The Survival of Phytophthora palmivora in a Cacao Plantation during the Dry Season

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ABSTRACT

A simple technique for estimating the inoculum potential of *Phytophthora palmivora* in soil is described. By the use of this method, it was shown that the fungus survived in soil throughout the dry season, lending support to the theory that the soil is an important source of primary inoculum for the black pod disease of cacao. The fungus was also detected in decaying leaves, twigs, pod pieces, and living and dead rootlets of *Theobroma cacao*. The survival of *P. palmivora* in infected pods left hanging on the trees fell to zero within 8 weeks, whereas infected pods contacting the soil contained viable fungus throughout the season. Phytopathology 61: 975-977.

Phytophthora palmivora Butl., incitant of the black pod disease of cacao (*Theobroma cacao* L.), is common in most areas where this crop is grown. Infectious propagules of this fungus exist in the soil (3, 4, 6), and it is generally assumed that the soil is the source of primary inoculum in the establishment of epiphytotics of the disease. That this might be true under Nigerian conditions has been suggested by Thorold (6) and Okaisabor (3). There is, however, little information regarding methods of estimating the inoculum level of *P. palmivora* in soil, although methods for studying the inoculum load of some other *Phytophthora* species in soil have been reported (1, 2, 7).

Little quantitative work has been done on the survival of *P. palmivora* during the dry season. Tarjot (5), working in the Ivory Coast, showed that the fungus was present in soil, in old dry pods, blackened cherelles, floral cushions, and peduncles from January to April. However, his method did not show any variation in the number of propagules in the soil as the season progressed.

Tsao (7) developed a serial dilution end-point method for estimating the disease potential of citrus phytophthoras in soil. This method is applicable to *P. palmivora* because when green cacao pod material is in contact with soil containing the fungus, recognizable symptoms are produced within 4-5 days under suitable environmental conditions. This paper reports a simple technique for estimating the inoculum potential of *P. palmivora* in soil; the use of this method to study the survival of the fungus over the dry season; and the survival of the fungus in dry pods on trees and in plant debris in contact with soil.

MATERIALS AND METHODS.—The technique used here for estimating the inoculum level of *P. palmivora* in soil is a modification of that developed by Tsao (7) for citrus phytophthoras. The definition of "Inoculum Potential Index" (IPI) is similar to that employed by him for "Disease Potential Index"; i.e., the highest dilution that resulted in infection under standardized conditions.

During the rainy season at Ile-Ife, five soil samples from the top 2.54 cm, rich in humus and organic matter, were collected from under ten randomly selected cacao trees in a plantation. The ten samples were combined and the soil was spread on a table to dry overnight in the laboratory and then sifted through a 0.18-cm mesh screen. Serial dilutions of the combined sample were prepared with sterile soil. Each dilution consisted of 150 g of soil which were then placed in a plastic bag. One hundred and fifty g each of nondiluted cacao soil and autoclave-sterilized soil were also weighed separately into plastic bags. Each bag was then vigorously shaken to thoroughly mix the soil, and the contents of each bag were divided equally into five petri dishes. Twelve ml of distilled water were added to each dish. Healthy, mature, green cacao pods, not previously sprayed with fungicide, were washed in detergent (Alcanox) and rinsed in distilled water to remove any propagules of *P. palmivora* that might have been present as contaminants. With a sterile knife, the pods were cut into 2.5-cm square pieces after the seeds had been removed. Into each petri plate, four randomly chosen pod pieces were placed with their inner surfaces downward. Pod pieces in autoclave-sterilized soil served as a control. The plates were then left in a dark incubator at ca. 25 C.

RESULTS.—Preliminary experiments showed that when green cacao pod material is in contact with moist soil containing *P. palmivora,* the host tissue selectively attacked by the fungus turns brown. Accordingly, after 5 days of incubation, the pod pieces in the petri plates were examined for this evidence of attack by *P. palmivora.* Some of the pod pieces had turned brown and there was a sparse growth of whitish mycelium on them. Examination of the brown pod pieces under a dissecting microscope revealed the presence of abundant lemon-shaped sporangia similar to those formed on pods infected with *P. palmivora.* When healthy pods were inoculated with tissue from these infected pod pieces, typical black pod symptoms occurred after incubation in a moist chamber for 3 days at 25 C. It was observed that the number of pod pieces infected per treatment was related to the dilution with sterilized

soil; the greater the dilution, the fewer the pod pieces attacked. The noninfected pod pieces remained green, although their margins were attacked by some saprophytic fungi and bacteria. None of the pod pieces in the control treatment was infected. The experiment was repeated twice with soil samples collected at intervals of 2 days in the same way from under the same 10 trees. The results of all three replicates of the experiment were remarkably similar. The highest dilution that gave infection of at least one pod piece in each case was 1/32. Thus, the IPI in each case was 32.

Comparison of the inoculum potential of P. palmivora in two soil samples containing known relative amounts of fungus propagules.—From a cacao meal:sand medium of *P. palmivora* (sample A) maintained at 21 C, a 1/8 dilution (sample B) was prepared with sterilized soil. The serial dilution end-point method was used to compare the IPI of samples A and B. Sterilized soil served as a control. Each treatment consisted of five petri plates, each containing four green pod pieces. None of the pod pieces in the control was infected. The greatest dilution that gave infection in sample B was 1/32; that in sample A was 1/256. The results indicate that the IPI of sample B was roughly 1/8 that of sample B; i.e., 32 to 256.

Inoculum potential of soil taken from different depths.—To determine the depth from which soil samples should be collected for future studies, the following experiment was carried out. Two sets of soil samples were collected from under five trees in September 1969. The first sample was taken exclusively from the top 2.54 cm; the second, from a depth of 0-7.62 cm. By means of the serial dilution method, the inoculum load of the two samples was evaluated. The highest dilution that gave infection in the 0-7.62-cm sample was 1/4 as compared to 1/32 for the top 2.54 cm; i.e., the respective IPI's were 4 and 32. The experiment was repeated 1 week later with similar results. The results were not unexpected, since it has been shown that *P. palmivora* produces little growth away from a food base (8). One expects to find the fungus mostly in the top layer rich in organic matter. All future samples were therefore collected from this zone.

Survival of P. palmivora in soil over the dry season.—Beginning from mid-September (the dry season starts in November), soil samples were collected every 2 weeks within a radius of 1 m under 15 randomly chosen trees. The samples were combined and the IPI was determined. At the same time, the IPI of soil samples collected from under three heaps of cacao pod husks discarded after bean extraction was also determined. These pod husks were heavily infected with *P. palmivora*. Sampling from the two areas continued throughout the dry season and into the rainy season in June 1970.

The fungus was detected throughout the dry season. although the inoculum level fell very low in the driest part of the season (Fig. 1). Leaving discarded pod husks in the plantation raises the inoculum level of the soil. Throughout the duration of the experiment, the IPI of the samples collected from under the decaying

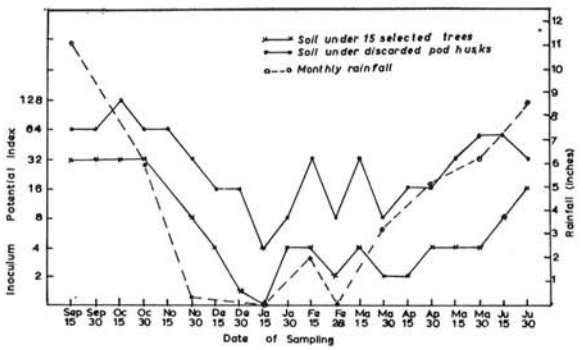

Fig. 1. Inoculum potential indices of cacao soil from September 1969 to June 1970.

pod heaps was consistently higher than that of soil collected under the 15 trees.

Phytophthora palmivora in plant debris and rootlets of Theobroma cacao.—When the IPI of soil collected under the 15 trees was at its lowest (Fig. 1), an experiment was conducted using a nonsieved soil. The IPI of the nonsieved soil was 4 while that of sieved soil was 0. This indicated that the fraction retained in the sieve contained a higher amount of the fungus than that which passed through. Apart from large soil particles, the sieve retained decaying leaves, small twigs, pieces of bark, pieces of decaying pods, and rootlets of *T. cacao*. The different types of plant debris were carefully separated from one another and from the rootlets. Decaying pod pieces in contact with soil were also collected from the three heaps of discarded pod husks mentioned earlier. Each group of plant material was washed many times in distilled water to remove all adhering soil particles. After drying on filter paper, 5 g of each were placed in a petri plate and 10 ml of distilled water were added. Four green pod pieces of the same size as those used in the estimation of IPI were put into each plate as bait. Five petri plates were set up for each type of plant material. Five plates containing only green pod pieces and distilled water were also set up as a control. The plates were incubated at 25 C for 5 days, then examined for infected pod pieces.

Phytophthora palmivora was isolated from decaying leaves, small twigs, rotting wood pieces, decaying pod pieces, and dead and living rootlets of *T. cacao*. This experiment was repeated from 8 January to June at monthly intervals. At each trial, sporangia were formed on the pod pieces used as bait, on the plant material tested, and at the bottom of the plate (Table 1).

Survival of P. palmivora in infected pods left on the trees.—In the first week of October 1969, 25 pods, each on a different tree, were inoculated by inserting into each an inoculum plug taken with a cork-borer from a recently infected pod. All 25 pods showed symptoms of infection within 3 days. Two weeks after inoculation, two inoculum plugs were taken from each pod. Each plug was then inserted into a green pod piece. The pair of green pod pieces with plugs from the same infected pod were put in a petri plate containing 15 ml of dis-

TABLE 1. Monthly sampling of plant residue and rootlets of *Theobroma cacao* for *Phytophthora palmivora* from January to June 1970[a]

Sampling date	No. plates showing infected pod pieces			
	Rootlets	Decaying leaves, twigs, and bark	Pod husks in contact with soil	Control[b]
8 Jan.	3	4	3	0
6 Feb.	5	4	2	0
5 Mar.	3	3	1	0
7 Apr.	4	4	2	0
5 May	2	3	2	0
4 June	4	5	2	0

[a] Figures in the table give number of petri plates in which one or more of the pod pieces used as bait were infected. Total number of plates for each kind of plant material was five.

[b] Petri dishes containing only green pod pieces and distilled water served as control.

tilled water. The plates were incubated for 5 days at 25 C. All the plates showed infected pod pieces.

When the experiment was repeated at 2-week intervals, the infection percentage declined progressively and reached zero within 8 weeks (Fig. 2). When these results are compared with those obtained from pod pieces in contact with soil (Table 1), it is clear that the survival of the fungus is higher in infected pod material in contact with soil. This observation is in agreement with results obtained by Waite & Diaz (9) in Costa Rica.

DISCUSSION.—The evaluation of the sensitivity of the serial dilution end-point method described here awaits the development of a quantitative method of estimating the actual numbers of propagules of *P. palmivora* in soil. However, the tests to which it was subjected indicate its reliability.

Recovery of *P. palmivora* in soil throughout the dry season supports the assumption that the soil is an important source of primary inoculum for the black pod disease. In this connection it is important to consider the IPI of zero for the soil sample of 15 January (Fig. 1). Certainly the fungus was not absent from the soil sample in question, as it was isolated from material retained in the sieve. It is probable that the soil contained such a low level of inoculum that the serial dilution method could not detect it.

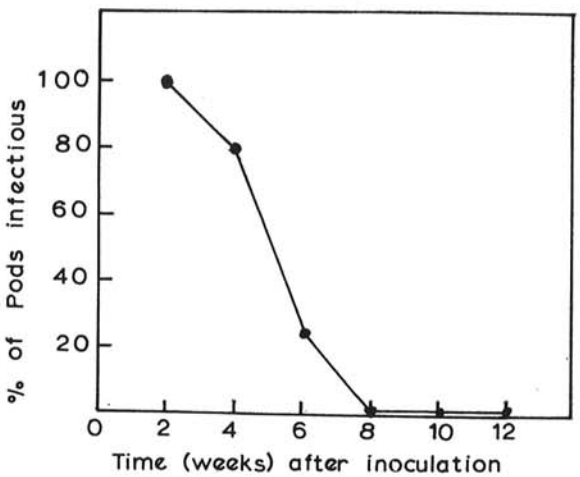

Fig. 2. Longevity of *Phytophthora palmivora* in infected cacao pods left hanging on trees.

The survival of *P. palmivora* was related to the amount of rainfall. The IPI was high at the end of the rainy season, fell to a low level during the driest part of the dry season, and was steadily rising with the progress of the rainy season at the termination of the experiment (Fig. 1). Moisture relationships may also account for the longer survival of the fungus in plant material in contact with soil than in material left on the trees as observed in this study and elsewhere (9). Zentmeyer & Mircetich (10) also found that the best recovery of *Phytophthora cinnamomi* was obtained from soil with a high level of moisture.

The results raise a number of practical points. Firstly, leaving discarded pod husks in the plantation is likely to raise the level of primary inoculum for the black pod disease. Secondly, any attempts to control *P. palmivora* by chemical soil treatment must take into account the abundance of inoculum in the decaying litter of leaves and twigs. Finally, the presence of the fungus in living rootlets indicates that its eradication with the ordinary soil chemicals will be futile. The elimination of the fungus from these roots will require a systemic fungicide.

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