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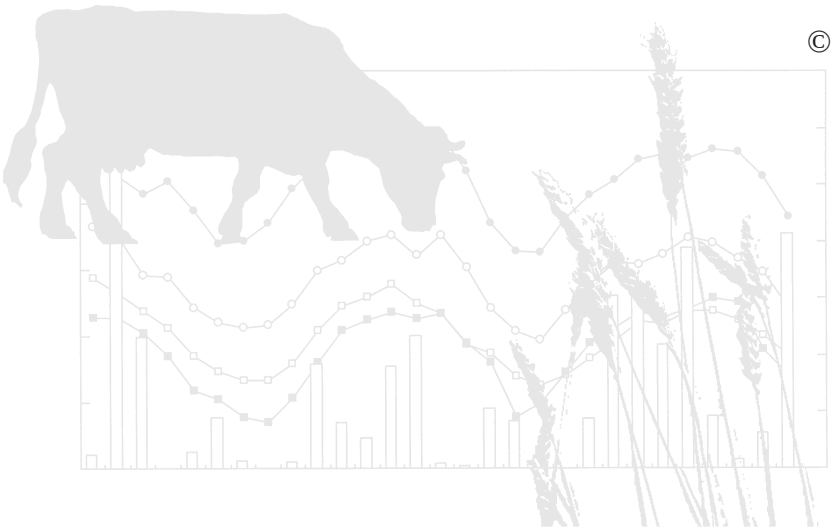

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Sampling procedures and damage thresholds for root-knot nematode (*Meloidogyne javanica*) on pineapple

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Abstract. The relationship between the population density of root-knot nematode (*Meloidogyne javanica*) and pineapple yield was studied by establishing different nematode densities in field plots at 3 sites. Differences in nematode populations between treatments were apparent 9–22 months after planting, but yields in the plant crop were similar, regardless of nematode density. In the ratoon crop, yields in treatments with less than 10 nematodes/200 mL soil at 9–22 months were reduced by about 10%. Yield reductions of more than 25% occurred when population densities were greater than 50 nematodes/200 mL soil. These results demonstrate that economically significant crop losses from root-knot nematodes can occur in pineapple when the population density at 12 months is greater than 1–5 nematodes/200 mL soil.

The sampling procedures required to obtain reliable estimates of *M. javanica* in pineapple fields were determined by studying nematode distribution in 2 fields in south-east Queensland. Nematodes were extracted from more than 100 individual soil cores on a 5 by 5 m grid and populations were found to have a clumped rather than random distribution. A composite sample of 41 cores in 1 field and 72 cores in the other gave a relatively precise estimate of the population of root-knot nematodes (i.e. standard error : mean ratio of 0.3). These data suggest that a 50-core sampling unit is appropriate when nematode population density is being estimated for decision-making purposes.

Introduction

Most of Australia's pineapples [*Ananus comosus* L. (Merr.)] are grown within 50 km of its eastern coast, between Brisbane in south-east Queensland and Yeppoon in Central Queensland. About 140 000 t of pineapples are produced each year, with 75% used for canning and the remainder destined for the domestic fresh fruit market. Instead of the plantation-style production systems that dominate the rest of the world's pineapple industries, the Australian industry consists of about 200 family farms, each producing between 500 and 5000 t of fruit each year. The crop is generally grown as a monoculture, with a cropping cycle of about 4 years. Pineapple crowns are planted into raised beds at a density of about 50 000 plants/ha and the first harvest (plant crop) is obtained 22–24 months later. A ratoon crop is harvested 38–42 months after planting. The crop is then ploughed out and the land left fallow for about 6 months before being replanted. Plant crop yields range from 100 to 120 t/ha, while ratoon crops produce about 80 t/ha. Productivity per person is high because the industry is highly mechanised and there is minimal use of hired labour.

Plant-parasitic nematodes are a major factor limiting production wherever pineapples are grown (Caswell *et al*. 1990). The nematode species found elsewhere are also found in Australia, namely root-knot nematode [*Meloidogyne javanica* (Treub) Chitwood], lesion nematode [*Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans], reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira) and spiral nematode [*Helicotylenchus dihystera* (Cobb) Sher]. However, lesion nematodes rarely increase to high population densities in Australia, reniform nematodes are not widely distributed and spiral nematodes cause little or no economic damage (Stirling 1993). Root-knot nematodes are therefore the most important nematode pest. They are widely distributed in all pineapple growing areas and when uncontrolled can cause yield losses of 30–60% in the ratoon crop (Stirling and Nikulin 1993). Growers therefore apply nematicides routinely as an insurance against crop losses. Ethylene dibromide (EDB) was widely used in the

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past, but since its withdrawal from the market in 1998, metham sodium is the only registered pre-plant fumigant. Many growers also apply foliar sprays of fenamiphos to both plant and ratoon crops.

Despite the widespread distribution of root-knot nematodes, crop loss assessment work has shown that their importance varies from field to field. In some fields, nematode populations increase rapidly to damaging levels while in others, populations remain low and responses to nematicides are not obtained (Stirling and Nikulin 1993). If these latter situations could be identified with some degree of certainty, nematicides could be applied strategically rather than routinely. Nematicide use would then be restricted to situations where there was a high probability of obtaining an economic response.

The principles behind the development of strategic decision making systems for nematode pests have been outlined by Duncan (1991) and McSorley and Phillips (1993). Basically, a decision to apply a nematicide (or some other control measure) is only made when the population density of the key pest is above the economic threshold. However, before such a system could be implemented in the pineapple industry, sampling and extraction procedures that reliably estimate nematode population density in a field must be available and the relationship between root-knot nematode density and yield must be understood. Both these issues are addressed in this study.

Materials and methods

Damage functions for Meloidogyne *on pineapple*

Experiments were undertaken in 3 pineapple fields (sites 1–3) in the Kallangur and Wamuran districts of south-east Queensland (Table 1). Root-knot nematode was not expected to cause problems at these sites because the nematode had either not been detected in the previous ratoon crop or was present at very low population densities. The area reserved for each experiment consisted of 5 beds, each about 25 m long. Beds were prepared ready for planting but were not fumigated with EDB, a practice that was common in the pineapple industry at the time the experiments were established. Each experiment had a latin-square design of 5 treatments by 5 replicates, with each plot being a 4.5–5.5 m length of bed.

Treatments consisted of different densities of root-knot nematode and were achieved by either increasing the natural nematode population by inoculation, or by reducing populations with a nematicide. Nematode inoculum was prepared by chopping tomato roots infested with *M. javanica* into pieces less than 1 cm long. The number of eggs per gram root material was quantified by counting eggs that had been removed from a subsample of roots with 1% NaOCl. Roots were then added to moist, sterile sand and this inoculum was sprinkled on the surface of the bed and incorporated with a rotary hoe to a depth of 10 cm. The amount of inoculum used was varied so that both a high and low number of nematodes were applied to 5 replicate plots at each site before planting (Table 1). Nemacur 10G (100 g fenamiphos/kg) was applied to a further 5 plots by sprinkling granules on the soil at 20 g/m^2 and then incorporating them by rotary hoeing. Pineapple crowns were then planted, with 10 of the 25 plots having received no inoculum or nematicide. Six months later, more inoculum was prepared and lightly raked into the surface of 5 of these untreated plots. Thus, 5 treatments were established: fenamiphos; a high nematode density at planting; a low nematode density at planting; a late inoculation with nematodes; and the natural nematode population. Specific details of each experiment are listed in Table 1.

Each crop was grown using the grower's standard management practices and yields of plant and ratoon crops were measured. Periodically during the experiments, 20 soil cores were collected from each plot at depths of 0–20 cm using a 2.4-cm-diameter sampling tube. Nematodes were extracted from 200-mL subsamples using a Baermann tray technique (Whitehead and Hemming 1965) known to have an extraction efficiency of about 40%. During the first 12 months, when nematode populations were low, each soil sample was also bioassayed by mixing 750 mL soil with 750 mL sterile sand and planting a tomato seedling (cv. Tiny Tim). To enable tomato seedlings to grow, activated charcoal was added to inactivate herbicides that were present in soil and dolomite was mixed with the soil to raise the pH to a level suitable for tomatoes. Bioassay plants were harvested after 1 month and the number of galls on each root system was counted. Since there was insufficient time for nematode reproduction to have occurred and nematode population densities were low, each gall indicated the presence of a single nematode. Thus, the number of galls/750 mL soil was used to estimate the number of root-knot nematodes/750 mL soil. Counts were corrected to nematodes/200 mL soil to allow comparisons with data derived by extracting nematodes directly from soil.

Sampling strategies for nematodes in pineapple fields

An 85 by 25 m area (site 4) was selected in a pineapple field on a grey, sandy loam soil near Donnybrook, Queensland. This area was marked as a 5 by 5 m grid and a soil sample was collected from each of the 108 grid intersection at a depth of 0–25 cm using a 2.4-cm-diameter sampling tube. Every third row of samples was taken in the furrow between beds so that a total of 36 samples were taken in the furrow and the remainder from the bed. A 50-mL subsample was retained from each sample and nematodes were extracted from the soil using the Baermann tray technique.

A similar sampling and extraction procedure was used in a crop growing in a sandy loam soil near Wamuran, Queensland (site 5). In this case, the sampling area was 155 by 15 m, and 128 samples were taken. Since the bed spacing differed from the first site, all samples were taken in the bed.

Results

Damage functions for Meloidogyne *on pineapple*

Meloidogyne javanica and *H. dihystera* were the only plant-parasitic nematodes recovered from the experimental sites. Data for *H. dihystera* are not presented, as the nematode is not considered economically important. With regard to *M. javanica*, only a small proportion of the eggs inoculated at each field site produced viable nematodes, as relatively few galls were produced on bioassay plants grown in soil collected before planting (Table 2). Nevertheless, different root-knot

Table 1. Details of nematode inoculum-density experiments on pineapple at three sites

	Site 1	Site 2	Site 3
Location of experiment	Kallangur	Wamuran	Wamuran
Plot size	5.5 by 0.7 m	4.5 by 0.75 m	4.5 by 0.6 m
Soil type	Sandy clay loam	Sandy loam	Sandy loam
Soil particle size analysis (%)			
Coarse sand	12	28	39
Fine sand	45	44	35
Silt	10	6	3
Clay	31	19	20
Planting date	Mar. 1993	June 1993	June 1993
Plant crop harvest	Feb. 1995	May 1995	May 1995
Ratoon crop harvest	Mar. 1996	Sept. 1996	Nov. 1995
Estimate of eggs added to plot/L soil			
High density (pre-plant)	400	1600	1600
Low density (pre-plant)	40	160	160
Late inoculation	400	1600	1600

nematode population densities were achieved in the 2 pre-plant inoculation treatments (Table 2). Subsequent results showed that these differences were maintained during the 9–15 months after planting (Table 3). At sites 2 and 3, nematode populations reached a peak at 15 months and then declined, probably because root volume had been reduced by nematode damage. Nematode populations were low in fenamiphos-treated and naturally infested plots at all sites for at least 22 months after planting (Table 3).

Data collected at plant crop harvest showed that there were few differences between treatments in either yield or average weight of canning fruit (Table 4). However, this situation changed in the ratoon crop, as heavy infestations of root-knot nematode (i.e. the high density treatments at all sites and the low density treatment at site 2) caused yield reductions of more than 25%. At sites 1 and 2, ratoon crop yields in the late-inoculation treatment did not differ significantly from the fenamiphos treatment, but there were suggestions that the relatively low nematode populations that occurred 12–22 months after planting may have reduced yields by about 10%. The same conclusion could be drawn for the low-density treatment at site 1. At site 3, the late-inoculation, natural and nematicide treatments, which had few root-knot nematodes at 22 months (Table 3), showed no signs of nematode damage at ratoon crop harvest. Ratoon crop yields were low in all 5 treatments, suggesting that factors other than root-knot nematodes were limiting yield at this site. Nevertheless, the nematode still had an impact, as yields of canning fruit in the ratoon crop were substantially reduced in the 2 treatments (high and low density) where root-knot nematodes were detected at 12 and 15 months.

Table 2. Pre-plant populations of root-knot nematode (nematodes/200 mL soil) as determined by bioassay, for five treatments in inoculum-density experiments at three sites

Data are presented as equivalent means with transformed means [ln (x + 1)] in parentheses

Treatment	Site 1	Site 2	Site 3
High density	59 (4.10)	15 (2.29)	28 (3.35)
Low density	2 (0.95)	1 (0.56)	1 (0.87)
Late inoculation[A]	3 (1.29)	0 (0)	0 (0)
Natural	0 (0.47)	0 (0)	0 (0)
Fenamiphos	0 (0.00)	0 (0)	0 (0)
l.s.d. (P = 0.05)	(1.53)	(0.42)	(0.87)

[A]This treatment had not yet been inoculated with nematodes.

The relationship between crop yield and nematode density 12 months after planting (D_{12}) was examined using linear regression of ratoon crop yield against ln (D_{12} + 1). The regression was significant (P = 0.001) at sites 1 and 3 but R^2 values were relatively low (54, 20 and 35% at sites 1, 2 and 3, respectively). The crop-loss model of Seinhorst (1965, 1998) was also fitted to each data set using Genstat. This model is described by

$$y = m + (1 - m)z^{(D - 1)}$$

where D is the nematode density (D_{12} was used in this work), y is the relative yield, m is the minimum yield, T is the value of D below which yield is not affected (i.e. the tolerance limit) and z is a constant (usually <1). Values for T of –0.3, –0.07 and 0 were obtained for sites 1, 2 and 3,

respectively. However, much of the variability in the data was not accounted for by the model, as R^2 values for the 3 sites were 51, 18 and 37%, respectively.

Sampling strategies for nematodes in pineapple fields

Meloidogyne javanica, *P. brachyurus*, and *H. dihystera* were present at both sampling sites. Data from site 4 showed that numbers of all plant-parasitic species were much greater in the bed than the furrow. Thus, only data from the 72 in-bed samples at site 4 were analysed and sampling was confined to the bed at site 5. For each nematode at both sites, the ratio of the variance to the mean was much greater than 1 (Table 5) indicating that the distributions of the populations were clumped rather than random. This is apparent in the distributions for root-knot nematode (Fig. 1).

The Maximum Likelihood Program (MLP) was used to fit Poisson, negative binomial and Neyman Type A distributions to each sample. Goodness-of-fit to each of these theoretical distributions was tested by means of a χ^2-test. All distributions at both sites were significantly (P<0.05) different from both the Poisson and Neyman Type A distributions, but did not differ from the negative binomial distribution. The k value (sometimes known as an aggregation index) for the negative binomial distribution fitted to each set of data is given in Table 6. Since low values are indicative of clumped populations and all k values were <1, there was a high degree of clumping for all nematode species at both sites.

The number of cores required to obtain a reliable estimate of nematode population density was calculated using the formula given by McSorley and Parrado (1982):

$$n = \frac{1}{E^2}\left(\frac{1}{\bar{x}} + \frac{1}{k}\right)$$

where n is the number of cores per single sample, $\bar{x}$ is the mean of all samples from a given field, k is a value from the negative binomial distribution and E is the standard error : mean ratio

$$\left(\frac{s/\sqrt{n}}{\bar{x}}\right)$$

Table 3. Root-knot nematode populations (nematodes/200 mL soil) at three sites, 9–22 months following planting

Populations were determined by extraction, except at 9 months (sites 2, 3) and 12 months (site 1), where bioassays were used
Data are presented as equivalent means with transformed means [ln (x + 1)] in parentheses

Treatment	No. of months after planting			
	9	12	15	22
		Site 1		
High density	—	29 (3.41)	59 (4.10)	157 (5.06)
Low density	—	1 (0.61)	4 (1.54)	24 (3.17)
Late inoculation	—	0 (0.21)	0 (0.32)	4 (1.26)
Natural	—	0 (0)	0 (0)	0 (0)
Fenamiphos	—	0 (0.05)	0 (0)	0 (0)
l.s.d. (P = 0.05)		(0.75)	(1.09)	(1.40)
		Site 2		
High density	96 (4.57)	506 (6.23)	1807 (7.50)	148 (5.00)
Low density	30 (3.42)	19 (2.99)	202 (5.31)	178 (5.18)
Late inoculation	1 (0.53)	2 (0.98)	9 (2.31)	147 (4.99)
Natural	0 (0)	0 (0)	0 (0)	3 (1.10)
Fenamiphos	0 (0)	0 (0)	0 (0)	4 (1.30)
l.s.d. (P = 0.05)	(0.61)	0 (1.29)	(1.59)	(1.62)
		Site 3		
High density	47 (3.86)	103 (4.64)	897 (6.80)	164 (5.10)
Low density	1 (0.47)	5 (1.72)	28 (3.35)	57 (4.04)
Late inoculation	0 (0.15)	0 (0)	0 (0)	1 (0.22)
Natural	0 (0)	0 (0)	0 (0)	0 (0)
Fenamiphos	0 (0)	0 (0)	0 (0)	1 (0.32)
l.s.d. (P = 0.05)	(1.02)	(0.93)	(0.90)	(0.71)

Table 4. Plant and ratoon crop yields in the presence of various nematode densities at three field sites

Treatment	Canning fruit weight (kg/plot)		Average fruit weight (kg)	
	Plant crop	Ratoon crop	Plant crop	Ratoon crop
		Site 1		
High density	57.7	35.4	1.67	1.32
Low density	58.1	45.9	1.71	1.40
Late inoculation	58.1	51.9	1.69	1.44
Natural	60.2	53.0	1.77	1.51
Fenamiphos	60.8	57.5	1.64	1.51
l.s.d. (P = 0.05)	n.s.	12.0	n.s.	0.09
		Site 2		
High density	31.4	42.7	2.07	1.61
Low density	31.0	42.9	2.21	1.73
Late inoculation	31.4	54.0	2.19	1.75
Natural	32.0	59.6	2.13	1.83
Fenamiphos	31.2	60.3	2.11	1.84
l.s.d. (P = 0.05)	n.s.	12.0	0.09	0.12
		Site 3		
High density	29.2	1.9	1.73	1.48
Low density	33.9	3.5	1.78	1.40
Late inoculation	33.5	8.4	1.76	1.26
Natural	34.3	10.1	1.79	1.10
Fenamiphos	36.8	10.8	1.78	1.12
l.s.d. (P = 0.05)	n.s.	5.4	n.s.	0.32

The results (Fig. 2) indicate that the number of cores required for any reasonable degree of precision is high as a consequence of the low means and low k values. Thus, for root-knot nematode, 41 and 72 cores were needed to obtain an estimate of the mean number of nematodes that was within 30% of the mean (Table 7). For greater precision (e.g. within 20% of the mean), 93 and 163 individual cores would be needed.

Table 6. Aggregation indexes (k) for nematode distributions that did not differ significantly (P>0.05) from the negative binomial

	Site 4	Site 5
Meloidogyne javanica	0.27	0.15
Helicotylenchus dihystera	0.17	0.04
Pratylenchus brachyurus	0.31	0.13

Discussion

One of the main objectives of this work was to obtain better information on the damage thresholds for root-knot nematode on pineapples. Similar studies with other crops have generally involved developing a relationship between crop yield and initial nematode density (McSorley and Phillips 1993), but it is questionable whether such an approach is appropriate for pineapple. Root-knot nematode populations at planting are often so low that the nematode is difficult to detect with standard nematode extraction methods (Stirling and Nikulin 1993). This detection and quantification problem, when coupled with the fact that the effects of nematodes are seen mainly in the ratoon crop and are not manifested until 36–42 months after planting, means that there is not likely to be a close relationship between pre-plant nematode densities and

Table 7. Number of cores required at two sampling sites to estimate mean numbers of root-knot nematodes with various levels of precision

	Precision (s.e./$\bar{x}$)		
	0.20	0.25	0.30
Site 4	93	59	41
Site 5	163	104	72

Table 5. Parameters describing nematode populations that were derived by collecting multiple soil cores from two pineapple fields

There were 72 samples of each nematode at site 4 and 128 samples of each nematode at site 5

Nematode sp.	Nematodes/50 mL		Mean	Variance	Variance : mean ratio
	Min.	Max.			
		Site 4			
Meloidogyne javanica	0	383	47	5822	124
Helicotylenchus dihystera	0	243	28	2436	94
Pratylenchus brachyurus	0	37	4	58	15
		Site 5			
Meloidogyne javanica	0	219	22	1622	73
Helicotylenchus dihystera	0	82	2	101	52
Pratylenchus brachyurus	0	52	2	53	26

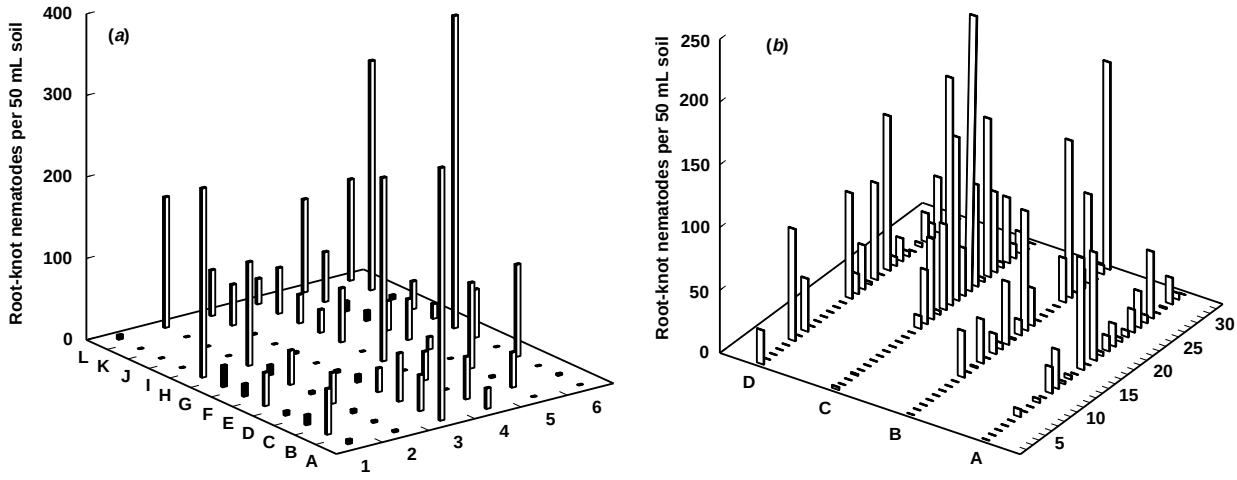

Figure 1. Distribution of root-knot nematode at 5 by 5 m intervals in a pineapple field. (*a*) Site 4, data are for 12 beds (A–L) and 6 sampling positions (1–6). (*b*) Site 5, data are for 4 beds (A–D) and 32 sampling positions (1–32).

ratoon crop yield. The results of our study suggest that nematode densities at the time most damage is occurring on roots (i.e. 9–22 months after planting), provide some indication of the impact that nematodes will have in particular fields or experimental plots.

Results of the 3 field trials confirmed observations by Stirling and Nikulin (1993) that the root damage caused by root-knot nematodes in the plant crop often has little impact on plant crop yields. Thus, at sites 2 and 3, where nematode populations in some treatments were very high and galls were observed on roots when the crop was 12 months old, yields of the plant crop were not reduced. Root-knot nematodes cause greater losses in the ratoon crop, with data from previous experiments (Stirling and Nikulin 1993) suggesting that significant losses can occur when population densities exceed 5–10 nematodes/

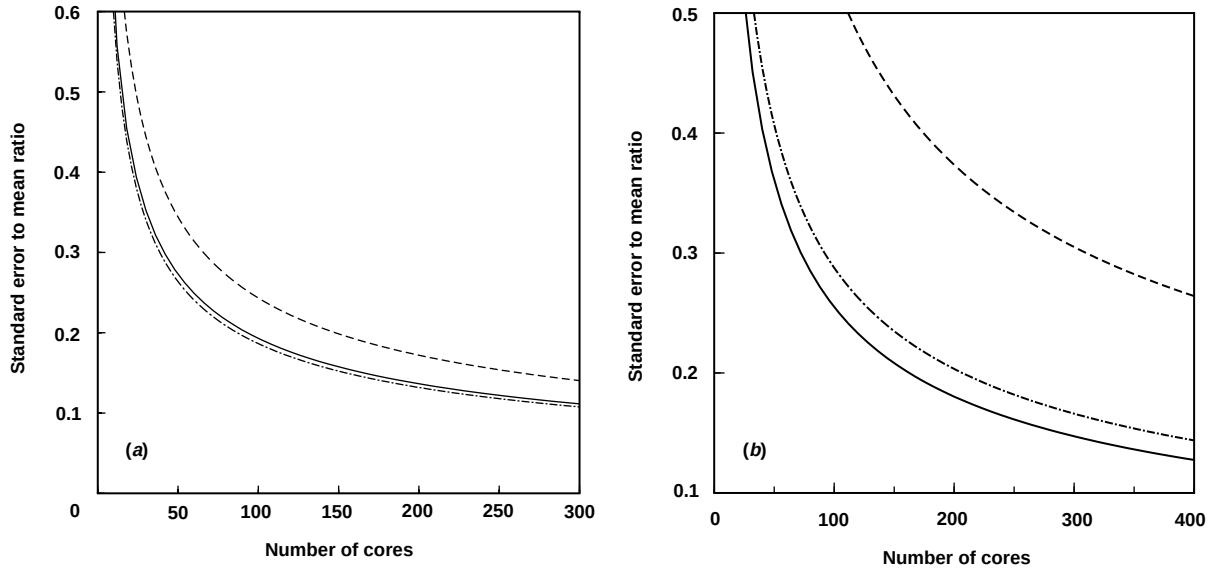

Figure 2. Number of cores required to estimate population densities of root-knot (—), spiral (— —) and lesion (– - –) nematodes with various degrees of precision at (*a*) site 4 and (*b*) site 5.

200 mL soil at 12 months. The results reported here confirm those observations as a 10% reduction in yield occurred when population densities 12–15 months after planting were less than 10 root-knot nematodes/200 mL soil. The tolerance limits determined using Seinhorst's model were also close to zero. Since a 10% yield reduction may cost a pineapple grower as much as A$3000/ha and nematicides cost A$1000–2000/ha, a damage threshold of 1–5 root-knot nematodes/200 mL soil at 12 months may therefore be appropriate for practical purposes. Thus, the nematode situation in Australia is similar to that in South Africa, where the presence of a single specimen of root-knot nematode is indicative of a potential nematode problem (Keetch 1982). The nematode control procedures that are applied before or soon after planting must therefore be used with the aim of maintaining populations of root-knot nematode below this threshold level. In cases where sampling at 12 months indicates that this has not been achieved, growers currently have the option of applying foliar sprays of fenamiphos.

Although our results indicate that few root-knot nematodes are needed at 12 months to cause economic damage, there was not a close relationship between nematode populations and ratoon crop yield. Linear regression and Seinhorst models accounted for less than 50% of the variability in our data, probably because of effects of various environmental factors and other root pathogens. Factors that influence water and nutrient supply and uptake of water and nutrients by roots are known to influence nematode damage functions (McSorley and Phillips 1993). Until their effects are better understood, the main value of a nematode count at 12 months is being able to predict that root-knot nematode will cause yield losses in the ratoon crop. The extent of those losses will depend on future environmental conditions and the standard of crop management.

The low yields recorded in all treatments at site 3 warrant some comment as they could not be explained as a response to nematodes. The whole of the unfumigated area used for this experiment grew poorly compared with the adjacent fumigated area, suggesting the presence of pests or pathogens that are normally controlled by EDB. Because yields at this site were much lower than is normal for the Queensland industry, data from this experiment need to be interpreted with caution. Nevertheless, the trends obtained at this site were similar to those obtained at sites 1 and 2.

Data on damage thresholds are of little practical value unless accurate estimates of nematode population density can be obtained. Results of our sampling studies suggested that 40–70 cores were needed from a 0.25-ha field to estimate root-knot nematode population density to within 30% of its true value. Since it takes 20–30 min to collect 50 soil cores in a pineapple field and a nematode count will cost A$50–80, it is impractical to obtain greater precision by sampling more intensively. Thus, a 50-core sample is probably the most appropriate sampling unit for quantifying nematodes on pineapple. Similar sampling intensities have been proposed for other crops (McSorley 1987).

The practical objective of this work was to use our understanding of nematode distribution, population dynamics and damage thresholds to develop a nematode-monitoring process which would be useful as a management tool. The results of this study have shown that monitoring provides a non-destructive way of determining whether damaging numbers of nematodes are present in a crop. The results of samples at 12–22 months will be particularly helpful, as they provide an indication of possible nematode problems before any above-ground symptoms are apparent. Since the nematicide fenamiphos will increase yields when it is used in a curative manner, monitoring is useful in identifying situations where a nematicide must be used. However, it is not as useful for predicting situations where nematicides are unnecessary. Root-knot nematode is often undetectable for the first 9–12 months after planting, but can then increase rapidly to high levels. Since 1–5 nematodes/200 mL soil at 12 months can reduce ratoon crop yields, the value of using monitoring as a decision-making tool at this time is diminished by the practical difficulties of detecting low nematode densities.

In some treatments at sites 2 and 3, root-knot nematode populations were relatively high at 15 months but had declined to much lower levels after 22 months, probably because roots were damaged and were no longer attractive to nematodes. This observation indicates that nematode population dynamics must be considered when nematode counts are used as a management tool. Results from a single sample can be difficult to interpret, but interpretation is improved if crops are sampled regularly.

Acknowledgments

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