Survival of *Phytophthora palmivora* in Soil and After Passing Through Alimentary Canals of Snails

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ABSTRACT

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Vertical distribution of propagules of *Phytophthora palmivora* morphological form (MF) 4 (cause of foot rot of *Piper nigrum*) in the field decreases with increasing soil depth and is highest at 0.5–15 cm and very low at 30–45 cm. Soil moisture and pH greatly affected survival of *P. palmivora* MF 4. The optimum soil moisture for survival was 25–45% water-holding capacity, at soil pH 6.5–7.0. The pathogen survived a maximum of 18 mo in its natural habitat, as assessed by continuous baiting. Fungal propagules were found in snail (*Achatina fulica*) feces in three pepper plantations. Sporangia survived and remained infective after passage through the alimentary canals of two snail species (*A. fulica* and *Hemiplecta crossei*).

Foot rot of pepper (*Piper nigrum* L.) caused by *Phytophthora palmivora* (Butl.) Butler occurs in Brazil, Ghana, India, Cambodia, Laos, Vietnam, Indonesia, Jamaica, Puerto Rico, Sarawak, Trust Territory of the Pacific Islands, and West Malaysia (T. K. Kueh, *unpublished data*). The disease is highly destructive to pepper vines and is the major factor limiting production of pepper in Southeast Asia. The pathogen is soilborne, and foot rot often recurs on pepper growing in a previously affected field.

This study was done to investigate the ability of *P. palmivora* morphological form (MF) 4 to survive in soil and in the digestive tracts of snails (*Achatina fulica* Bowdish and *Hemiplecta crossei* Pfeiffer), because snails play a role in the transmission of foot rot (10).

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MATERIALS AND METHODS

The plant materials used were *Piper nigrum* 'Kuching,' which is extremely susceptible to foot rot. Mature leaves of this cultivar can be used successfully as bait for *P. palmivora* MF 4 (T. K. Kueh, *unpublished data*).

Infested soil. Soil was collected at 0.5–15, 15–30, 30–45, and 0.5–30 cm depths around underground stems and roots of pepper vines newly infected by *P. palmivora* MF 4. The 498 soil samples were from plantations with foot rot throughout the major pepper-growing regions of Sarawak. Water-holding capacity and pH were determined.

Each 80-g soil sample was suspended in 80 ml of distilled water. Five pepper leaves were partially dipped, petiole end down, in the suspension. Each sample was replicated twice and kept at room temperature (26 ± 2 C).

The numbers of lesions per leaf and of leaves infected were recorded daily for 10 consecutive days. Tissues were cut from the edges of lesions, placed in Petri's solution and examined for sporangia immediately and after 24 and 48 hr.

Rating of inoculum levels. The total number of Phytophthora leaf spots for all infected leaves was divided by the total number of leaves in a soil sample. The average number of spots per leaf was graded on a 0–5 scale, in which 0 = no spots and 5 = five or more spots. The scores from all soil samples in a soil pH range or water-holding capacity range were averaged to obtain the inoculum level rating.

Assessment of survival of *P. palmivora* MF 4 by baiting techniques. For the study with continuous baiting, 12 infected pepper vines were randomly selected at three plantations. Ten mature pepper leaves were partially buried in a mound of infested soil in each of three concentric circles; the inner, middle, and outer circles were 5, 20, and 35 cm, respectively, from the basal stem. The leaves were removed for lesion counts, and fresh leaves were inserted weekly.

In the study using single baiting, 49 infected vines were randomly selected at three pepper plantations. Ten mature pepper leaves were partially buried at random in soil around a stem. The mounds were considered free of the pathogen if the leaves remained uninfected after 3 wk.

Survival in snail feces. Feces of the giant African snail (*A. fulica*) collected from infected vines were made into a paste with sterile distilled water. The paste was inserted into four holes (5 mm diameter, 15 mm deep) in an apple. Firm, brown necrotic tissue that developed around the hole was cut and treated with Petri's solution for disease evaluation.

For the leaf inoculation test, 10 pepper leaves were dipped in the suspension and checked daily for infection.

Snails (*A. fulica* and *H. crossei*) collected from areas free of foot rot were divided into groups of 15 and starved for 24 hr. After the snails were fed with *P. palmivora* MF 4 freshly cultured on oatmeal agar, they were washed twice with distilled water, dried, and placed in cages for feces collections. Smears of fresh droppings were examined microscopically, and a portion of the feces was treated with Petri's solution and examined microscopically at 8-hr intervals.

Table 1. Inoculum level ratings of *Phytophthora palmivora* MF 4 obtained by leaf baiting at different soil pH and water-holding capacities

pH	Inoculum rating[a]	Water-holding capacity (%)	Inoculum rating[a]
3.0–4.5	0.2 a	15–25	0.1 a
4.5–5.5	1.2 b	25–35	0.9 c
5.5–6.5	2.3 c	35–45	0.9 c
6.5–7.0	3.1 d	45–55	0.3 b

[a] Means in the same column followed by the same letter are not significantly different ($P=0.05$), by Duncan's multiple range test. 0 = no spots per leaf; 5 = 5 or more spots per leaf.

RESULTS

Infested soil. Of the 498 soil samples, 35.5% contained sufficient inoculum to infect mature pepper leaves. The percentages of positive isolations were 38.2, 8.1, and 0.1 at soil depths of 0.5–15, 15–30, and 30–45 cm, respectively. The fungus was isolated from all of the composite soil samples at 0.5–30 cm depth.

Of the soil samples that were positive for *P. palmivora* MF 4, those with pH 6.5–7.0 had the highest inoculum level rating (Table 1). The inoculum level ratings were progressively lower with lower pH levels. Inoculum levels were highest in soil samples with 25–45% water-holding capacity.

Assessment of survival of *P. palmivora* MF 4 by baiting techniques. The maximum survival of *P. palmivora* MF 4 in infested soil was 18 mo, as shown by the continuous baiting technique, and 16 mo, according to the single baiting technique (Table 2).

The inoculum level was highest in soil nearest the stem, as indicated by percentage of leaf infection (Table 3). Inoculum concentration, expressed as percentage of leaf infection at three plantations, closely followed the pattern of monthly rainfall recorded at Tarat Agricultural Station (Fig. 1).

Survival in snail feces. *P. palmivora* MF 4 was present in snail feces collected from the field and remained infective, as shown by studies with apples and pepper leaves. Direct microscopic examination of feces indicated that the ingested sporangia and hyphae of *P. palmivora* MF 4 were not changed by passage through the digestive tract of either snail species. The sporangia and hyphae excreted by the snails were viable after 24-hr treatment with Petri's solution.

DISCUSSION

In a soil of fairly uniform texture, the concentration of the fungi decreases with depth (4), as was observed for *P. palmivora* MF 4 under field conditions. This vertical distribution of propagules may be attributed to the fact that *P. palmivora* MF 4 was active in the upper 15 cm of soil because adequate oxygen (11) and light (1,5,7,12) stimulate sporangial production by *Phytophthora*. Lower inoculum levels deeper in the soil could be due to both the lack of oxygen and the clayey soil in Sarawak that may have prevented free downward movement of sporangia and zoospores.

The optimum condition for *Phytophthora* to develop in nutrient solutions has been reported to be pH 6.0 (2,10). Sporangial production of *P. palmivora* MF 4 was also maximal at pH 6.0 (10). The high inoculum level in infested soil at pH 5.5–6.5 and 6.5–7.0 was therefore expected. High inoculum levels in soil with 35–45% water-holding capacity indicated that, although *Phytophthora* species are regarded as water molds (3), appropriate soil moisture is required for optimum growth and sporulation. Excessive soil moisture deprives the soil of oxygen, which may hinder growth and sporulation of the fungus.

Results of the continuous and single baiting techniques both indicate a long survival ability of *P. palmivora* MF 4 in its natural habitat. A continuously maintained pepper plantation would allow comparably long survival because the host plants would provide appropriate food for the pathogen. In Sarawak where pepper is commonly planted on the same field year after year, the long survival of the pathogen could pose a considerable problem for disease control in a newly replanted pepper field.

High inoculum levels in soil close to the stem were attributed to the effect of root exudate of the host plants. The inoculum concentration of the pathogen in the mound soils from the three areas closely followed the monthly rainfall pattern, with higher levels during the wet months

Table 2. Survival of *Phytophthora palmivora* MF 4 in soils of pepper plantations

Site	Continuous baiting[a]: Survival (mo) on plant no. 1	2	3	4	5	Single baiting[b]: Plants (no.)	Isolations (no.)	Survival (mo)
Balang	12	12	18	18	3	11	6	16
27½ mile	14	14	15	...[c]	...	10	7	14
Tarat	13	13	13	12	...	28	24	9

[a] In continuously maintained pepper plantations.
[b] In abandoned pepper plantations.
[c] Not tested.

Table 3. Mean percentage of leaf infection in mound soil at bases of infected pepper stems

Distance from base of stem (cm)	Leaf infection[a] (%) at Tarat	27½ mile	Balang
5	73.1 a	54.8 b	33.9 b
20	71.5 a	47.5 b	25.8 a
35	69.6 a	35.6 a	22.0 a

[a] Means in the same column followed by the same letter are not significantly different ($P=0.05$), by Duncan's multiple range test.

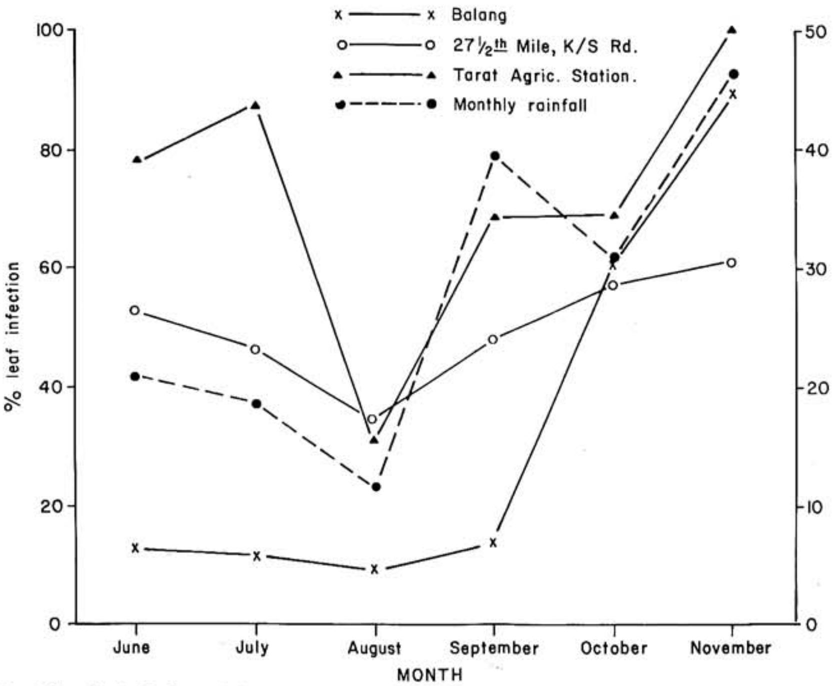

Fig. 1. Survival of *Phytophthora palmivora* MF 4 in mounds of soil around infected vines at three pepper plantations.

of October and November. This phenomenon was also observed previously (8,9). Water allowed better growth and sporulation of the fungus, and the decrease in temperature caused by sudden heavy rain could stimulate large-scale release of zoospores that in turn might give rise to higher inoculum levels. This could explain the greater incidence of foot rot in Sarawak during the rainy season than during the dry season.

Isolation of *P. palmivora* MF 4 from snail feces collected in the field indicated the important role of snails in spread of the disease. Snails are common pests on pepper plantations in Sarawak, and they may help to establish small foci of infection that could lead to epidemics when environmental conditions are suitable. The sporangia and hyphae in the feces confirmed that propagules of *P. palmivora* MF 4 are resistant to the digestive secretions of the two snail species. No attempt was made to determine whether degradation of cell walls of sporangia and hyphae occurred, but an earlier report (6) indicated that oospores of *P. erythroseptica* and *P. cactorum* have thinner walls and ready germination ability after passage through the alimentary canal of the snail species *Helix aspersa*.

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