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Phytophthora Leaf Disease in Malaysia

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Since its first confirmed occurrence in 1966, Phytophthora *leaf fall has spread slowly in the north of West Malaysia on clones of high susceptibility, particularly RRIM 600, PR 107 and PB 86. The pathogen predominantly associated with the disease, profusely sporulating in culture, is regarded as a new species. Clones have been screened for their inherent susceptibility to* Phytophthora *leaf disease by dipping detached petioles in a zoospore suspension and measuring the length of the resulting lesion.*

In a survey of the occurrence of Phytophthora *in the soil of rubber plantations, the pathogen has been found, mainly in the top 2 in., only in areas affected by leaf disease or black stripe.*

Survival and spread in soil has been studied in the laboratory and in the field. Mycelium from agar inocula or infected petioles resting on non-sterile soil in petri dishes spread $\frac{1}{2}$ *– 1 cm in different soil types; from mycelial or zoospore inocula, without a food base, no spread was detected. The pathogen could not be re-isolated a month after soil had been inoculated with mycelium but infected petioles buried in soil still yielded* Phytophthora *after three months. Zoospores or sporangia or structures produced by them remained viable for six weeks. Direct isolations from naturally infected soil have indicated that the chlamydospore is the principal survival structure. These findings are discussed in relation to possible disease control methods.*

Following the outbreak of *Phytophthora* leaf fall and pod rot in Malaysia in 1966 (CHEE *et al.*, 1967), much has been learnt about the fungus and the disease it causes. Strains of *P. palmivora* (Butl.) Butl. associated with the tapping panel disease 'black stripe' are widespread and presumably indigenous in Malaysia. The new leaf fall pathogen, which cannot be identified with any known *Phytophthora* species, is here referred to as the new species though previously (CHEE *et al.*, 1967; CHEE AND GREENWOOD, 1968) as *P. (?) meadii* because it has certain characteristics of *P. meadii* McRae. It is restricted to leaf fall areas, its geographical distribution suggesting that the species entered Malaysia from Thailand.

Just as *P. palmivora* exists as three strains — rubber, cacao and atypical (TURNER, 1960), there are three similar strains — two complementary and an atypical — of the unnamed species. An investigation of the pathogenicity of all six strains to ten *Hevea* clones being reported elsewhere (CHEE, 1969) indicates some instances of clone and strain interaction. The present paper records tests carried out on a wide range of clones, using the (–) strain of the unnamed species associated with *Phytophthora* leaf fall in Malaysia. In the limited tests already referred to, this strain appeared the most virulent of the three.

Considerable progress has been made in the recent years in studies on the ecology of *Phytophthora* spp. in soil, but little is known of the behaviour of those that infect *Hevea.* In investigating the source of *Phytophthora* spp. infecting tapping panels and leaves of *Hevea,* over forty isolates were obtained from soil under rubber trees in West Malaysia, and the distribution in soil and survival of the several species were studied.

MATERIALS

The soil for laboratory experiments was from four soil series: Sungei Buloh (freely drained sand, pH 4.9), Selangor (poorly drained silty clay loam, pH 4.6), Kuantan (free draining clay loam, pH 4.8), and peat (pH 3.9) (OWEN, 1951). It was air-dried, sieved through

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a 20-gauge mesh, autoclaved, and moistened to about 50% of its moisture-holding capacity. This moisture level was maintained throughout the experiments. Plates were incubated at ambient laboratory temperature (about 24°C). Isolations were made by inserting test samples in holes on unripe cacao pods, which function as a differential medium for the fungus (CHEE AND FOONG, 1968).

The four organisms studied were: an atypical strain of *P. palmivora* isolated from the tapping panel, an atypical strain from an infected petiole, a (+) and a (−) strain of the unnamed species, also from infected petioles. Sporangial suspensions were obtained by lightly scraping the spores from the surface of a 6-day-old culture on 2.3% Difco lima bean agar. To obtain zoospores, sporangia were chilled for 5 minutes and then returned to room temperature; zoospores were separated by filtration through Whatman No. 1 filter paper.

EXPERIMENTAL METHOD AND RESULTS

Clonal Susceptibility

Twenty-five uniformly aged young leaves (eight weeks old) of each of 304 clones were collected from a budwood nursery. The leaf blades were removed and the ends of the petioles were sealed with paraffin wax. The petioles, supported in groups of 250 by a wire rack, were held erect in plastic trays filled with a zoospore suspension (20 000 spores/ml) of the unnamed species to a depth of $\frac{1}{2}$ in., thoroughly sprayed with distilled water and enclosed in a moist polythene bag. After four days at 22°C, the intensity of infection was determined by measuring the extent of the discolouration which developed above the immersed end of the petioles. The test was repeated on a second occasion with all clones, and the forty in which agreement was least good were tested on a third occasion.

A preliminary experiment using leaves of different ages showed that the severity of infection varied with the age of the petiole, young material being more susceptible. Severity was also related to the zoospore concentration, but there was no appreciable difference in disease development with more than 1000 spores/ml.

Statistical analysis of the length of the discolouration showed highly significant differences in susceptibility between clones, which have been listed (*Table 1*, see page 82) according to their geographical origin. The minimum significant difference ($P = 0.05$) between any two clones chosen at random being about 11 mm, the clones have been grouped in 6 mm intervals of lesion length, so that a clone is of significantly different susceptibility from any clone in the next group but two.

Geographical Distribution

A total of 866 soil samples was collected over two and a half years from rubber plantations throughout West Malaysia *(Figure 1)*, their state of origin being: Perlis 54, Kedah 147, Perak 250, Kelantan 18, Pahang 4, Selangor 7, Negri Sembilan 14, Malacca 22 and Johore 350. Each sample was composed of 5 to 10 scoops of soil made with a trowel to a depth of 2 inches over an area of 2 to 5 square yards, after the litter and humus layers had been scraped away. About 10% of the samples were taken from low-lying terrain with a high soil moisture content. Of the 866 samples, 27 yielded *P. palmivora*, 4 yielded the unnamed species, 2 yielded *P. heveae* Thompson, and 5 yielded *P. nicotianae* var. *parasitica* Waterhouse. *P. palmivora* is the pathogen mainly associated with black stripe, the unnamed species with leaf fall and pod rot (CHEE *et al.*, 1967; CHEE, 1969). *P. heveae* and *P. nicotianae* var. *parasitica* are not known as parasites of rubber, except for one record of the association of *P. heveae* with black stripe and pod rot (THOMPSON, 1929).

One hundred and eighteen samples were collected under or near trees affected by leaf fall, black stripe or patch canker (CHEE, 1968); it was only from among these that *Phytophthora* was isolated. Recovery of the pathogen did not appear to be affected by soil type or soil moisture content.

Vertical Distribution

Soil pits 18 in. square and 18 in. deep were dug under trees affected by black stripe; one vertical face was carefully smoothed and thin soil slices removed at the surface and at 2 in. intervals from 2 to 12 in. below the surface.

Figure 1. States of West Malaysia.

Each sample was placed in holes in cacao pods. *P. palmivora* was isolated from 6 out of 20 pits; there were 5 positive isolations at the soil surface, 4 at 2 in. and 1 at 8 in. The pattern of vertical distribution resembles that of *P. cinnamomi* Rands, under trees infected with littleleaf disease, where the fungus was most abundant at the 2- and 3-inch depths, less at 6 and 7 inches, and more frequent again at 8 and 9 inches (CAMPBELL, 1951).

Direct Isolation

Isolation of the fungus was attempted by the direct soil plating method (OCANA AND TSAO, 1966): 0.5 g of naturally infested soil was sprinkled on the surface of a Difco cornmeal agar medium containing pimaricin, vancomycin and pentachloronitrobenzene (PCNB) at 10, 200 and 100 p.p.m. respectively, flooded with sterile distilled water and incubated for 24 hours at 21°C. Plates were then washed under a slow stream of tap water to remove soil particles, the germinated spores remaining attached to the medium by hyphae. Spores were pin-pointed on the dish with a pen and examined 48 to 72 hours later, by which time hyphae and sporangia of *Phytophthora* spp. had formed.

Of the 59 resulting colonies of *P. palmivora*, 50 originated from chlamydospores, 7 from sporangia, and 2 possibly from oospores. This suggests that *P. palmivora* survives in soil mainly in the form of chlamydospores, confirming other recent work (HENDRIX AND KUHLMAN, 1965; MCCAIN *et al.*, 1967).

Growth from a Food Base

Phytophthora spp. may spread in the soil by mycelial growth or by spores swimming in the soil water. The rate of mycelial growth of both the atypical strain of *P. palmivora* and the (—) strain of the unnamed species were determined in various soil types in the following manner: non-sterile soil was smoothly packed in petri dishes and 5 mm plugs of agar inoculum of the fungus placed in the centre of each. At weekly intervals, small samples of soil were taken at distances of 5, 10, 15 and 20 mm from the inoculum and tested for the presence of the fungus.

The rate of recovery of the two *Phytophthora* species was identical in the same soil type. The fungus was re-isolated from Kuantan series soil at the 5 mm mark at the first and fourth samplings, whereas the Selangor series isolation was negative at this mark. Growth of the fungus in peat or Sungei Buloh series soil was less restricted; it was recovered at the 10 mm mark at the second and third samplings respectively; but, at the fourth sampling (after which the experiment was terminated), the fungus could not still be isolated from any soil type at the 15 mm mark *(Table 2)*. Inoculum size was not critical; growth from a 2 cm disc was similar to that from $\frac{1}{2}$ or 1 cm.

Table 2 also shows that, using a 1 cm length of diseased petiole artificially infected with an atypical strain of *P. palmivora* as inoculum, *Phytophthora* was recovered at the 5 mm mark in peat as well as in the Selangor soil type. Growth of the fungus was detected at the 10 mm distance with the Kuantan and Sungei Buloh series soils respectively at the second and third samplings. Only in Kuantan and

TABLE 1. SUSCEPTIBILITY OF *HEVEA* CLONES TO *PHYTOPHTHORA* LEAF DISEASE IN LABORATORY TESTS

Mean length of lesion (mm)*	Origin of clone					
	Africa	America	Ceylon	Indo-China	Indonesia	Malaysia
8.1 to 14.0		FX 649 IAN 2867			PR 249	61C/441 PB 202
14.1 to 20.0	Har 1	IAN 2750, 2921, 3646, 3714, 3787, 6500 FX 3925	RLD 45 Nab 20	TR 1549, 3702 OY 1 IRCI 6 BS 4	GyX 20900, 20908 AVROS 1502, 1591	RRIM 508, 511, 615, 620, 703, 709, 717, 720, 722, 724 61C/240, 61C/414 Ch 137 60C/219
20.1 to 26.0		IAN 710, 2570, 2668, 2744, 2829, 2877, 2892, 3763, 3795, 3819, 3827, 6165, 6167, 6497, 6584, 6640, 6641, 6753, 6754, 6757, 6834 FX 349, 505, 614, 637, 2261, 3482, 3810, 3899 Ford 4542	Nab 12 RRIC 1,7,22,41	TR 1406, 3645 IRCI 2, 9	PR 247, 248, 257, 258 AVROS 385, 470, 1279, 1349, 1350, 1518, 1712, 1734, 2012 GyX 20899 Pat 190	RRIM 513, 515, 519, 520, 526, 603, 608, 609, 617, 619, 622, 625, 628, 629, 630, 632, 701, 702, 705, 708, 711, 712, 715, 723 TM 30, 34, 35 PB 86, 206, 217, 28/59 48E-96, 48E-109, 48E-267, 48E-275, 48E-277 56A/3, 56A/58, 56A/205 61C/49, 61C/364 60H/11, 60H/221 58B/174, PBT 207, Sog 56, Sab 24, 51D/11, ES 1, Ch 32, CISG 170

TABLE 1. (continued)

26.1 to 32.0		IAN 2489, 2879, 2890, 2897, 3460, 3803, 3828, 3893, 6163, 6166, 6499, 6585, 6586, 6587, 6645, 6755, 45–873 FX 516, 567	RRIC 3, 4, 5, 6, 16, 36, 37, 52 Nab 15, 17	IRCI 1, 7, 10 TR 1542	AVROS 157, 308, 427, 529, 1735, 2037 PR 107, 228 LCB 1320 Waring 4 Tjir 1 GT 1 GyX 20909	RRIM 505, 510, 512, 600, 601, 602, 604, 610, 613, 621, 623, 624, 627, 633, 636, 637, 638, 700, 704, 706, 710, 714, 725 PB 5/63, 49, 186, 203 56A/137, 56A/181, 56A/238 61C/307, 61C/329, 61C/417 48E-118 ES 2, 8 Ch 153, 57A/397, 58B/142, TM 1, Rub 393, 51D/102, SPert 3, G1 1, PBT 157
32.1 to 38.0		IAN 2667, 2887, 2928, 2933, 3457, 3711, 3892, 45–717 FX 4056	RRIC 14, 21, 28, 42		AVROS 1191, 1447 PR 226, 231 WR 101	RRIM 501, 516, 605, 606, 607, 611, 612, 614, 616, 618, 626, 631, 634, 635, 707, 719 Ch 26, 30, 31, 123, 148 48E-137, 48E-175, 48E-355 ES 4, 6, 7 51D/91, 51D/100 PB 5/51, 213 Sog 39
38.1 to 44.0		FX 1042, 2831, 4098 IAN 2903, 3702, 6756		IRCI 3	LCB 870	ES 3, 5, 9 TM 2

* S.E. of a clone mean (average of two complete trials) ±3.947 Min. sig. diff. (P = 0.05) between two clones chosen at random 10.98

TABLE 2. RECOVERY OF *PHYTOPHTHORA* AT VARIOUS DISTANCES FROM A FOOD BASE IN SEVERAL SOIL TYPES

Soil type	Food base	Distance from inoculum (mm)		
		5	10	15
Kuantan	agar	+	−	−
	petiole	+	−	−
Sungei Buloh	agar	+	+	−
	petiole	+	+	−
Selangor	agar	−	−	−
	petiole	+	−	−
Peat	agar	+	+	−
	petiole	+	−	−

Selangor series soils was the rate of recovery improved by using the petiole inoculum.

Growth without a Food Base

Dense zoospore and sporangial suspensions of the unnamed species and of atypical *P. palmivora* were placed in small central depressions in sterile and non-sterile soil plates. Tested by cacao pod inoculation, samples taken at weekly intervals 5, 10, and 15 mm from each depression were all negative, even though the inocula were still viable up to the end of the 51-day trial period. Thus the fungi were able to survive a long period in soil but failed to spread in the absence of an additional food base.

Longevity of Phytophthora *in Soil*

The capacity of the unnamed species to survive in soil was studied by mixing a macerated pea-broth culture of the fungus with sterile and non-sterile soil at the rate of 1 to 100 by volume. The inoculated soil was placed in 6 in. diameter clay pots and kept in a glass-house (23–32°C), where half the number of pots were watered daily and the others were allowed to dry out. To maintain soil temperature and moisture conditions comparable with those in the field, a second set of pots was buried to the brim in soil under mature rubber trees; samples were taken at 10-day intervals for ten weeks. *Phytophthora* was not recovered from pots containing sterile or non-sterile soils after one month in the field. In the glass-house, the fungus was recovered after thirty days in non-sterile soil which had been kept moist, compared with twenty days in the dry soil. Recovery was extremely poor from sterilised soil, the fungus being re-isolated after ten days only from moist soil. From the sterilised soil the propagules of several saprophytic organisms were isolated: *Trichoderma, Penicillium, Aspergillus* and *Mucor;* possibly these were present in sufficient quantity to be inhibitory to *Phytophthora.*

Saprophytic Survival in Petioles

In order to observe the saprophytic survival of *Phytophthora,* naturally and artificially infected petioles (the latter having the atypical strain of *P. palmivora*) were buried in non-sterile soil in pots in the laboratory. Petioles were removed weekly and tested for the presence of *Phytophthora* by inoculations into cacao pods. The fungus could be recovered up to four months, by which time the petioles had almost completely rotted away; it could also be recovered over the same period from soil within 10 mm of the petioles.

Survival of Spores in Soil

During the *Phytophthora* leaf fall season, it is to be expected that sporangia and zoospores would be washed into the soil from fallen leaves. To study their survival, non-sterile soil samples were moistened to 50% m.h.c. while others were either air-dried or saturated with

water. The soil samples were plated in sterile petri dishes, sporangia and zoospores of the unnamed species were added separately to each, and the contents of the dish were thoroughly mixed. Viability was tested at intervals by cacao pod isolation. After six weeks, no recovery was achieved from the air-dried mixtures containing sporangia or zoospores. In the moistened and water-saturated plates, both sporangia and zoospores, or structures produced by them, were still viable after six weeks; thereafter re-isolation was no longer possible.

Saprophytic Colonisation in Soil

Petri dishes containing non-sterile Sungei Buloh series soil were centrally inoculated with agar discs of the two *Phytophthora* spp. After twelve days' incubation, a piece of freshly detached autoclaved rubber leaf was placed over the inoculum, and a similar piece on the soil surface 2 mm from the inoculum, and left for a further fifty-four days. Leaves were lifted at intervals for the cacao pod test for *Phytophthora*. The fungus was re-isolated from the leaf sections placed above the agar inoculum, but not from those on the soil surface, showing that the *Phytophthora* in the soil was unable saprophytically to colonise the leaves under the competitive conditions prevailing in the non-sterile soil. The unnamed species was recovered over its inoculum for the whole of the incubation period; *P. palmivora* was not recovered beyond the first nine days.

DISCUSSION

One factor which must be expected to affect the occurrence of *Phytophthora* leaf fall is the susceptibility of petioles to colonisation by zoospores. The laboratory test described, using a standardised zoospore suspension and petioles of standard age, has given reproducible results which show marked clonal differences. That other factors also affect clonal susceptibility is however clearly shown by information available on field performance (severely limited though, by the restricted occurrence of *Phytophthora* leaf fall), for this differs in several important respects from the *in vitro* susceptibilities. Thus RRIM 600, PB 86 and Gl 1 are given the same susceptibility rating, whereas in the field the latter clone is the least susceptible. A possible reason for the laboratory test not providing a good forecasting behaviour in the field is the great difference in the amount of seed set; green pods are highly susceptible to infection by *Phytophthora*, which sporulates freely on them and from there infects the leaves; this is only a partial answer, however, for defoliation has been seen in the virtual absence of seed pods, infected or healthy. An additional factor affecting the field behaviour of certain clones is their different reaction to the six pathogenic strains of *Phytophthora* (CHEE, 1969).

Another determining factor is the soil, which is believed to be the reservoir of the fungus. *Phytophthora* is invariably found in the top 2 in. of soil under or near rubber trees affected by one of the *Phytophthora* diseases. The correspondence between infested soil and diseased trees raises the question of the significance of infection by rain-splash of soil — considered important in the spread of black pod disease of cacao (*Theobroma cacao* L.) (TURNER, 1965) and of fruit rot of papaya (*Carica papaya* L.) (HINE *et al.*, 1965) caused by *P. palmivora* and *P. parasitica* Dastur respectively. Heavy tropical rains frequently splash soil particle several feet up the trunk, and it is a common observation that the frequency of black stripe increases as the tapping cut nears ground level.

More research is needed to establish how important the soil is in *Phytophthora* infection; it may be possible to manage infested soils in such a way as to reduce the inoculum level and thus minimise infection. Chemical treatment may be a useful approach in new outbreaks over a small acreage, provided that the pathogen occurs mainly in the surface layer of soil, and that soil infection follows rather than precedes infection of the panel and leaves.

That it is so, and the activity of the pathogen in soil is very limited, is shown by the experiments described. There is negligible radical extension even when a large inoculum of the fungus is backed by a food base. The fungus rather rapidly loses its viability in soil, its decline being hastened by prolonged drought. The situation is thus similar to that of *P. palmivora*

infecting cacao, for this pathogen has also been shown to have a low soil viability (TURNER, 1965); there is an important difference, however, in that the reservoir of inoculum in the soil is constantly being added to by the production of infected pods. Thus frequent harvesting of cacao and removal of diseased pods help to reduce the disease incidence in cacao plantations (WHARTON, 1958). A similar removal of diseased rubber leaves and pods does not appear from the present studies to be attractive, for four reasons: (1) the fungus does not sporulate on the petioles; (2) it survives for only four months in infected petioles buried in soil; (3) the principal survival structure of *P. palmivora* is the relatively thin-walled chlamydospore, a structure which germinates readily in water and hence would not be expected to be long persistent; (4) there is no evidence of any autochthonous saprophytic growth by the utilisation of substances contained in soil. Natural decline in viability would eventually result in the eradication of the pathogen, in the absence of susceptible hosts. Infected leaves, as well as the fungus developed from them in the soil, would be expected to have disappeared or been reduced to an insignificant infectious level before the return of the next monsoon when conditions once again are favourable for leaf fall to occur. The conditions for black stripe disease to occur are, however, less critical; field observations show that a week of continuous rain is enough to bring about an infection in a susceptible clone. In this way the survival of the pathogen may be prolonged by the reactivation of the fungus as a black stripe pathogen.

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DISCUSSION

Chairman: Dr. J. W. Blencowe

This paper was discussed together with the preceding paper (PERIES, 1969).*

Prof. F.J. Newhook confirmed that the *Phytophthora* fungus described by Dr. Chee was distinct from *P. meadii* and that he knew Miss G.M. Waterhouse, an authority on the taxonomy of the group, agreed. *P. meadii* and *P. palmivora* might prove to be synonymous, but the new fungus differed also from *P. palmivora* which caused black stripe in Malaya and leaf fall in several countries. The actual pathogen should be determined in each case. It was misleading to group all *Phytophthora* leaf fall diseases together because of superficially similar symptoms. The new species should be named by publication of a description as soon as possible. Dr. N.W. Simmonds added that a new common name was required for the leaf fall disease caused by the new *Phytophthora* species in north Malaya and south Thailand to avoid confusion with leaf fall induced by *P. palmivora* elsewhere, so that the incidences of the different diseases and susceptibilities of clones to them could be recorded separately.

Dr. P. de T. Alvim believed that the same fungus *P. palmivora* attacked both trunk and foliage in Bahia, whereas Mr. W. Partosoedarso said there appeared to be no relationship between the incidences of black stripe and leaf fall in Java. Dr. O.S. Peries remarked on the much heavier production of *Phytophthora* sporangia on *Hevea* fruit in Ceylon than in north Malaya. Mr. K. Jayarathnam added that the fruit were completely covered with sporangia in India also, where oospores had been found on fruit in the field.

Mr. R. Shepherd recommended selection of clones bearing few fruit because of their role in spore dispersal; the new clone Prang Besar 252 (PB 86 × PB 30/36) flowered profusely but set no fruit. Dr. Chee attributed differences in *Phytophthora* leaf fall incidences on either side of the border between north Malaya and south Thailand to the cultivars planted in the respective areas. Susceptible clones such as RRIM 600 were planted extensively in northeast Malaya.

*See page 73.