Development of Sampling Strategies for *Pachymetra chaunorhiza*, a Sugarcane Root Pathogen

R. C. Magarey

Research plant pathologist, Bureau of Sugar Experiment Stations, P.O. Box 566, Tully, Queensland, Australia 4854
Accepted for publication 23 June 1989 (submitted for electronic processing).

ABSTRACT

Magarey, R. C. 1989. Development of sampling strategies for *Pachymetra chaunorhiza*, a sugarcane root pathogen. Phytopathology 79:1306-1309.

Sampling procedures associated with an assay for *Pachymetra chaunorhiza*, a root pathogen of sugarcane in Queensland, Australia, have been established and sampling strategies were investigated. A computer simulation model was used to assess two field-plot sampling strategies using spatial pattern data for *P. chaunorhiza*. A sequential strategy was developed for sampling laboratory-processed soils using Iwao's patchiness regression model. Assay variation when thoroughly mixed soils were repeatedly subsampled and assayed also was investigated. Only one assay per soil was found necessary to provide representative results.

Additional keywords: soilborne pathogen, soil oospores, sugarcane root rot.

Pachymetra chaunorhiza Croft & Dick, an oomycete associated with sugarcane poor root syndrome in the coastal districts of Queensland, Australia, is a recently described pathogen (2) and is considered a major factor leading to the development of poor root systems in affected fields (4). The pathogen causes a flaccid rot of primary shoot roots resulting in reduced sugarcane yield and stool anchorage problems. An assay has been developed for *P. chaunorhiza* based on the direct count of extracted soil oospores (4).

All assays attempting to quantify field inoculum density require sampling strategies. Sampling in the assay for *P. chaunorhiza* is undertaken at the following points in the assay procedure: 1) soil sampling in field plots, 2) subsampling bulked soil collected from field plots for laboratory processing, and 3) subsampling laboratory-processed soil samples for oospore counts.

This paper outlines the research undertaken in the development of sampling procedures for the assay for *P. chaunorhiza*. The objectives of the research were to minimize the error (variability) and costs associated with sampling procedures employed with the assay. Soil sampling in field plots was investigated through computer simulation modeling using field spatial pattern data. Subsampling bulked soil cores was investigated by undertaking analysis of variance on repeat assays from thoroughly mixed soils. Subsampling laboratory-processed soil samples was investigated through the fitting of several mathematical models to data and using one of the models to predict the number of oospore counts required to achieve desired precision levels.

MATERIALS AND METHODS

Field-plot sampling. The objective when sampling field plots must be to obtain the minimum quantity of soil (minimizing costs associated with sampling) with characteristics representative of those within the plot (minimizing errors). A practice commonly used in plot sampling is to take a predetermined number of soil cores from the plot using a sampling scheme (usually random selection), bulk these soil cores, and subsample the composited soil cores to obtain soil for assay. The number of cores required per plot to provide a representative soil sample depends upon the spatial pattern of the organism under study.

One approach to determine the details of a satisfactory field-sampling procedure is to do replicated plot sampling and analyze the variation in assay results versus the number of cores taken per plot. With this approach it is assumed that the standard deviation of the mean on replicated (repeat) samplings from a single plot will decrease asymptotically as the number of cores per composite sample is increased. The number of cores taken per plot in the selected strategy should be the minimum number leading to minimal variability, at reasonable cost. This approach is time consuming and limiting with regard to analyses that may be undertaken using assay data.

A second approach is to create a computer simulation of replicated plot sampling, using spatial pattern data. This method has the added advantage of facilitating the analysis of alternative sampling strategies without increasing the required field and laboratory work. This alternative approach was adopted for investigating field-plot sampling for *P. chaunorhiza*.

To provide a basis for computer simulation studies, spatial pattern data for *P. chaunorhiza* were obtained from infested fields in major Queensland cane-growing districts in the following way. Experimental plots (0.009 ha, 4 rows × 15 m) were marked out at field trial sites in northern (Mourilyan), central (Walkerston), and southern (Gooburrum) Queensland. This plot size is representative of those regularly sampled for assay for *P. chaunorhiza*. The regions where the experiments were located cover a large part of the geographical range of *P. chaunorhiza*. Sugarcane had been grown at each site for a number of years, and sites were known to be infested with the pathogen. After the destruction of the previous crop, the land had been prepared for planting, by plowing and rotary hoeing, before sampling began. To obtain data on the spatial pattern of the pathogen, plots were sampled in a uniform manner at 2-m intervals along the planting line (each row, 28 core samples per plot). At the Mourilyan site, samples were taken using a 40-mm soil corer, whereas at the Walkerston and Gooburrum sites, samples were taken using a 40-mm-diameter Edelman (Eijkelkamp, Netherlands) auger. Sampling depth was to 450 mm (5); core samples were placed in separate labeled plastic bags for transport to the laboratory. Each sample was thoroughly mixed and assayed using the technique described elsewhere (4).

Two commonly used plot sampling strategies are selecting cores completely at random within plots and a stratified random strategy where at least one core is randomly selected from each row of the plot. A computer program was written that facilitated a simulation of each of these strategies using the field data. When the spatial pattern data had been obtained, the standard deviation of the mean in the simulation oospore count was calculated when 100 replications of 1, 2, 3, or 4, etc., cores per plot, up to 28 cores per plot, were chosen in a completely random fashion. A stratified random sampling strategy was simulated by calculating the mean oospore count when 100 replications of 4 (1 core per

© 1989 The American Phytopathological Society

row), 8 (2 cores per row), 12 (3 cores per row), etc., up to 28 cores, were chosen per plot. With both sampling methods, a "with replacement" strategy was employed (that is, core data could be selected more than once in each "composite" sample). The standard deviation of the mean in the simulation was calculated for each number of cores per composite sample, and comparisons were made between sampling methods.

Subsampling composite samples. The variability in spore counts associated with subsampling bulked soil samples was determined by taking replicated subsamplings from the same composite soil. In established field experiments in northern (Babinda and Mourilyan districts) and central (Walkerston) Queensland, plots were sampled (6 cores per plot), and composite samples were mixed thoroughly by hand. That is, soils were laid out on plastic sheeting, spread out in a thin layer, heaped up, and spread around. This process was repeated a number of times.

Five repeat subsamples were taken from each composite soil, and five oospore counts were made per subsample. Results were analyzed using analysis of variance.

Subsampling laboratory-processed soil samples (oospore counts). Several mathematical models were examined to determine which best described the variability within oospore count data. The model describing the variability most adequately was used to determine the number of oospore counts required per assay to give fixed levels of precision and fixed-precision-level "stop lines," which indicate when data have reached a fixed level of precision.

Between 1985 and 1988, many assays were conducted on soil samples collected from the experimental sites described above. With each sample assayed, five oospore counts were conducted. From these data, counts performed on 100 representative samples were analyzed using the mathematical models.

Statistical analysis. Means and variances were calculated for each set of five oospore counts per assayed sample. Dispersion indices were calculated using the Poisson and negative binomial distributions, Taylor's power law (7), and Iwao's patchiness regression (3). If the data follow the Poisson distribution (that is, random pattern), the mean oospore count ($\bar{x}$) equals the variance (s^2). Departures of $s^2/\bar{x}$ from unity were tested by calculating the index I:

$$I\ [s^2(n-1)]/\bar{x}]$$

in which n is the number of oospore counts comprising a sample. I has an x^2 distribution with $n - 1$ degrees of freedom when the Poisson distribution holds.

Two parameters, the mean and the dispersion parameter k, describe the negative binomial distribution. The parameter k is a measure of the amount of clumping and often has values around 2, becoming larger as the distribution approaches the Poisson. The value of k was calculated for each assay sample using the moment method described by Southwood (6).

The Taylor power law relates the variance to mean oospore count through $ln\ (s^2) = a + b\ ln\ (x)$, where the intercept is a sampling factor and b, the slope, is an index of aggregation. Distributions are uniform when $b < 1$, random when $b = 1$, and aggregated when $b > 1$ (7). Ordinary least squares were used to estimate a and b.

The method described by Iwao (3) uses a linear regression of mean crowding ($\mathring{m}$) on the mean through the model $\mathring{m} = \alpha + \beta\ \bar{x}$, where α and β are regression constants. The slope β, the density-contagiousness coefficient, takes on values of $\beta < 1$ for uniform distributions, $\beta = 1$ for random distributions, and $\beta > 1$ for aggregated distributions. In this analysis α and β were calculated using ordinary least squares by first calculating $\mathring{m}$ as $\bar{x} + [(s^2/x) - 1]$. Using the model of best fit, a sequential sampling strategy for oospore counts was developed so that counts could be terminated when the desired precision level was achieved.

RESULTS

Field-plot sampling, computer simulation. Analysis of variance of field spatial pattern data suggested that at the Mourilyan site,

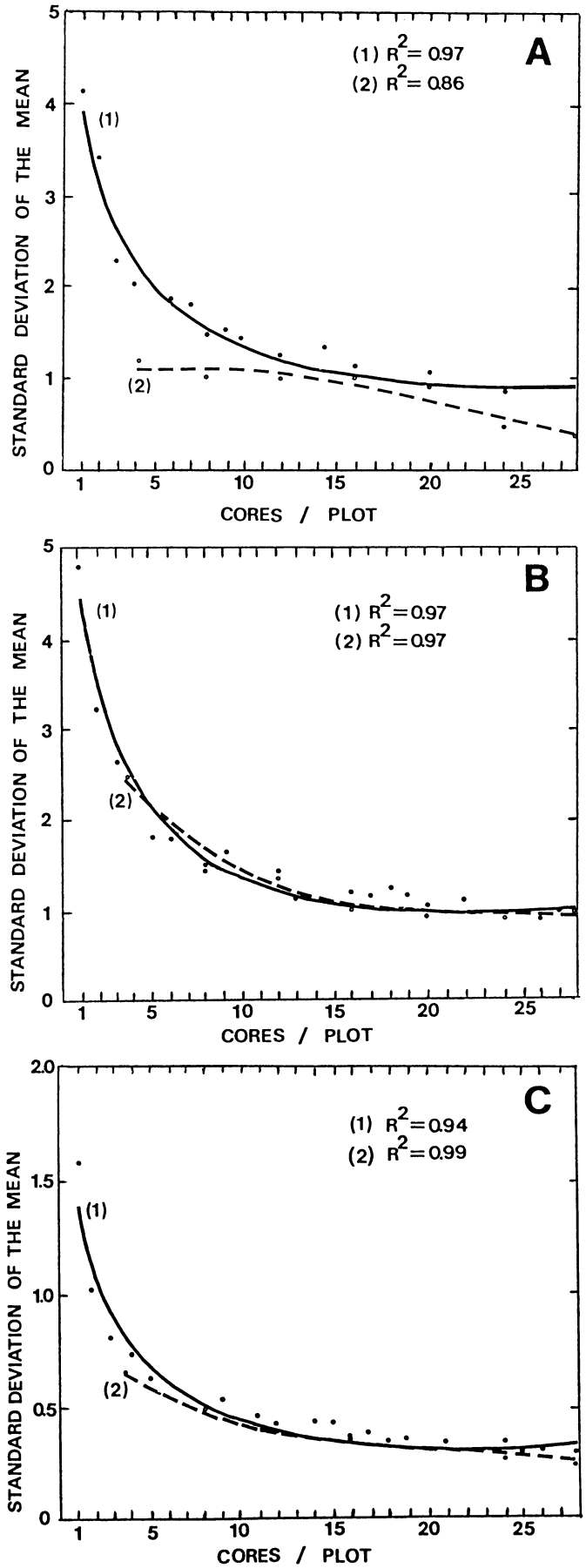

Fig. 1. Computer simulation of the relationship between the standard deviation of the mean oospore count (100 replications) and the number of cores per plot for completely random (1) and stratified random (2) sampling strategies using spatial pattern data from three field sites: **A,** Mourilyan; **B,** Walkerston; and **C,** Gooburrum.

but not the Walkerston and Gooburrum sites, inoculum density varied significantly ($P < 0.05$) among rows within each plot. Accordingly, simulated sampling suggested that only with the Mourilyan data was the stratified sampling strategy more efficient than the completely random strategy in reducing sampling variation. This effect was noted particularly when 4 or 8 cores were selected per plot. At all three sites, sampling variation declined rapidly up to sampling intensities of 10–12 cores per plot, whereupon increasing the number of cores per plot led to a relatively small decrease in sampling variation. The results are presented in Figure 1.

Subsampling composite soil samples. Analysis of variance indicated that only one assay per soil was required ($P < 0.05$). A single 100-g sample thus is representative of a set of composited soil cores as long as the cores are thoroughly hand mixed.

Subsampling laboratory-processed soil samples. The analyses undertaken using each of the models indicated that oospore counts within each assay sample were distributed randomly. The I statistic indicated that the variance was not different from the mean (with a probability greater than 95%). With the negative binomial analysis, k was estimated at 56 ($P = 0.001$) suggesting a random distribution. A value of 1.04 for b ($P < 0.001$) was obtained using Taylor's power law; the t statistic indicated that b was not significantly different from 1.0 ($P < 0.001$). The R^2 value in the regression was 0.53. Iwao's patchiness regression showed a good fit to the data. A value of 1.02 was calculated ($P < 0.001$), suggesting a random distribution. The R^2 value was 0.99.

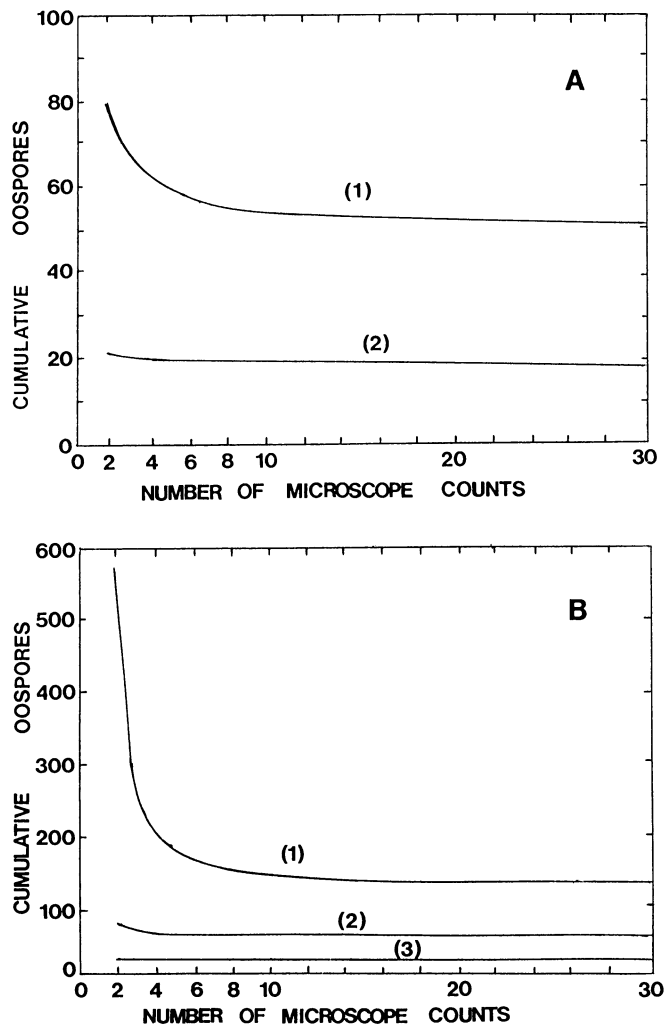

Fig. 2. The relationship between the cumulative number of oospores counted and the number of microscope counts, and precision-level "stop lines," in the sequential sampling strategy for oospore counts in the assay for *Pachymetra chaunorhiza*. **A,** Precision levels of 0.15 (1) and 0.25 (2) of the standard deviation of the mean. **B,** Precision levels of 0.10 (1), 0.15 (2), and 0.25 (3) of the standard deviation of the mean.

A sequential sampling strategy for oospore counts was developed using Iwao's patchiness regression model (3) and the following equation:

$$Tn = \frac{\alpha + 1}{D^2 - [(\beta - 1)/n]}$$

where Tn = total number of oospores counted, α and β are calculated regression constants, D = standard error of the mean, and n = number of microscope counts. Stop lines were calculated for precision levels $D = 0.10$, 0.15, and 0.25 (Fig. 2). Using these data, the relationship between the number of oospore counts per assayed sample and the number of oospores per microscope count, and the stop lines were established. These results are illustrated in Figure 3.

DISCUSSION

Sampling strategies are an important component of any assay procedure and need to be researched so that the precision of assay results can be assessed (1). The variation associated with assay procedures has been reported in only a few studies (1). This paper reports on sampling strategies and associated variation for the assay for *P. chaunorhiza*.

Field sampling was investigated in fields prepared for planting. The spatial pattern of the pathogen will vary according to field conditions and cropping history, and field sampling strategies should be further researched if sampling is undertaken in situations other than those described.

Computer simulation of field sampling using spatial pattern data proved useful in this study and demonstrates the benefit of having relevant spatial pattern data. At the three sites where field sampling was investigated, relatively few cores were required per plot to reduce the variation associated with field sampling to minimal levels. The stratified random strategy, as expected, increased the sampling efficiency only at the site where significant differences in row populations were noted. The benefit of the strategy under these circumstances warrants its use as a standard technique when sampling fields for *P. chaunorhiza*.

Only one subsample from soil composites mixed thoroughly by hand is required when conducting assays for *P. chaunorhiza*. Hand mixing of soils should be the procedure adopted with the assay for *P. chaunorhiza*.

Sequential sampling techniques minimize costs associated with generating data of a known level of precision. Such a technique proved particularly useful when applied to sampling soil-oospore suspensions. It is estimated that, for each assay sample, field-plot sampling (12 cores per plot) and mixing of composite samples

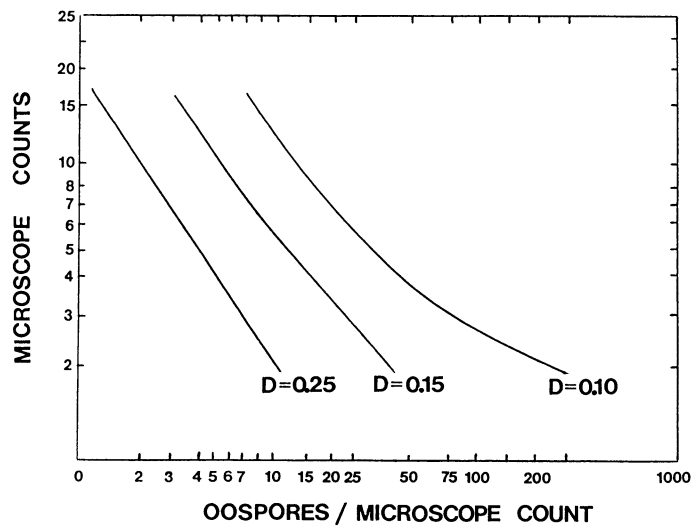

Fig. 3. Log-log plot of the relationship between the number of microscope counts and oospores per microscope count, and precision level (D = fraction of the standard deviation of the mean) in the assay for *Pachymetra chaunorhiza*.

takes approximately 15 min, and each oospore count (approximately five counts are undertaken per assay sample) takes 8 min. Minimizing the number of oospore counts required per assay to achieve the desired level of precision in the data therefore is important in reducing labor input and hence costs associated with the assay.

No fixed number of oospore counts can be recommended for all assays for *P. chaunorhiza*. The number required will depend on the precision required in the data and the number of oospores per count which is a reflection of field inoculum density.

The data presented in Figure 2 should be considered not only when assays are in process but also when selecting sites for experiments, if full benefit of the strategy is to be achieved. Treatments leading to small changes in inoculum density will require a high level of precision for treatment effects to be significant. Experiments incorporating such treatments should be located at field sites with relatively high inoculum density if the number of oospore counts is to be kept within practical limits and costs kept to a minimum. Conversely, treatments leading to large changes in inoculum density may require a much lower level of precision to show significant differences among treatments and could well be located at sites with much lower inoculum densities (provided epidemiological considerations are favorable). The data presented provide a quantitative basis for assessing the suitability of field sites.

The time taken and costs associated with the assay for *P. chaunorhiza* depend on the treatments included in the experiment, the inoculum density prevailing at the field site, and the precision required in the data. Research reported here provides a basis for quantifying the variation associated with each sampling technique in the assay procedure and for minimizing costs. The results will be used to further investigate the spatial pattern of *P. chaunorhiza* in whole fields in an attempt to identify underlying environmental factors influencing Pachymetra root rot.

LITERATURE CITED

1. Campbell, C. L., and Noe, J. P. 1985. The spatial analysis of soilborne pathogens and root diseases. Annu. Rev. Phytopathol. 23:129-148.
2. Dick, M. W., Croft, B. J., Magarey, R. C., de Cock, A. W. A. M., and Clark, Gillian. 1988. A new genus of the Verrucalvaceae (Oomycetes). Bot. J. Linn. Soc. 99:97-113.
3. Iwao, S. 1968. A new regression method for analyzing the aggregation pattern of animal populations. Res. Popul. Ecol. 10:1-20.
4. Magarey, R. C. 1989. Quantitative assay of *Pachymetra chaunorhiza*, a root pathogen of sugarcane in Australia. Phytopathology 79:1302-1305.
5. Reghenzani, J. R. 1984. Northern Poor Root Syndrome—Its profile distribution and the effects of temperature and fallowing. Proc. Aust. Soc. Sugar Cane Technol. 1984 Conf. pp. 79–86.
6. Southwood, T. R. E. 1978. Ecological Methods with Particular Reference to the Study of Insect Populations. 2nd ed. Chapman & Hall, New York. 524 pp.
7. Taylor, L. R. 1961. Aggregation, variance and the mean. Nature 189:732-735.