Evaluation of a petroleum jelly slide technique for monitoring *Oidium mangiferae* conidia in mango orchards

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A petroleum jelly slide technique was evaluated for use in monitoring *Oidium mangiferae* conidia in a mango orchard. Conidia were deposited in clumps and the area read on a slide had a significant influence on the accuracy of conidia counts. Variation in the number of conidia trapped on slides short distances apart was not significant, thus single slides would be adequate for estimating deposition of conidia over time in an orchard.

Key words: fungal disease, mango, *Oidium mangiferae*, petroleum jelly-coated spore trap.

Powdery mildew, caused by the fungus *Oidium mangiferae* Berthet, is an important disease of mangoes (*Mangifera indica* L.) in most mango-growing areas of the world, including South Africa (Palti et al. 1974). Losses caused by the disease are mainly due to blossom infections, although young tissue is also susceptible. In severe cases the entire panicle becomes infected and no fruit is set (Ruehle & Ledin 1956).

Current control measures include the application of fungicides at the onset of infection, usually at the 50 % flowering stage (Vermeulen et al. 1990), but frequent instances of poor disease control suggest that this may be too late. Conidia of *O. mangiferae* are disseminated by wind (Palti et al. 1974), and monitoring the aerial conidial load in an orchard provides a method for determining the optimum time for fungicide applications.

Spore traps fall into three general categories, namely impactors (Gregory 1951; Hirst 1952; Asai 1960; Romig & Dirks 1966; Roelfs et al. 1968), impingers (Faulkner & Colhoun 1976), and sedimentary samplers (Gregory & Stedman 1953; Ostry & Nicholls 1982). Commercial impactors and impingers are expensive and consequently not widely used in the industry. An inexpensive sedimentary spore trap was therefore sought that could be 1) used at various sites, and 2) easily operated and maintained by growers. The present paper considers the number and configuration of petroleum jelly-coated slide traps in an orchard and the refinement of counting methods.

Material and methods

Spore traps

The sedimentary spore trap evaluated was modelled on that of Ostry & Nicholls (1982). Microscope slides, coated with petroleum jelly (Vaseline®) on the upper side, were held horizontally with clothes pegs attached to the ends of two metal strips fixed at right angles to one another on top of a 1 m-high pole (Fig. 1). Each spore trap therefore comprised four slides. A wire was arranged above the slides to prevent birds alighting on the traps. To test for conidial impaction, slides were attached in a vertical position on some traps (see below). A hardboard canopy (80 × 80 × 5 mm) was mounted 10 cm above the slides to prevent sedimentation.

Field layout

Three traps were erected 7 m apart in a straight line in the middle of an unsprayed section of a one hectare, 17-year-old mango orchard (cv. Tommy Atkins) at Nelspruit, Eastern Transvaal. Slides were mounted horizontally on two of the traps and vertically on one, with the four slides pointing NNE, ESE, SSE and NNW respectively. Slides were replaced weekly from 26 June 1989 to 25 June 1990, and stained with 0.1 % trypan blue in 50 % lactophenol (Fig. 2).

Determination of sample size

It was impracticable to count all conidia on a slide, particularly when the disease was well established. As a consequence, reliable sampling

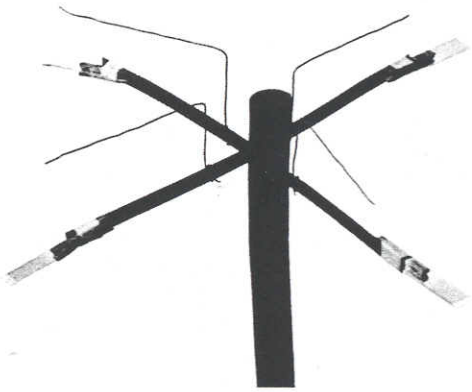

Fig. 1. Horizontal spore trap for determining airborne conidia of *O. mangiferae*.

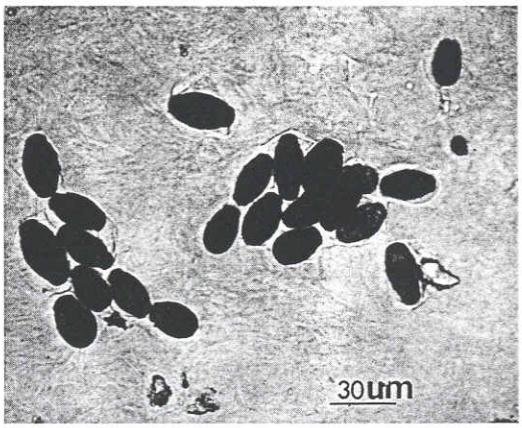

Fig. 2. *Oidium mangiferae* conidia trapped on a petroleum jelly-coated microscope slide.

procedures had to be sought, taking into account the strong tendency for conidia of *O. mangiferae* to be deposited in clumps.

The basic sampling unit was a transect (25.0 × 0.7 mm) across the width of a slide, at ×312 magnification (Ostry & Nicholls 1982), with 80 transects covering the petroleum jelly-coated area.

Van Ark's (1981) procedure was used to determine the number of samples required for the stabilisation of the standard error of the mean. This involved counting 80 transects on each of two slides; one with a low mean conidia count per transect (1.8) and one with a high mean per transect (42.6). Increasing numbers of random samples were chosen from each slide, the standard error of the mean calculated and plotted against the number of samples.

Elliott's (1977) procedures were used to determine the mathematical distribution of the samples and the number of samples required. Conidia in 20 transects on each of the four slides of a trap were counted for each of 12 weeks, from the beginning to the peak of disease development. For each set of twenty transects, the mean and variance were calculated. At the beginning of the season, when means per slide were below 0.6 conidia per transect, the variance was equal to the mean, suggesting a Poisson distribution tested for by the χ^2 variance to mean ratio test. When counts were higher, the variance exceeded the mean, indicating a negative binomial distribution. The parameter k was calculated by the first moment estimate for small samples and the t-statistic computed for agreement with a negative binomial distribution (Elliott 1977). The formula

$$n = \frac{t^2}{D^2}\left(\frac{1}{\bar{x}} + \frac{1}{k}\right), \qquad (1)$$

where n equals the sample size, $\bar{x}$ and k are constant parameters for the distribution and D is the index of precision, was used to calculate the sample size. For samples fitting the Poisson distribution, the formula reduces to:

$$n = \frac{t^2}{D^2\,\bar{x}} \qquad (2)$$

D values of 0.2 and 0.4 gave precision levels of 60 % and 80 % respectively.

Variation of counts between traps and between slides

Analysis of variance was used to assess variation between traps and between slides on a trap, between horizontal and vertical orientation, and with regard to vertical traps, the effect of wind direction. During the logarithmic and peak phases of disease development, which lasted five weeks, six transects were counted once a week on all slides from three traps. Data were normalised by transformation to $\log(x + 1)$.

Results

The curve of the standard error of the mean versus sample size stabilised at a sample size of seven for the slide with a high mean conidia count per transect (42.6) and at approximately 40 for the

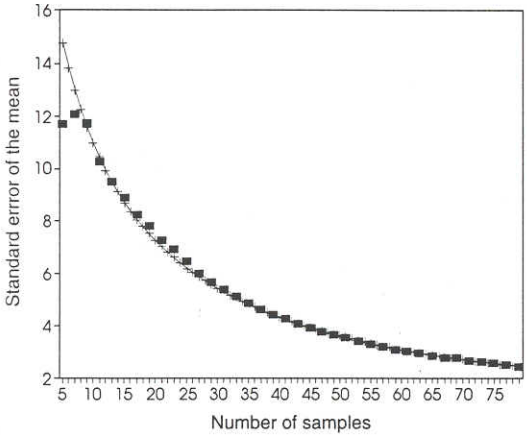

Fig. 3. Relationship between standard error of the mean and number of samples for a slide with a high mean number of *Oidium mangiferae* conidia per transect.

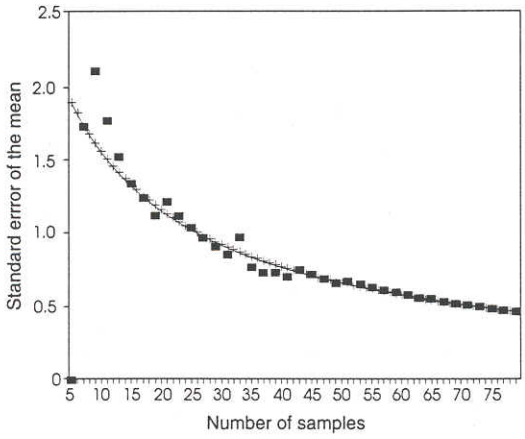

Fig. 4. Relationship between standard error of the mean and number of samples for a slide with a low mean number of *Oidium mangiferae* conidia per transect.

Table 1. Analysis of variance for conidia counts of *Oidium mangiferae* for three posts, four positions (NNE, ESE, SSE & NNW) and two orientations.

	F-ratio	Significance level
Among posts[a]	2.617	0.074
Among slides	0.590	0.622

[a]Slides on two posts oriented horizontally, and on one post vertically.

slide with a low mean per transect (1.8) (Figs 3, 4).

A common *k(Kc)* could be calculated by regression of the data fitting the negative binomial distribution as there was no relationship between x and $1/k$ and therefore no trend or clustering (Elliott 1977). The value of *Kc* was 10.63 for the data set and this was used to calculate the number of transects required for various means and two levels of precision (Fig. 5).

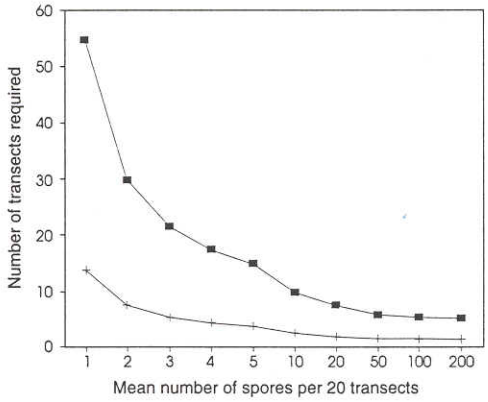

Fig. 5. Relationship between the number of transects needed for 60 % (+) and 80 % (■)accuracy and the mean number of *Oidium mangiferae* conidia trapped per 20 transects.

Disease appearance

The first conidia were detected five weeks before disease symptoms were noted. Two weeks before symptom expression, the mean number of conidia exceeded one and mean counts increased to more than ten when symptoms first became evident.

Variation in counts between traps and slides

There was no significant difference in conidia numbers between horizontal and vertical slides and no differences between slides on a trap (Table 1). In addition, the effect of wind direction on the vertical slides was not significant.

Discussion

Petroleum jelly-coated slides are useful for monitoring conidia levels during disease development and can be used to improve control recommendations. This is in agreement with Romig & Dirks (1966) who found that the number of conidia of *Puccinia graminis* and *P. recondita* on microscope slides were correlated with the general development of rust in their study area.

The number of transects read on spore traps varies greatly (1–20 per slide) in the literature (Bromfield et al. 1959; Peries 1962; Sutton & Jones 1976) with no indication of the level of precision attained. The present study showed that the number of transects read has a marked influence on the accuracy of conidia counts. Plots of the standard error of the mean against the number of samples (transects) showed that more samples (40) were required when the mean conidia count was low (1.8), while fewer samples (7) were required when the mean was high (42.6). This is in agreement with the findings of Elliott (1977) who used a theoretically more accurate procedure.

In practice it will be necessary to determine the required sample size several times during the progress of the disease. To obtain counts of a desired precision, 20 transects (samples) should be read on a slide and the mean number of conidia per transect calculated. The required number of transects can then be read off the graph and counting continued, if necessary. As the disease progresses and the mean number of conidia increases, the procedure must be repeated to establish the number of samples required for the following weeks. In general, the higher the mean number of conidia, the fewer transects have to be read on a slide, and vice versa.

The method used in the present study is inexpensive yet effective, and lends itself to application at many field sites simultaneously, for example in comparative studies between different climatic regions. However, its efficacy is dependent upon the unambiguous identification of conidia on a slide, such as those of *O. mangiferae* in the present study.

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