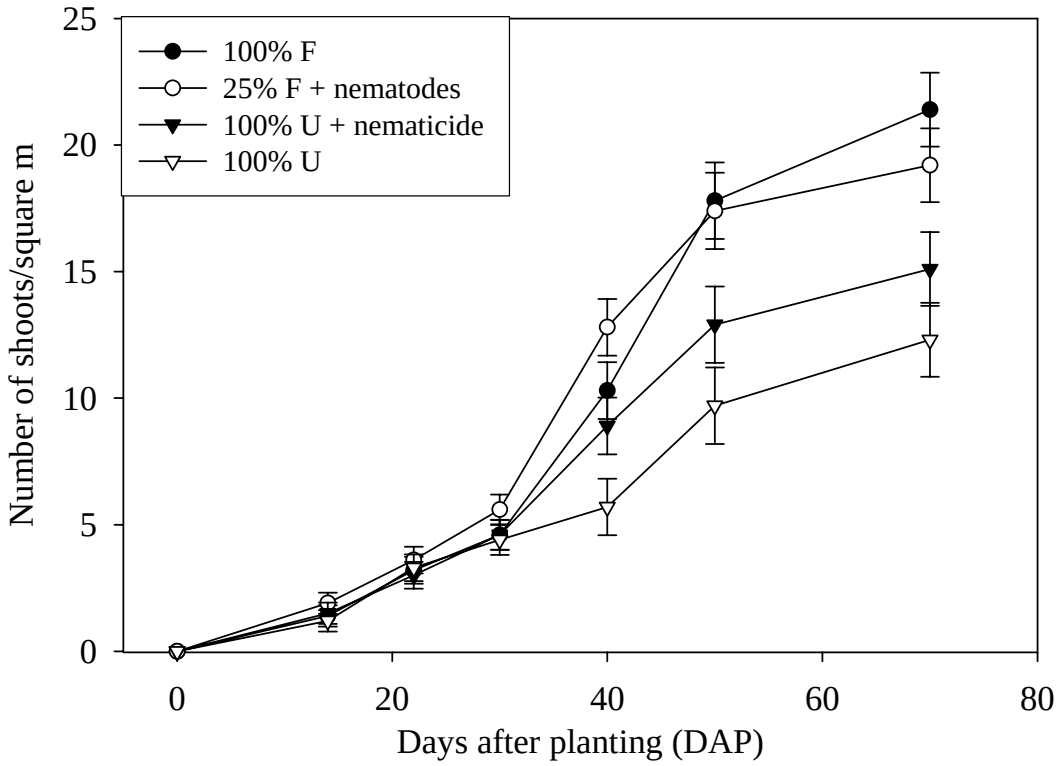
Appendix 9.3.3a and 9.3.3b: Effect of sett root pruning and soil treatment on number of shoots emerging from the soil in Experiment 1 (U = untreated, F = fumigated, LSD bars represent P=0.05).





Appendix 9.3.4a and 9.3.4b: Effect of sett root pruning and soil treatment on number of Q117 shoots emerging from the soil in Experiment 2 (U = untreated, F = fumigated, LSD bars represent P=0.05).





Appendix 9.3.4c and 9.3.4d: Effect of sett root pruning and soil treatment on number of Q138 shoots emerging from the soil in Experiment 2 (U = untreated, F = fumigated, LSD bars represent P=0.05).

Appendix Plate 9.2: Representative ‘old’ (left) and ‘new’ (right) buds.

Appendix Plate 9.3.5: Representative unshaved (above) and 100% shaved (below) stem cuttings.

Appendix Plate 9.3.6: Setts with 75% of root primordia removed, showing root growth only from the unshaved area.

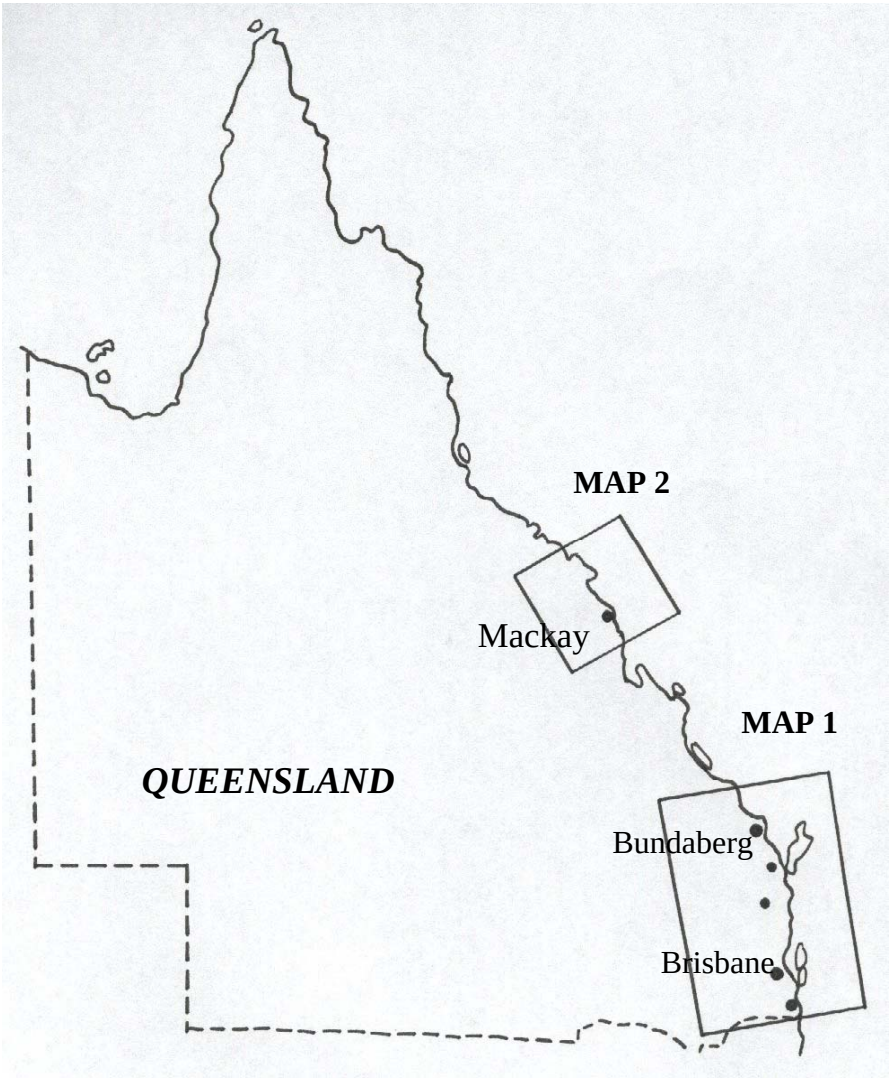


Plate 10.2.1: Regions of sugarcane production in south (see Map 1) and central Queensland (see Map 2) where crop losses were assessed.

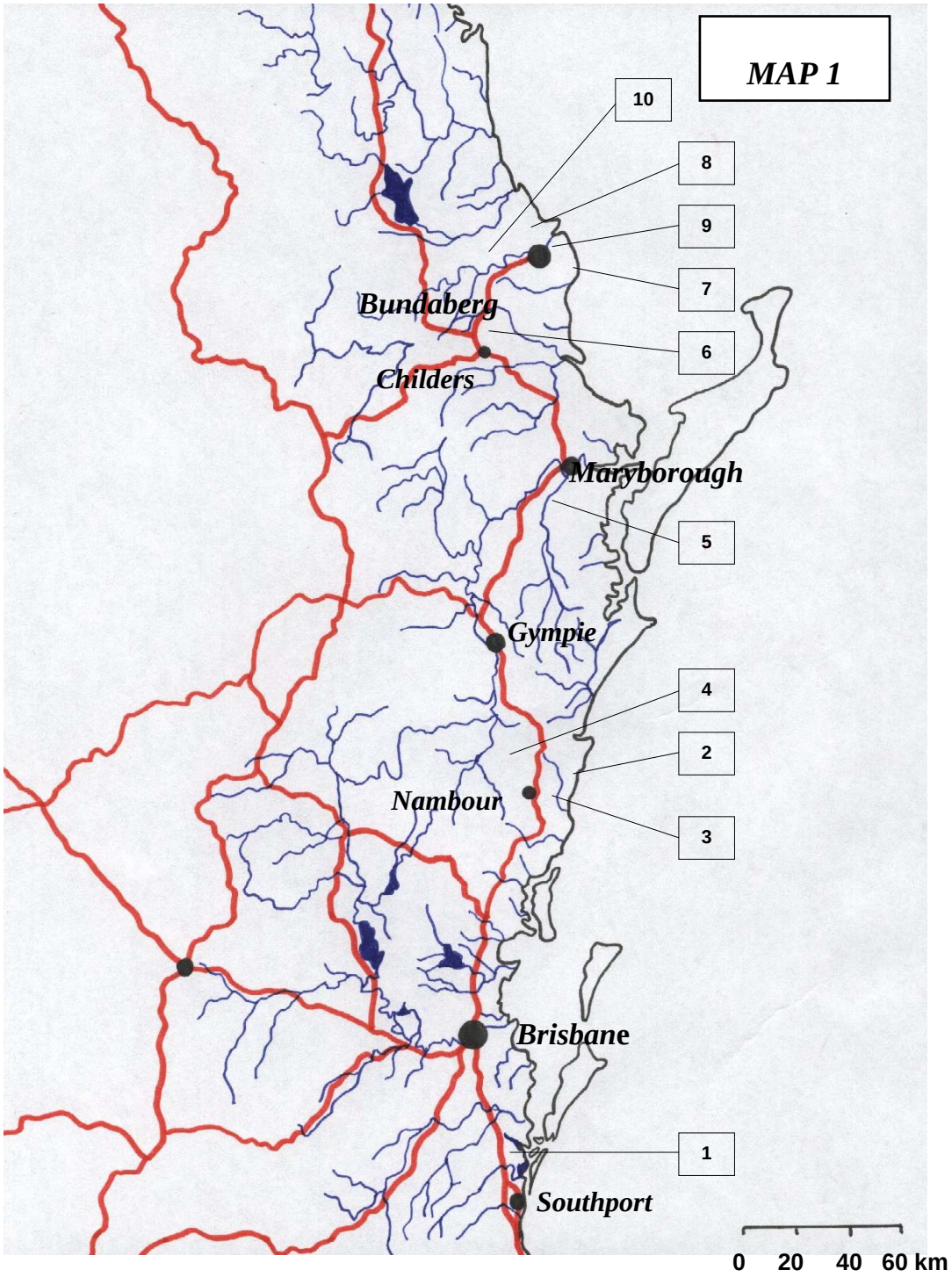


Plate 10.2.2: Sites where crop losses were assessed in south Queensland.

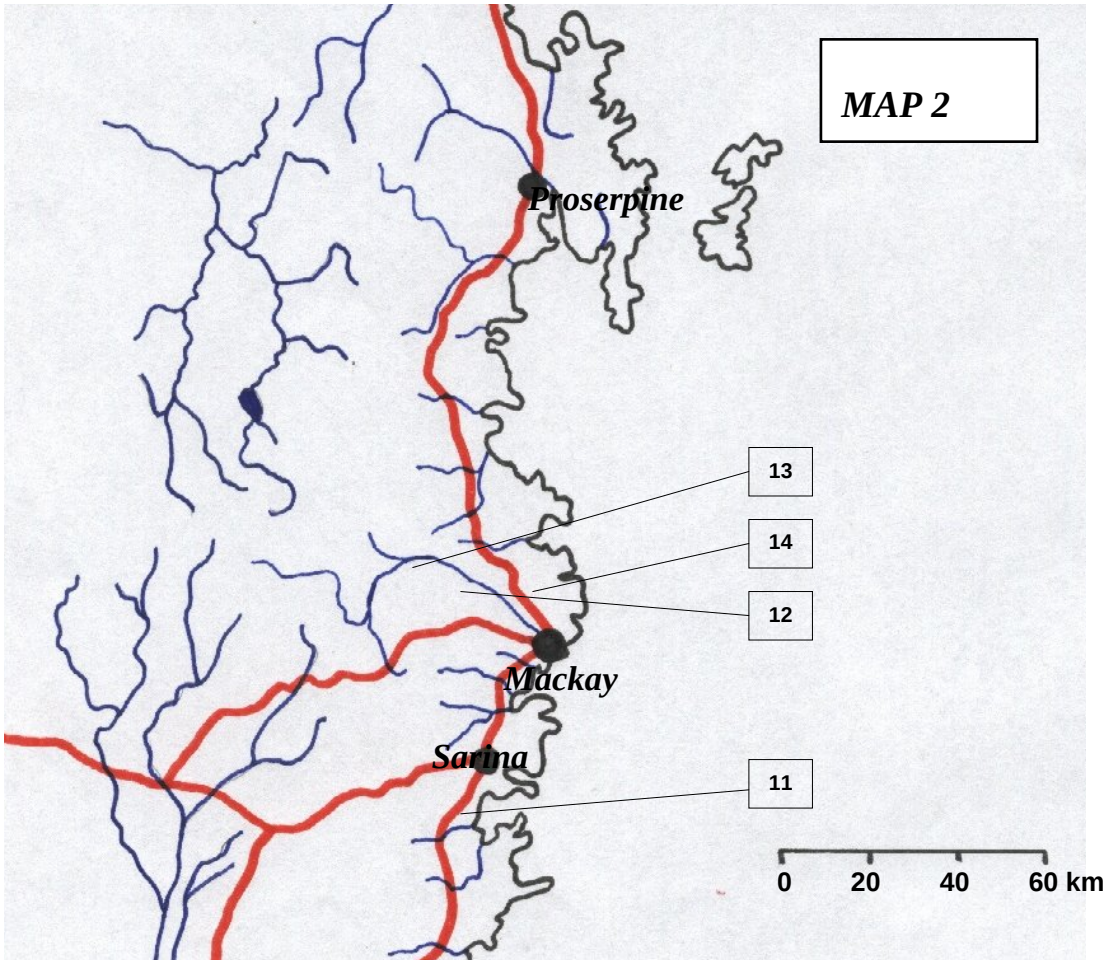


Plate 10.2.3: Sites where crop losses were assessed in central Queensland.

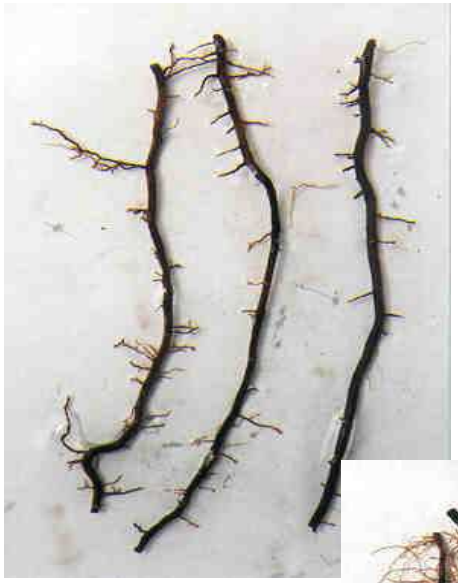
Appendix 10.2.3: Details of when aldicarb (A) or fenamiphos (F) were applied at the field sites, and where the nematicide was placed in relation to the trash blanket.

Region or mill area	Nematicide treatments (days after planting) in the plant crop – aldicarb (A), fenamiphos (F)					Nematicide treatments (days after ratooning) in the 1st ratoon – aldicarb (A), fenamiphos (F)					Nematicide treatments (days after ratooning) in the 2nd ratoon – aldicarb (A), fenamiphos (F)			
	0-20	21-60	61-110	111-200	201-240	0-20	21-70	71-130	131-210	211-270	0-10	11-20	21-70	71-200
1. Rocky Point	A	F	F	A,F	A		F	A	F	A				
2. Coolum	A	F	A	F			A	F	A					
3. Maroochydore	F	A	A	F	A		F	A	F					
4. Yandina	F	A	A	F	A	F		A	F					
5. Maryborough	A			F	A		A[1]		F[1]		A[2]	F[2]	A[2]	
6. Childers	F	A	F		A		F[1]		A[1]		A[2]	F[2]	A[2]	
7a. Elliot Heads	F	A	F	A	F	A	F	A						
7b. Elliot Heads	F	A	F	A	F	A	F	A						
8. Fairymead	F	A	F	A	F		A	F						
9. Fairymead	F	F		F	F	A[1]		F						
10. Bingera	F	A	F		A				F[1]	A[1]	A[1]			F[1]
11. Plane Creek	A	F	A			A	F	A						
12. Mirani	F	F	F	F										
13. Racecourse	A	F		A	F	A[2]	F[2]							
14a. Farleigh	F	F		F										
14b. Farleigh	F	F		F										

Experiment not continued in this crop class

[1]Nematicide placed under the trash blanket. [2]Nematicide placed on top of the trash blanket prior to forecasted rain or irrigation.

1



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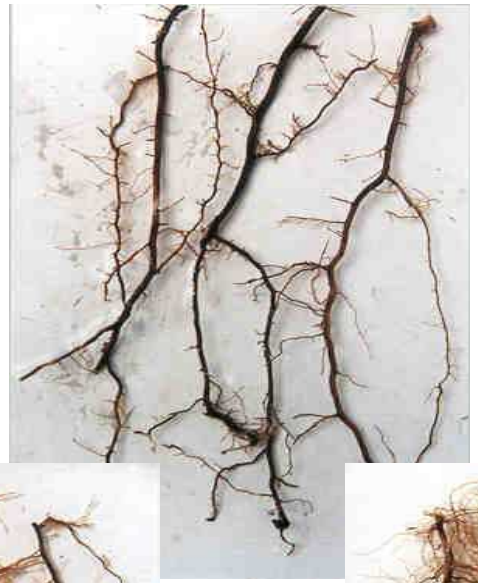

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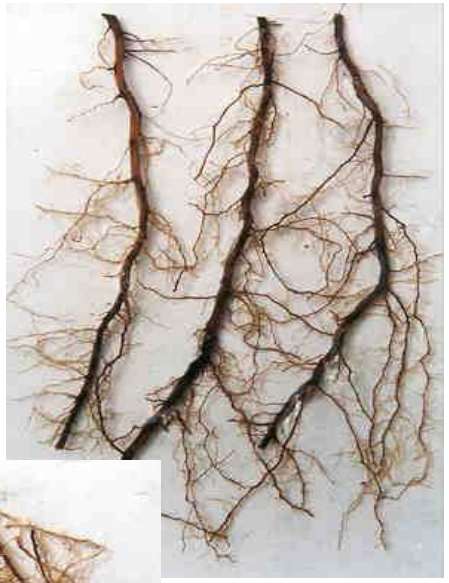

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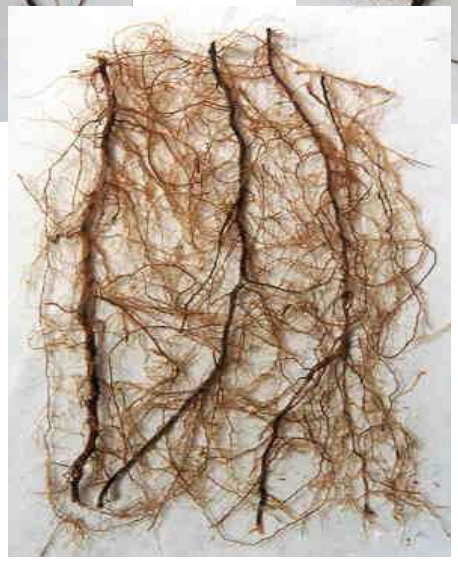

Appendix 10.2.4: Root health ratings used, according to root growth.