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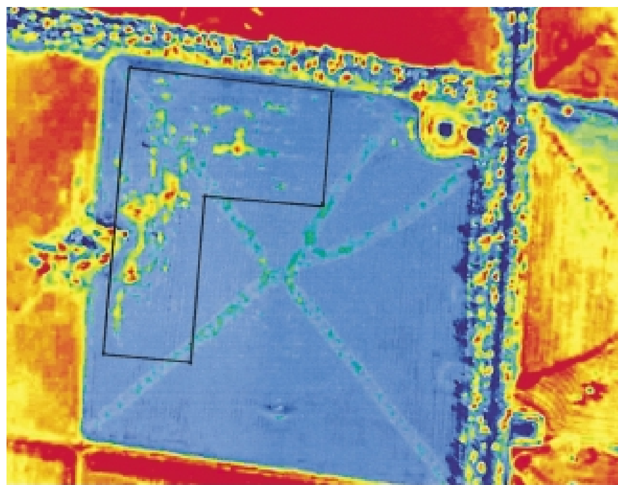

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A sampling strategy to assess banana crops for damage by *Radopholus similis* and *Pratylenchus goodeyi*

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Abstract. The economic threshold of burrowing (*Radopholus similis*) and lesion nematode (*Pratylenchus goodeyi*) on banana may be used to determine whether it is economic to apply nematicide. However, to use such a threshold, a sampling strategy is essential to determine the severity of root damage caused by the nematode. Ten banana crops in south-eastern Queensland and northern New South Wales and 10 in northern Queensland were sampled several times over several years to determine the disease index (percentage cortical root damage caused by *R. similis* and *P. goodeyi*) and nematode populations in roots. The negative binomial distribution and Taylor's power law analysis were used to determine the relationship between the mean and variance of the disease index and nematode populations. Taylor's power law gave the better fit, and was therefore used to determine fixed-precision stop lines for sequential sampling for precision at 20–30% for disease index and 20–40% for nematode populations. Twenty samples per crop were sufficient to achieve 25% precision when assessing nematode infestations using disease index but only 40% precision when using nematode populations.

Introduction

In Australia, bananas are grown mainly in tropical northern Queensland and subtropical south-eastern Queensland and northern New South Wales, with smaller industries in Western Australia and the Northern Territory. In northern Queensland, 96% of the area is infested with *Radopholus similis* (Cobb) Thorne, burrowing nematode (Broadley 1977 in Schipke and Ramsey 1994), which is recognised as the most important nematode pest of bananas worldwide (Gowen 1995). A survey in 1997 (J. M. Stanton and J. A. Cobon unpublished data) found that 53% of crops in the subtropical region were infested with *R. similis* and 8% of crops were infested with *Pratylenchus goodeyi* Sher & Allen, lesion nematode. Both of these nematodes cause cortical root necrosis, plant toppling, reduced bunch weight and increased time between successive bunches.

A rating scale (Broadley 1979) quantifies damage caused by *R. similis* and *P. goodeyi* on banana roots by splitting roots lengthwise and estimating the percentage of the cortex of each root occupied by lesions; values are: 0, no lesions; 1, 1–25% of root cortex occupied by lesions; 3, 26–50%; 5, 51–75%; 7, 76–100%. These ratings are then used to calculate the disease index using the following equation,

$$\text{Disease index} = \frac{\sum \text{Ratings} \times 100}{\text{Total number of roots} \times 7} \qquad (1)$$

To prevent yield loss due to *R. similis* and *P. goodeyi*, growers have used routine applications of non-volatile nematicides which are toxic and expensive (A\$1600–1900/ha.year), and subject to enhanced microbial degradation (Pattison *et al.* 2000). To reduce the frequency of nematicide application required for control, the decision to apply a nematicide could be based on the economic threshold of nematode damage in a crop or on nematode populations as has occurred in Costa Rica and West Africa (Gowen 1995).

However, to determine and use an economic threshold, a sampling strategy is essential to determine the severity of root damage caused by the nematode. In order to use disease index or nematode populations to estimate the nematode status of a crop, it is necessary to obtain a representative sample. Accurate sampling relies on understanding the distribution of the nematode and associated damage. Nematode distribution is typically aggregated and has often been described by the negative binomial distribution, where the variance (s^2) is greater than the mean (x) of the population (McSorley 1987).

However, Allsopp (1990) compared several models for determining the relationship between the mean and variance of nematode counts on sugarcane in Queensland and found that, in general, Taylor's power law (Green 1970) gave a better fit than the other models tested, including the negative binomial distribution.

Our study aimed to assess the ability of the negative binomial distribution and Taylor's power law to describe disease index and nematode distribution. This allowed us to determine the number of samples required to provide a reasonable estimate at a set precision of the nematode status

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of banana crops in the tropical and subtropical banana-growing regions of Australia.

For advisory samples, 25% precision (standard error : mean ratio) is considered a useful guideline (Southwood 1978 in McSorley 1987). Allsopp (1990) found that 25% precision was appropriate for nematodes in sugarcane crops but that 10% precision was impractical. Hutchinson *et al.* (1988) tested 20, 25, 30 and 35% precision while Elliott *et al.* (1997) tested 10, 25 and 40% precision. Both groups found that 25% precision was appropriate when sampling insect populations and that the sample sizes required for greater precision were impractical.

Materials and methods

Ten crops each in subtropical south-eastern Queensland and northern New South Wales (latitude 26°30′ to 28°30′S) and in tropical northern Queensland (latitude 17°15′ to 18°S) were sampled 1–7 times at 3-month intervals. In each crop, 20 plants at the bract fall stage of bunch development were selected on a grid pattern. A grid pattern was considered easier for growers than random sampling. The bract fall stage was chosen for sampling because it is easily recognised by the abscission of bracts covering the hands and lasts for a short time; 2 weeks in the tropics. It is also the time when that pseudostem produces no more roots and older roots have not yet decayed. All crops sampled were of the Cavendish group cv. Williams (AAA genome group) except for 2 farms in the subtropics which were cv. Ladyfinger (AAB) and 1 farm which was cv. Goldfinger (AAAB).

At each sampling, a soil block, 25 by 25 by 25 cm, was cut with a spade next to the bunching pseudostem and lifted. All functional roots were removed from the soil block, split lengthwise and rated for cortical damage using the scale developed by Broadley (1979) as described above. The disease index was then calculated for each plant using equation 1. Nematodes were extracted from all roots for 7 days in a misting chamber and counted.

Statistical analyses

Data for disease index and nematode numbers at each sampling time for each crop were tested using Genstat 5 (1998) for fit to the negative binomial distribution (using a χ^2-test) and to Taylor's power law (using regression analysis).

The negative binomial distribution is described by 2 parameters; the mean (x) and the aggregation coefficient (k). The mean and the variance of the disease index or nematode population can be used to determine the aggregation coefficient (k) as described in equation 2 (Barker *et al.* 1986):

$$k = \frac{x^2}{s^2 - x} . \quad (2)$$

For each set of data that fits the negative binomial distribution, mean and aggregation coefficient of disease index were determined. Linear regression, using Genstat 5 (1998), tested the relationships of k with time of sampling and disease index or nematode populations. A 2-sample t-test examined the effect of region on k.

Aggregated nematode populations are characterised by a low aggregation coefficient and require intense sampling to reduce sampling error (Barker *et al.* 1986). As the nematode population becomes more dispersed, the aggregation coefficient increases (McSorley 1987). For practical purposes, Barker *et al.* (1986) suggested that a population with an aggregation coefficient >8 could be considered as having a near random distribution. The aggregation coefficient and mean may be used to develop a relationship between the number of samples (n) and required precision (D = standard error/mean) by using equation 3 (McSorley 1987):

$$n = \frac{\left(\frac{1}{k} + \frac{1}{x}\right)}{D^2} . \quad (3)$$

Once the number of required samples has been determined above, the error of subsequent sampling can be estimated by rearranging equation 3.

Taylor's power law relates the variance to the mean by equation 4, where a is a sampling factor and b is an index of aggregation. b is constant for a certain species/habitat and ranges from <1 for uniform distributions, to 1 for random distributions and to >1 for aggregated distributions:

$$s^2 = ax^b . \quad (4)$$

Fixed-precision stop lines were calculated using equation 5 where T_n is the cumulative disease index, D is the precision required, n is the number of sampling points, and a and b are the Taylor's power law coefficients (Green 1970):

$$\ln T_n = [(\ln(D^2 / a)) / (b-2)] + [(b-1)/(b-2)][\ln n] . \quad (5)$$

Stop lines for 20, 25 and 30% precision were calculated for disease index and lines for 20, 25, 30 and 40% precision were determined for nematode populations.

Results

The χ^2-tests of the negative binomial distribution showed that, of the 51 and 44 sets of data on disease index from subtropical crops and tropical crops, respectively, 26 and 34 fit the negative binomial distribution. The mean aggregation coefficients (k) for the subtropical and tropical crops were 1.18 and 1.08, respectively. With later sampling k decreased (R^2 = 0.2581, $F_{1,19}$ = 6.61, P = 0.019 in the subtropics, and R^2 = 0.3919, $F_{1,31}$ = 19.98, P<0.001 in the tropics), i.e. the distribution of nematode symptoms became more aggregated, and increased with increasing disease index (R^2 = 0.2948, $F_{1,19}$ = 7.94, P = 0.011 in the subtropics and R^2 = 0.5285, $F_{1,31}$ = 35.86, P<0.001 in the tropics), i.e. the distribution of nematode symptoms became less aggregated. However, region had no effect on k (P = 0.777).

The χ^2-tests of the negative binomial distribution showed that, of the 51 and 44 sets of data on nematode numbers from subtropical crops and tropical crops, respectively, 8 and 9 fit the negative binomial distribution. The mean aggregation coefficients (k) for subtropical and tropical crops were 0.33 and 0.66, respectively. There was no correlation between k and time of sampling (R^2 = 0.4343, $F_{1,7}$ = 4.6, P = 0.076 in the subtropics and R^2 = 0.2652, $F_{1,7}$ = 2.16, P = 0.192 in the tropics) or nematode populations (R^2 = 0.4761, $F_{1,7}$ = 5.45, P = 0.058 in the subtropics and R^2 = 0.1665, $F_{1,8}$ = 1.4, P = 0.275 in the tropics) and was unaffected by region (P = 0.105).

A regression analysis of the disease index data for the tropics and subtropics (weighted for the number of samples used to calculate the mean disease index) using Taylor's power law (ln s^2 against ln x; 2 subtropical data points were omitted because both s^2 and x were zero) indicated that there

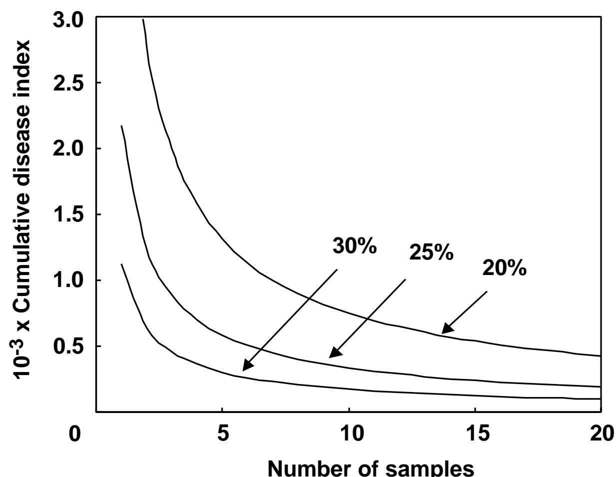

Figure 1. Stop lines for fixed-precision sequential sampling of disease index on banana crops in south-eastern Queensland and northern New South Wales. Arrows indicate the precision.

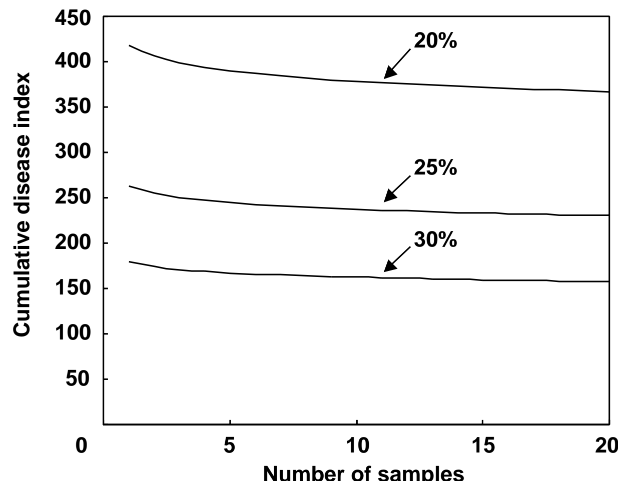

Figure 2. Stop lines for fixed-precision sequential sampling of disease index on banana crops in northern Queensland. Arrows indicate the precision.

were significant differences between the slopes (b) of the regression lines for the 2 regions ($F_{1,91}$ = 6.78, P = 0.011) and hence the data are best represented by separate, non-parallel lines. For both regions, Taylor's power law gave significant linear regressions (tropics, $F_{1,42}$ = 68.77, P<0.001; subtropics, $F_{1,49}$ = 345.5, P<0.001) and in both cases the intercepts differed significantly from zero (tropics, t_{42} = 7.88, P<0.001; subtropics, t_{49} = 10.69, P<0.001). The equation for the tropics is:

$$s^2 = 12.988x^{1.042} \ (R^2 = 0.621) \tag{6}$$

and for the subtropics:

$$s^2 = 4.319x^{1.449} \ (R^2 = 0.876). \tag{7}$$

Using Taylor's power law, Figures 1 and 2 show fixed-precision stop lines for 20, 25 and 30% precision for disease index in the subtropical and tropical regions based on equations 5, 6 and 7. To assess whether the disease index determined using a stop line was different from that when using all samples collected from a crop, all sets of data were tested using the appropriate stop line to determine the mean disease index when the cumulative index reached the stop lines for 20, 25 and 30% sampling precision (Table 1). This showed that, for all data sets, the disease index obtained at the stop line was consistent with that obtained using all samples collected at a particular farm/sampling time. More tropical than subtropical data sets were terminated by 20, 25 and 30% stop lines (Table 1) probably because the disease index was generally higher in the tropics, i.e. there were few low data values in the tropics. However, of those data sets that were terminated by the stop lines, disease index was similar for the tropics and subtropics.

Taylor's power law for nematode populations in roots showed no significant difference between regions for either slope, b ($F_{1,87}$ = 1.12, P = 0.293), or intercept, ln a ($F_{1,88}$ = 1.69, P = 0.197). Thus, a single power law

Table 1. Summary of total data sets (farm × sampling time) and root samples terminated at the 20, 25 and 30% precision stop lines (Figs 1 and 2) for subtropical and tropical banana crops

	Total for farm × sampling time		20% precision stop line		25% precision stop line		30% precision stop line	
	Subtropics	Tropics	Subtropics	Tropics	Subtropics	Tropics	Subtropics	Tropics
Mean disease index for all samples	6.9	14.6	—	—	—	—	—	—
No. of data sets	51	44	1	12	4	26	17	35
Mean no. root samples/data set terminated by stop line	—	—	18	17	16	14	13	11
Mean disease index for data sets terminated by stop line	—	—	30.0	24.8	18.0	21.0	14.2	19.0
Mean disease index for data sets not terminated by stop line after 20 samples	—	—	6.6	10.7	6.2	7.1	3.7	5.1

regression (equation 8) was used to relate s^2 to x of nematode populations for both regions ($F_{1,89}$ = 1694.5, P<0.001).

$$s^2 = 18.029\,x^{1.723} \quad (R^2 = 0.950)\,. \qquad (8)$$

Stop lines were calculated as for the disease index data. Stop lines for 20 and 25% precision for nematode numbers required unrealistic numbers of samples so only stop lines for 30 and 40% precision are shown (Fig. 3).

The negative binomial distribution failed to model the disease index data for 35% of the crops and failed to model nematode data for 82% of the crops. Therefore, since the Taylor's power law analysis incorporated all the data and gave significant regressions, it was chosen as the better descriptor of the relationship between the mean and the variance.

To compare the precision of disease index and nematode populations for assessment of nematode severity, we compared the number of data sets that would have crossed the 30% precision stop line. When using disease index, 52 of 95 data sets crossed the 30% precision stop line (Table 1) while, when using nematode populations, only 1 of 97 data sets crossed the 30% precision stop line. A χ^2-test indicated a strong association between the method of assessment (disease index or nematode populations) and numbers of samples crossing the 30% precision stop line (χ_1^2 = 69.27, P<0.001). Thus, sampling is more likely to achieve 30% precision if the disease index, rather than nematode population, is used to assess nematode severity.

In the tropics, there was a significant correlation between disease index and nematode populations in roots with the following relationship:

Disease index = nematodes/100 g roots/71.3 (R^2 = 0.316).

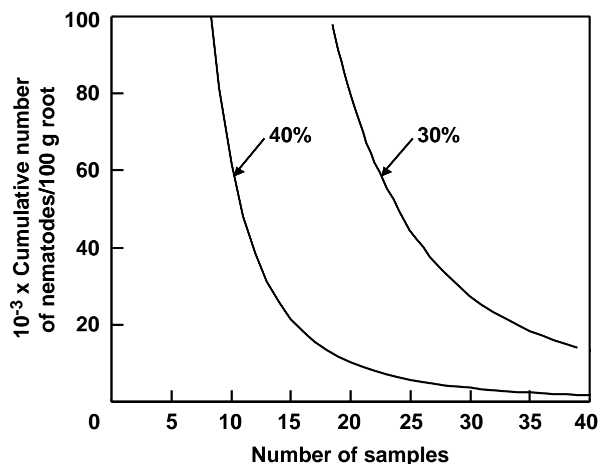

Figure 3. Stop lines for fixed-precision sequential sampling of numbers of *Radopholus similis* and *Pratylenchus goodeyi* on banana crops in Queensland and New South Wales. Arrows indicate the precision.

Precision of 30 and 40% (50 of 97 data sets crossed the 40% precision stop line) in nematode populations in 20 samples required mean nematode populations of 4038 and 508/100 g root, respectively (Fig. 3), which correspond to respective disease indices of about 57 and 7 in the tropics.

Discussion

Depending on the nematode decline between successive banana crops, we recommend monitoring disease index early in the life of the new crop when nematode damage is low. The aim is to determine when nematicide treatment is first required. If monitoring continues every 3 months until the disease index reaches the economic threshold, our study shows that it is unlikely that the economic threshold will be significantly exceeded between sampling times.

To use the fixed-precision stop line obtained from Taylor's power law, cumulative disease index is plotted successively against the sample number until the stop line is crossed. At this stage, the mean disease index is estimated as T_n/n. If it is not practical to calculate disease index following collection of each successive sample, then 20 samples can be collected initially and excess samples discarded once the stop line has been crossed.

This study showed that, using disease index to assess nematode severity, 20 samples per crop were sufficient for 25% precision, which is considered acceptable (Southwood 1978 in McSorley 1987). If the resulting disease index is less than the economic threshold, sampling should be repeated every 3 months. This procedure is likely to detect crops requiring nematode control within one bunching cycle but with a practical sample number.

Where 20 samples were not sufficient to terminate sampling at the 30% precision stop line, the resulting mean disease index was very low (3.7 for the subtropics and 5.1 for the tropics) (Table 1; Figs 1 and 2) and unlikely to cause economic loss. This assessment is based on economic thresholds of about 10 in the tropics (A. B. Pattison and J. M. Stanton unpublished data) and 20–45 in the subtropics (J. M. Stanton and J. A. Cobon unpublished data). It would also be considered very low in Costa Rica where the economic threshold is estimated at 10 000 (Araya *et al.* 1996) to 20 000 (Pinochet 1987) *R. similis*/100 g roots and in Ivory Coast where 1000 *R. similis*/100 g roots are considered to cause economic loss (Guerout 1972).

If disease index is overestimated, nematicide may be applied before it is required but costs will be no greater than if nematicide was applied routinely. If disease index is underestimated, then nematicide may not be applied when it is first required and sampling will continue every 3 months. However, because disease index increases very slowly in the tropics (0.5 per month, A. B. Pattison and J. M. Stanton unpublished data) and not appreciably in the subtropics (J. M. Stanton and J. A. Cobon unpublished data), little extra

damage is likely to result from delaying nematicide application for 3–6 months.

A sampling strategy is most likely to be used by farmers or consultants if it is simple and quick. Sampling and assessing 20 plants per crop takes about 90 min, which is practical. There is currently a trend in northern Queensland toward monitoring disease index by using 20 samples per banana crop with more than 3000 ha in northern Queensland being tested for disease index before applying nematicide. This is usually undertaken by paid consultants while visiting the farms for other reasons so cost is very low. Even if monitoring occurs during a dedicated visit to the farm, the cost is still lower than routine application of nematicide. In addition, monitoring avoids unnecessary application of nematicide with its attendant health and environmental risks.

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