Quantitative Assay of *Pachymetra chaunorhiza*, a Root Pathogen of Sugarcane in Australia

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ABSTRACT

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A quantitative assay based on the direct count of oospores extracted from soil was developed for *Pachymetra chaunorhiza*, a recently described pathogen associated with poor root syndrome of sugarcane in Australia. The efficiency of oospore recovery and the size distribution of oospores in a naturally infested soil were investigated. The relationship between soil oospore density and *P. chaunorhiza* root rot was investigated with a susceptible sugarcane cultivar Q90 growing in pasteurized soil inoculated with cultured oospores, in naturally infested field soils diluted with pasteurized soil, and in assayed soils infected with *P. chaunorhiza*. The results suggest a strong relationship between oospore population and root rot and indicate that the assay is a useful method for quantifying *P. chaunorhiza* in field soils.

Additional keywords: soilborne pathogen, sugarcane root rot.

Poor root syndrome (PRS) is a root disease affecting sugarcane in the coastal districts of Queensland, Australia. Decreased yield and harvesting and milling problems created by the syndrome have caused serious concern (6,9). Two pathogenic oomycete fungi are commonly associated with diseased root systems. One, a fungus recently described (8) and known only from Queensland cane fields, is *Pachymetra chaunorhiza* Croft & Dick, and the other has been identified as *Pythium graminicola* Subr. *P. chaunorhiza* causes a flaccid rot of primary shoot roots in susceptible sugarcane cultivars (Pachymetra root rot) and is considered responsible for much of the stool anchorage problems associated with PRS. The effect of *P. chaunorhiza* on yield also is thought to be considerable, and experiments investigating this effect are in progress. Oogonia of *P. chaunorhiza* are produced in large numbers in diseased roots and their large blunt projections (Fig. 1) are of reliable diagnostic value (6). They vary in size from 30 to 60 μm, with a mean of 48.1 (8). Investigations into the taxonomy of *P. chaunorhiza* and the etiology of Pachymetra root rot suggest that zoospores usually are not produced by the fungus (8,12). Oospores are the only propagules of the pathogen that have been identified (12). An assay for the pathogen was needed to study the effect of various environmental factors and control measures on soil inoculum levels. The characteristic appearance of the oospore and the apparent lack of other types of propagule suggested that direct counting of oospores could be used to quantify soil inoculum density. Many assays have been developed to measure soil inoculum densities for species of *Pythium* and *Phytophthora* (4,7,10,11,13-19,21,22), but few assays for pathogenic oomycetes rely on the direct observation of propagules from soil (3,4,13,15).

This paper describes an assay for *P. chaunorhiza* based on the direct count of oospores extracted from soil. The relationship between soil oospore populations and *P. chaunorhiza* root rot is examined in soils infested with cultured oospores and naturally infested soils supporting a range of oospore populations.

MATERIALS AND METHODS

Soil samples from 32 fields affected by PRS in north and central Queensland were collected in January to March 1984 from land growing sugarcane. Soil was removed to a depth of approximately 25 cm, dry sieved (0.5-cm-diameter aperture), and mixed thoroughly by hand, and a 100-g sample was collected from each for assay. Oven dry weight was determined for each soil, and additional soil was stored for glasshouse experiments.

Oospore count assay. A 100-g sample of each soil was blended for 60 sec in 1 L of water, then washed carefully through a set of Endecott sieves (with apertures of 500, 250, 125, 63, and 38 μm) (Endecotts Ltd., London, England). After thorough rinsing of each sieve, soil particles and oospores collected on the 38-μm sieve were suspended in 300 ml of water, to which 2.5 ml of sodium hypochlorite solution (3.5% available chlorine) was added, and the flask containing the suspension was sealed. After 24 hr, oospore suspensions were rinsed in the 38-μm sieve and resuspended in 100 ml of water containing 1.6 ml of a 2.5% (w/v) solution of methyl blue in lactophenol. After staining for 4–7 hr, the suspensions were again rinsed in the 38-μm sieve, resuspended in 300 ml of water, and held in 500-ml Erlenmeyer flasks. Uniform stirring of suspensions was ensured by using a magnetic stirrer operating at a set angular velocity. Oospore counts were performed by taking 1-ml subsamples of the resulting suspension and counting the oospores in a Hawksley eelworm counting chamber (Hawksley & Sons, Lancing, England) under a microscope at ×100 magnification. Oospores of *P. chaunorhiza* stained bright blue in contrast to the nonstaining soil particles and were distinguished by their characteristic morphology. Use of sodium hypochlorite as a bleaching agent was necessary in the assay procedure so that oospore morphology could be observed clearly. The bleaching and staining procedures allowed oospores of *P. chaunorhiza* to be readily distinguished from soil and organic matter particles of similar shape and size.

Recently the staining method has been simplified. After the sodium hypochlorite treatment, oospore suspensions are made up to 300 ml in a 500-ml beaker and 0.5 ml of methyl blue stain is added. With no intermediate rinsing, the oospore suspension is subsampled after 12–48 hr, and counts performed as before.

Oospore density and Pachymetra root rot versus soil particle size. How soil oospore density and Pachymetra root rot relate to soil particle size was investigated using a Spanos series soil from the Mourilyan district of northern Queensland which was naturally infested at a high level with *P. chaunorhiza*. A 100-g sample of soil was blended and sieved according to the assay procedure, and deposits on the standard five sieves and an additional 20-μm-aperture sieve were collected and thoroughly mixed into 1.4 kg (dry weight) of pasteurized (70 C, 30 min) soil from the same site. For each particle-size fraction, 13 clay pots (15-cm-top diameter) were infested in this way. Single-bud cuttings of the susceptible sugarcane cultivar Q90 were germinated in University of California potting mix type BII (1), grown until the plants were 10–20 cm high, then transplanted into the clay

pots. Each pot contained one pregerminated plant and was fertilized with 0.343 g of K_2HPO_4 and 0.153 g of NH_4NO_3. At 4 wk, 0.115 g of urea was applied to each pot. Plants were maintained for 6 wk in air-conditioned benches (16) operating between 25 and 30 C in a glasshouse. Pots were subirrigated using clay saucers 2 cm deep. At harvest, roots were washed free of soil, and the percentage of rotted primary shoot roots was determined as a measure of Pachymetra root rot. Particle-size fractions associated with Pachymetra root rot were assayed for oospores (repeated five times).

Recovery of oospores. To determine the efficiency of the assay procedure in recovering oospores, known numbers of cultured oospores were added to field soil free of *P. chaunorhiza* (Spanos series from the Mourilyan district), and an assay was undertaken. Assays on checks confirmed that oospores of *P. chaunorhiza* were not present before inoculation. *P. chaunorhiza* was cultured at 20 C in the dark in 300-ml-capacity aluminium foil trays containing 150 ml of Difco corn meal agar (Difco Laboratories, Detroit, MI), sealed within clear polypropylene bags. Oospores were harvested by blending (Instablend, General Electric Co., Fairfield, CT) the cultures in distilled water for 60 sec and sieving the resulting suspension through 63- and 38-μm-aperture sieves. Oospores collected on the 38-μm sieve were used in the experiment. Soils were infested at a rate of 182 oospores/g, and the experiment was repeated five times.

Development of *P. chaunorhiza* infectivity bioassay. Pasteurized (70 C, 30 min) PRS-affected soil (Thorpe series soil from the Babinda district of northern Queensland) was infested with cultured oospores of *P. chaunorhiza* at rates ranging from 0 to 200 oospores/g dry weight of soil. Oospores were mixed by hand into infested soils. In repeat experiments, soil from the same site and from a site in the Mourilyan district (Spanos series soil) was pasteurized (70 C, 30 min) and infested at rates ranging from 0 to 80 oospores/g dry weight of soil. For each rate of inoculum, five to seven clay pots (15-cm-top diameter) were filled with inoculated soil and planted with a single pregerminated plant of the cultivar Q90. Trial maintenance procedures were the same as those described above. At harvest, the percentage of rotted

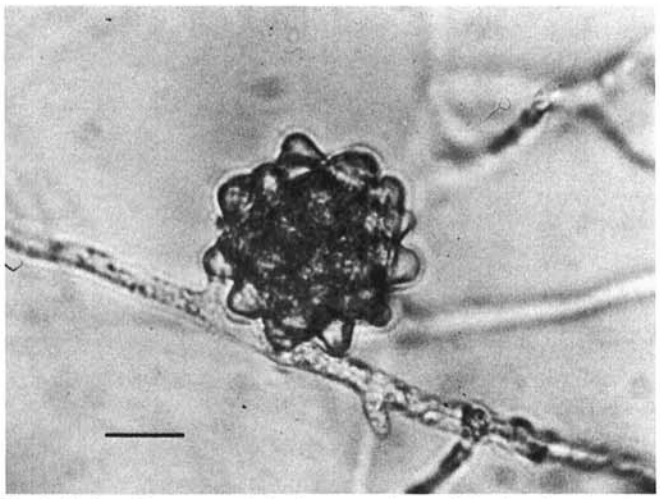

Fig. 1. Cultured oospore of *Pachymetra chaunorhiza* showing the characteristic projections of the oogonial wall. Scale bar represents 20 μm.

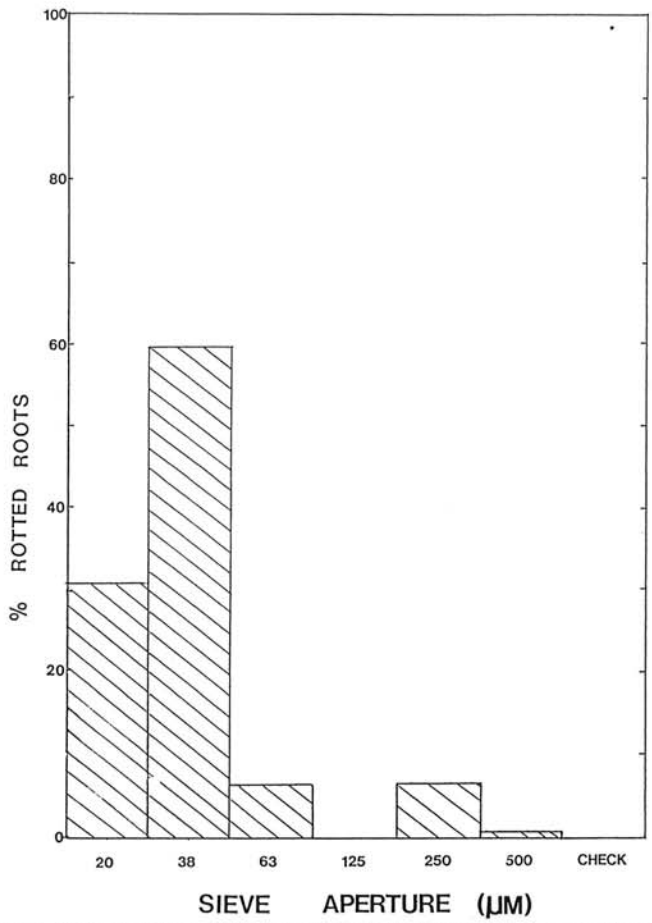

Fig. 2. Soil particle size (sieve deposits) versus the percentage of primary roots with Pachymetra root rot in the sugarcane cultivar Q90.

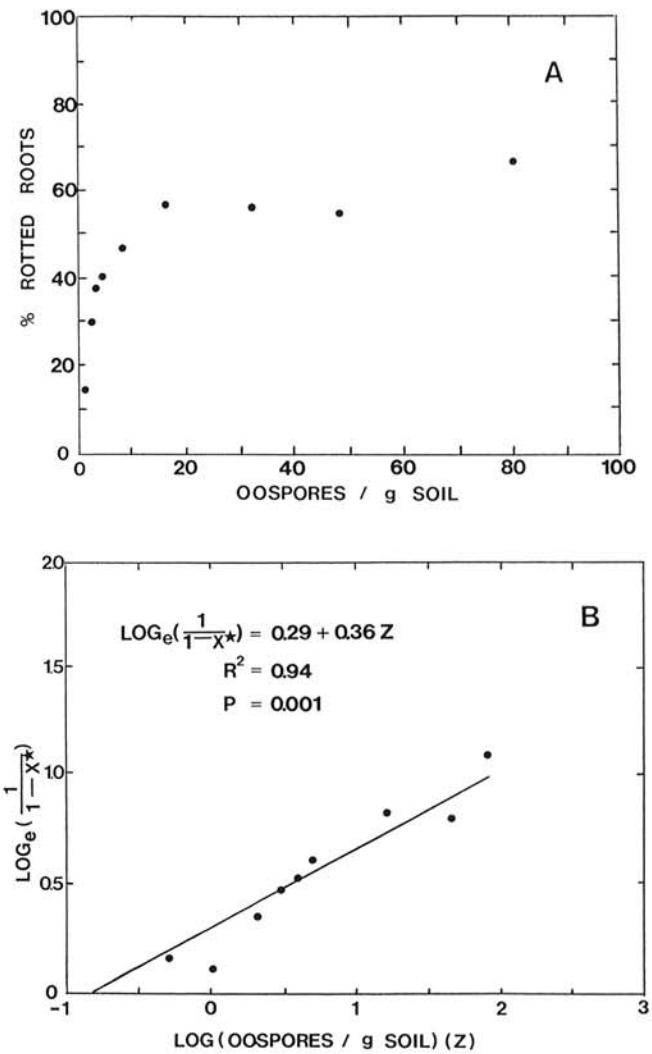

Fig. 3. Relationship between percent rotted primary shoot roots of the sugarcane cultivar Q90 and oospore density of *Pachymetra chaunorhiza*. Soil infested with cultured oospores. X^* is the fraction of rotted primary shoot roots; Z is the log of oospores per gram of soil. **A,** Arithmetic plot. **B,** Multiple infection plot.

primary shoot roots was determined as a measure of Pachymetra root rot.

The relationship between soil oospore density and root rot also was investigated in soils naturally infested with *P. chaunorhiza*. Infested soil was diluted with pasteurized (70 C, 30 min) soil from the same site to give oospore populations ranging from 0 to 400 oospores/g dry weight of soil.

Assay of field soils for *P. chaunorhiza* root rot. For each of the 32 soils infested with *P. chaunorhiza*, five clay pots were filled with approximately 1.4 kg dry weight of soil and planted with the susceptible cultivar Q90. At harvest, the percentage of rotted primary shoot roots was determined as a measure of Pachymetra root rot.

Statistical analysis. The relationship between oospore density and Pachymetra root rot in naturally infested and inoculated soils was examined after transformation of the data to account for multiple infections (2) and after a logarithmic transformation of inoculum data (oospores per gram). Standard linear regression analysis was used to describe the relationship. Slopes of the regression lines were compared using the *t* statistic (20, p. 173).

RESULTS

Soil oospore populations and Pachymetra root rot versus soil particle size. Pachymetra root rot was associated with several particle-size fractions, but the greatest amount was associated with the fraction of 38–63 μm (Fig. 2). Root rot was consistently observed in the fractions of 20–38 and 63–125 μm (at lower levels) and sporadically observed in the fractions of 250–500 and > 500 μm where only two and one pots, respectively, in 13 replications exhibited Pachymetra root rot.

Assays conducted on the three smallest fraction sizes indicated that 76.7% of oospores contained within the three fractions were in the particle-size range of 38–63 μm, 20.2% in the range of 20–38 μm, and 3.1% in the range of 63–125 μm. Oospores observed in the last-mentioned samples usually were clumped, a probable reason why they were not washed onto the sieve with the 38-μm aperture. The very sporadic incidence of root rot in the fractions of 250–500 and > 500 μm was most likely due to the same reason, that is, a clumping of oospores and subsequent failure to wash through to the smaller aperture sieves.

Recovery of oospores. The mean percent recovery rate of cultured oospores in assays conducted on five inoculated soils was 93% (95% confidence interval = ±7.1%). This result compares favorably with the recovery of sclerotia of *Macrophomina phaseolina* (Tassi) Goid. from soil by sieving as reported by Campbell and Nelson (5).

Pachymetra root rot bioassay. The relationship between oospore density and disease in soils infested with cultured oospores, in naturally infested soils diluted with pasteurized soil, and in the 32 field soils is represented in Figures 3A, 4A, and 5A. There was a significant linear relationship between oospore density and percent rotted shoot roots (Figs. 3B, 4B, and 5B). The densities required for an ID_{50} were 3.5 (soil inoculation,

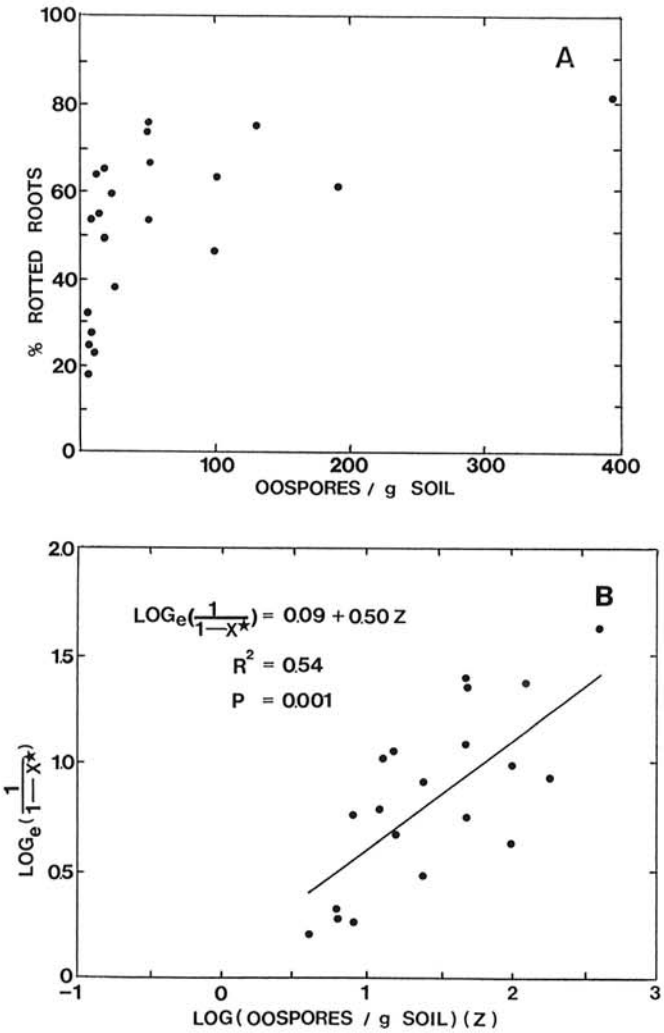

Fig. 4. Relationship between percent rotted primary shoot roots of the sugarcane cultivar Q90 and oospore density of *Pachymetra chaunorhiza*. Naturally infested soil diluted with pasteurized soil. X^* is the fraction of rotted primary shoot roots; Z is the log of oospores per gram of soil. **A,** Arithmetic plot. **B,** Multiple infection plot.

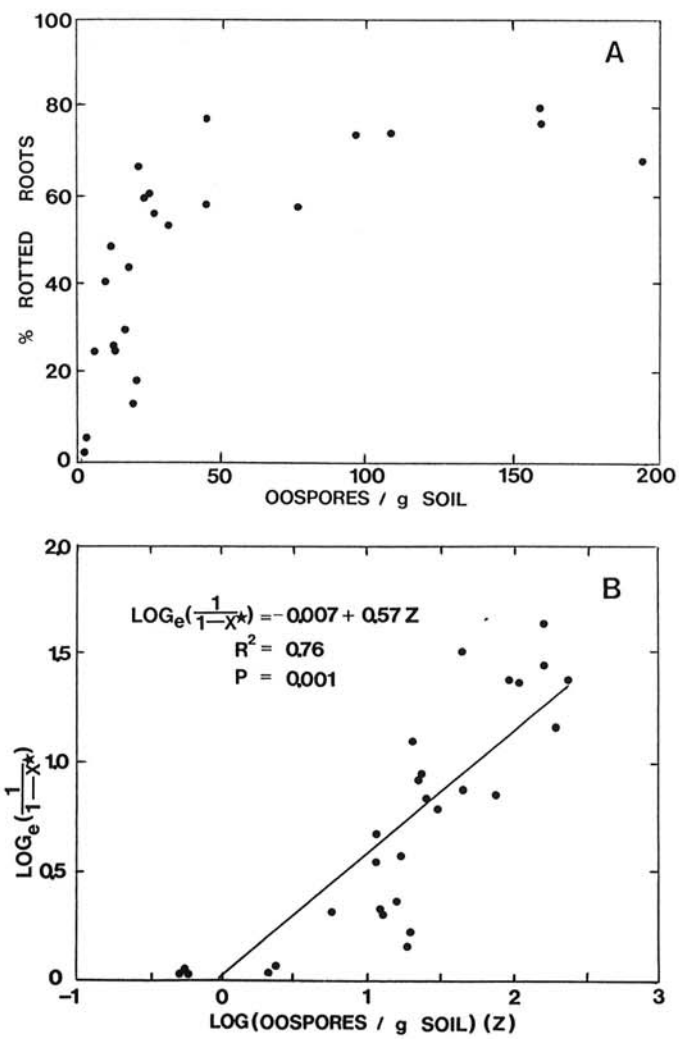

Fig. 5. Relationship between percent rotted primary shoot roots of the sugarcane cultivar Q90 and oospore density of *Pachymetra chaunorhiza* in 32 naturally infested soils. X^* is the fraction of rotted primary shoot roots; Z is the log of oospores per gram of soil. **A,** Arithmetic plot. **B,** Multiple infection plot.

cultured oospores), 21.3 (dilution of naturally infested soils), and 11.9 oospores/g dry weight of soil (32 field soils).

The slope of the regression line (transformed data) in the 32 naturally infested soils (0.57) was not significantly different ($P > 0.05$) from 0.67, the expected slope for model II (nonmobile inoculum invaded by a mobile infection court) suggested by Baker (2). The slope also was not significantly different ($P > 0.05$) from the slope of the regression analysis of the naturally infested soils diluted with pasteurized soil (0.50) but was significantly greater ($P < 0.05$) than the slope of the regression analysis of the soils infested with cultured oospores (0.36).

DISCUSSION

Pachymetra root rot of sugarcane is characterized by the production of soft and flaccid roots that are filled with the typical oospores. Consequently, an assay for *P. chaunorhiza* based on the direct count of soil oospores provides a cumulative assessment of the amount of disease previously caused by this pathogen. This assay technique is being used to assess the influence of cultivar resistance on the epidemiology of the disease, particularly in relation to the level of resistance required to stabilize or reduce disease levels at varying inoculum densities. It also is being used to investigate the effect of environmental conditions, for example, cultural practice, soil type, and moisture, on the disease.

The results presented in this paper suggest that there is a strong relationship between soil oospore density and the level of disease caused by *P. chaunorhiza*. A root rot bioassay could be used to quantify inoculum levels but has a number of disadvantages compared with direct counts. For example, it requires an extended time period for glasshouse experiments, a number of soil dilution treatments with appropriate statistical strategy, much larger soil samples for assay, and considerably greater expense. The assay based on the direct count of soil oospores is relatively quick (48 hr) and requires only small quantities of soil.

Not all oospores in naturally infested soils were recovered in the assay procedure using only the deposits in the 38-μm-aperture sieve. In a repeat experiment using 16 naturally infested soils, the inclusion of additional oospore counts made on soil fractions of 20–38 and 63–125 μm did not improve the relationship between Pachymetra root rot and soil oospore density. Considering the extra time required to do these counts and their questionable benefit, it is recommended that only oospores on the 38-μm-aperture sieve be considered in the assay procedure.

Linear regression analyses of the transformed data suggest that the disease caused by *P. chaunorhiza* may best fit model II as described by Baker (2). The slope in the regression analyses of the inoculated soils was significantly less than that in the naturally infested soils. The reason for this is not readily apparent. The greater ID_{50} values in the naturally infested soils likely reflect lower oospore viability as compared to the cultured oospores and the competitive effects exerted on *P. chaunorhiza* by antagonistic organisms. For example, the presence of *P. graminicola* has been shown to reduce the amount of disease caused by *P. chaunorhiza* (6). The ID_{50} values reported in this paper are within the range reported by researchers working with other soilborne diseases (19).

The assay has shown that soils most affected by Pachymetra root rot have oospore densities up to 700/g dry weight of soil. The buildup of high densities of oospores beneath susceptible cultivars also has been identified. The assay currently is being used to assess the effect of *P. chaunorhiza* on sugarcane yield in susceptible cultivars through a regression of inoculum densities on yield data.

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