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Fruit Rot of Durian Caused by *Phytophthora palmivora*

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ABSTRAK

Suatu reput buah yang teruk pada durian telah dijumpai di Bentong dan Raub di Pahang; Dengkil di Selangor; Muar dan Kluang di Johor disebabkan oleh Phytophthora *sp. Selain daripada buah durian, asingan-asingan patogen juga berupaya menjangkiti akar, batang dan daun pokok durian. Asingan-asingan dikenalpasti sebagai* P. palmivora, *'cross-inducing' (heterotalik) dan jenis perpasangan A1, mengeluarkan oospora apabila dipasang dengan jenis perpasangan A2* P. palmivora *yang piawai dan suatu lagi perpasangan A2 daripada koko dan bukan dengan jenis perpasangan A1* P. parasitica.

ABSTRACT

A severe fruit rot of durian fruits in Bentong and Raub in Pahang; Dengkil in Selangor; Muar and Kluang in Johore was found to be caused by a Phytophthora *sp. Besides fruits, isolates of the pathogen were also capable of infecting roots, stem and leaves of durian. The isolates were identified as* Phytophthora palmivora, *cross-inducing (heterothallic) and of the A1 mating type, producing oospores when paired with a standard A2 mating type of* P. palmivora *and another A2 isolate from cocoa but not with an A1 mating type of* P. parasitica.

INTRODUCTION

Like most fruit crops, the durian, *Durio zibethinus* Murr. is susceptible to attacks by pests and disease-causing microorganisms at all stages of the crop phenology. Among the latter group is the fungus *Phytophthora palmivora* which can cause devastating losses at both the seedling and adult tree stages. The fungus infects the roots, causing root rot (Navaratnam, 1966; Tai, 1970); the trunk, causing patch canker (Thompson, 1934; Navaratnam, 1966; Tai, 1970); leaves and shoots, causing leaf blight, defoliation and die-back (Tai, 1970). Recently, durian fruits were also found to be infected by a *Phytophthora* sp. Severe fruit rots were found in several fruit orchards in Bentong and Raub in Pahang; Dengkil in Selangor, and Muar and Kluang in Johore. Fruits on high branches were also attacked in several orchards. Affected fruits lost their marketability completely.

In cognizance of the seriousness of the disease, investigations were undertaken i) to identify and characterize the causal fungus through cultural studies; ii) to establish pathogenicity on fruits and other parts of the durian plants; and iii) to study the symptomatology of the fruit rot.

MATERIALS AND METHODS

Isolations

Diseased fruit tissues (5 × 3 × 1 mm) from advancing margins were obtained with a steri-

lised scalpel after peeling off the epidermis. Four pieces of such tissues were embedded in 2% water agar or Pimaricin Vancomycin PCNB media (Ocana and Tsao, 1966). Single sporangial cultures of two representative isolates designated PDFD (isolate from Dengkil), and PDFK (isolate from Kluang) were obtained and maintained on Vegetable Juice (V8) Agar (VJA) (Miller, 1955) slants at 24°C and used for the studies.

Cultural Studies

Six mm mycelial discs taken from the advancing edge of five-day old VJA cultures of the two isolates were separately placed in the centres of carrot agar (CA), Difco cornmeal agar (CMA), Difco potato dextrose agar (PDA) and VJA plates in two sets of four replicates. One set was incubated in continuous darkness at 28 ± 1.5°C and the colony characteristics were compared after seven days. The other set was used to assess growth rates at the same temperature where the colony diameter measurements were taken at right angles on alternate days for a duration of seven days. Data were subjected to statistical analysis (ANOVA).

The procedures were repeated for temperature-growth relations using CMA as the medium and temperature regimes of 16, 20, 24, 28, 32 and 36°C.

Spore Characteristic Studies

Measurements of sporangium and chlamydospore dimensions; apical thickening; pedicel length and width of exit pore of sporangia were based on 100 spores of the test isolates in separate experiments.

To study sporangium dimensions and depth of apical thickening, the test isolates were grown on CMA plates under flourescent light for seven days at 28 ± 1.5°C and harvested for zoosporangia as follows: a standardised amount of sterile distilled water was added to the plates and a bent glass rod was used to dislodge the zoosporangia. Drops of sporangium suspension were mounted on glass slides in half-strength lactophenol blue to facilitate measurements of sporangium length, breadth and depth of apical thickening.

The procedures were repeated for measurement of chlamydospores from another batch of CMA plates after fourteen days incubation.

To study sporangium caducity, a modified method of Al-Hedaithy and Tsao (1979) was used. Mycelial mat colonies of the test isolates were established in 90 mm petri dishes containing 20 ml of vegetable juice made up of 10% Campbell's V-8 juice and 0.2% calcium carbonate and incubated in the light at $28 - 15$.°C. After five days incubation, the mats were washed five times and transferred to petri dishes containing 25 ml of sterile distilled water. After 48 hours of incubation, the plates were observed for naturally and mechanically detached sporangia and their pedicel lengths were measured.

To measure the sporangium exit pore, a separate sporangium suspension was subjected to chilling treatment at 6°C for 25 min. (Lee and Varghese, 1974), and then returned to room temperature to facilitate release of zoospores. Sporangia and zoospores were studied using half-strength lactophenol blue and the width of the exit pores of the sporangia were measured.

Compatibility Type and Oospore Production

Both test isolates were paired among themselves, between each other, separately between standard Al mating type of *P. parasitica* (IMI 268688), A2 mating type of *P. palmivora* from cocoa and an A2 mating type of *P. palmivora* (IMI 203533) on VJA medium and incubated in the dark at 28 ± 1.5°C. All compatibility testings were made in four replicates and examined for oospore production after two weeks. A hundred oospores were measured per plate.

In addition, induction of oospore production of the test isolates was determined using the *Trichoderma* method of Brasier (1974). Five-day old VJA cultures of the isolates were inverted over five-day old PDA culture of *T. koningii* and sealed together with paraffin and cello-

phane tape. The combined plates were likewise incubated and examined for oospore production after two weeks.

Pathogenicity Studies

Artificial inoculations of the roots, stem, leaves and fruits were carried out. For the first three plant parts, six two-month old polybag seedlings were used per test isolate and check treatment and three mature, large fruits were likewise used for fruit inoculation. Inoculation of the test isolates was repeated on cocoa seedlings and fruits.

Zoospore suspensions prepared as described for the sporangium caducity studies were used for the root, leaf and fruit inoculations. The concentration was adjusted to 5000 spores per ml with the help of the Neubauer haemocytometer. Root inoculation was done by drenching the loosened soil of the polybag with 200 ml of zoospore suspension. Check plants were drenched with 200 ml of sterile distilled water. The plants were covered with polyethylene bags for 48 hours and were kept water-logged for three days by placing them on clay saucers filled with water. The plants were watered daily and after three months, the plants were removed and the roots washed and evaluated for the degree of root infection. Leaf inoculation was accomplished by spraying the seedlings with 50 ml of the zoospore suspension using the Desaga spray gun. Check plants were sprayed with sterile distilled water. The plants were again kept for 48 hours in moist polybags. Leaves were examined for disease development after five days. For fruit inoculation, three shallow circular plasticine wells (10 mm diameter, 6 mm deep) were constructed on the fruit surface which had been sterilised with alcohol. The wells were filled with 0.5 ml of the zoospore suspension. The wells on the check fruits were filled with sterile distilled water. The fruits were kept in moist chambers and observed for lesion development up to a week.

Stem inoculation was carried out by pricking the tender green stem of the plant to a standard depth of 2 mm with the tip of a needle carrying mycelial fragments taken from a seven-day old VJ broth culture of the test isolate. The wound was covered with moist cotton wool and bounded with transparent polyethylene strips. Check plants were wounded with a clean sterile needle. The plants were kept moist as described above. Length of canker lesions were regularly measured for a period of two weeks.

Reisolations were made on PVP medium from all positive inoculations.

RESULTS

Isolations consistently yielded the same *Phytopthora* sp. Representative isolates, PDFD and PDFK, both produced stellate and striate colonies with sparse aerial mycelia and well defined margins on CA, VJA and CMA; whilst on PDA, colonies were more fluffy and margins were slightly irregular. Growth on VJA was the fastest, while PDA registered the slowest growth rate (Table 1). Both isolates grew at temperatures of 16°C to 32°C, with optima at 28°C and both failed to grow at 36°C. Isolate PDFD exhibited higher growth than isolate PDFK *(Fig. 1.)*

Both isolates produced papillate, caducous, ovoid to ellipsoid, rounded-based sporangia with a mean of 53.68×35.28 μm *(Fig. 2)*. Isolate PDFK produced larger sporangia than PDFD on CMA under light incubation (Table 2). The length: breadth ratio of PDFD was 1.48 and that of PDFK was 1.78. The sporangia possessed short, hyaline pedicel, occluded by a plug; the mean pedicel length observed was 2.61 ± 0.10 μm and 2.56 ± 0.09 μm for PDFD and PDFK respectively. The mean apical depth recorded for PDFD was 2.76 ± 0.12 μm and for PDFK was 3.59 ± 0.14 μm; whilst the mean width of the exit pore measured was 5.72 ± 0.14 μm and $6.12 + 0.14$ μm for PDFD and PDFK respectively.

Both isolates produced large, spherical, chlamydospores in abundance after two weeks of incubation. Chlamydospores of PDFD ranged from $31.72 - 43.92$ μm (mean 36.37 ± 0.21) in diameter while those produced by PDFK were smaller and ranged from $30.50 - 39.04$ μm (mean 34.39 ± 0.18 μm).

TABLE 1
Growth rate of *Phytophthora palmivora* isolates from durian fruits on selected media at 28 ± 1.5°C

Agar medium	Isolate PDFD	Isolate PDFK
	Mean growth rate (mm/day)	
Carrot agar	11.95* a ++	12.33 a
Cornmeal agar	11.63 a	11.58 a
Vegetable juice agar	12.11 a	12.62 a
Potato dextrose agar	7.20 a	6.93 b

*Each value is an average of 4 replicate plates.

++Mean value in column followed by similar letters denote no significant differences at P = 0.05 as determined by New Duncan Multiple Range Test.

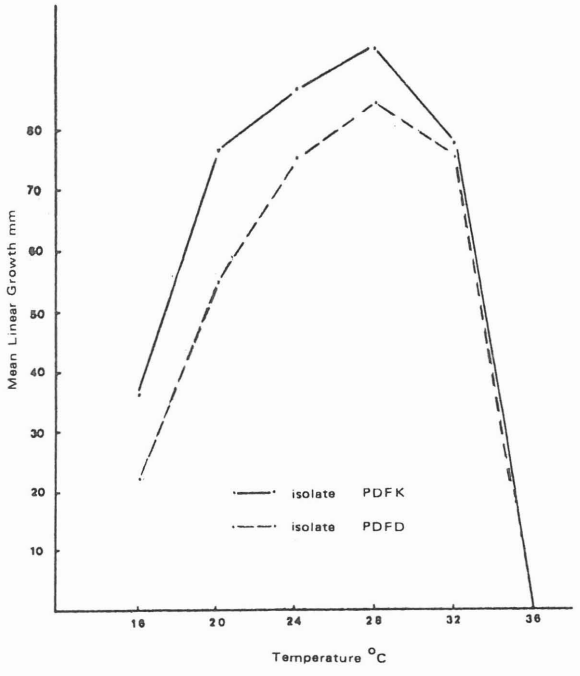

Fig. 1: Effect of temperature on mycelial linear growth of ***Phytophthora palmivora*** *isolates from durian fruit on cornmeal agar.*

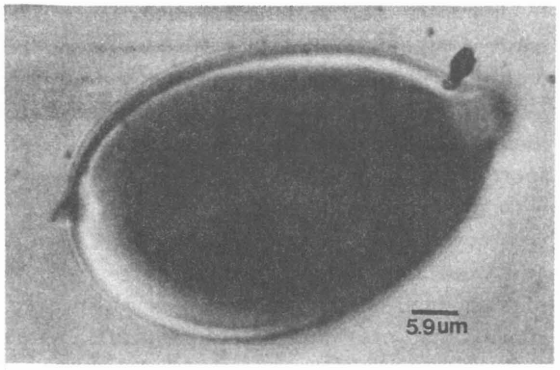

Fig. 2: A typical papillate, caducous, ellipsoid sporangium of ***Phytophthora palmivora*** *from durian (isolate PDFK) with a short pedicel.*

No oospores were formed when the isolates were selfed, paired between each other or paired with the Al mating type of *P. parasitica*. Abundant oospores with amphigynous antheridia were observed when the isolates were separately paired with a *P. palmivora* isolate from cocoa and a standard A2 isolate of *P. palmivora*. Diameters of oospores produced ranged from 20.74 ± 30.50 μm (Table 3). No oospores were formed by the *Trichoderma* induction method. However, abundant chlamydospores and morphogenic changes such as the thickening of the colony margin of the isolates, vacuolation of the cell contents and hyphal lysis were observed.

Both isolates were capable of infecting the roots, stem, leaves and fruits of the durian, but were avirulent on cocoa. They caused a reduction in the amount of rootlets, necrosis of the rootlets and tap roots of seedlings. Both isolates were equally virulent on the durian stem (Table 4), causing extensive canker lesions which girdl-

TABLE 2

Sporangium morphology of *Phytophthora palmivora* isolates from durian on cornmeal agar after 7 days light inoculation at 28 ± 1.5°C

Isolate	Range of length (μm)	Mean length* (μm)	Range of breadth (μm)	Mean breadth* (μm)	Length/ breadth ratio (μm)	Mean pedicel length* (μm)	Mean apical depth* (μm)	Mean width of exit pore* (μm)
PDFD	39.04 – 73.20	52.94 ± 1.29	26.84 – 46.36	35.92 ± 0.83	1.48	2.61 ± 01	2.76 ± 0.12	5.73 ± 0.14
PDFK	46.36 – 68.32	56.46 ± 0.94	24.40 – 41.48	36.06 ± 0.63	1.78	2.56 ± 0.09	3.59 ± 0.14	6.12 ± 0.14

*Average of 100 spores.

TABLE 3
Dimension of oospores obtained from pairings between *Phytophthora palmivora* isolates from durian (PDFD, PDFK) and A_2 mating types on vegetable juice agar

Crossings	Range (μm)	Mean diameter (μm)*
PDFD × PC1 (*P. palmivora* A_2 cocoa isolate)	21.96 – 30.50	25.54 ± 0.39
PDFK × PC1 (*P. palmivora* A_2 cocoa isolate)	20.74 – 28.06	25.14 ± 0.30
PDFD × A_2 *P. palmivora* (IMI 203533)	20.74 – 26.84	23.91 ± 0.23
PDFK × A_2 *P. palmivora* (IMI 203533)	20.74 – 26.84	23.18 ± 0.23
PDFK × A_1 *P. parasitica* (IMI 268688)	0	0
PDFD × A_1 *P. parasitica* (IMI 268688)	0	0

*Average of 100 oospores count per replicate plate.

TABLE 4
Pathogenicity of *Phytophthora palmivora* durian isolates on durian seedlings

Isolate	Number of days after inoculation			
	3	6	10	14
		Mean stem lesion (mm)		
PDFD	10.75* a**	110 a	176.5 a	dieback
PDFK	8.50 a	102.3 a	186 a	dieback
Blank agar	0 b	0 b	0 b	0

*Average of 4 replicates consisting of 6 durian seedlings per replicate.

**Mean values followed by the same letter in each column are not significantly different at P = 0.05 as determined by New Duncan Multiple Range Test.

ed the stems, leading to dieback of the seedlings. When inoculated on the leaves, symptoms were visible after three days. Leaf lesions were characterised by small off-coloured, water-soaked spots which darkened and coalesced into larger patches. Infected leaves drooped and aborted prematurely. On fruits, symptoms first appeared as hydrotic patches which turned brown and then dark-brown to black. A whitish bloom of mycelia and sporangia formed on the lesion after five days *(Fig. 3)*.

The test isolates were reisolated from all positive inoculations. All check inoculations did not produce disease symptoms.

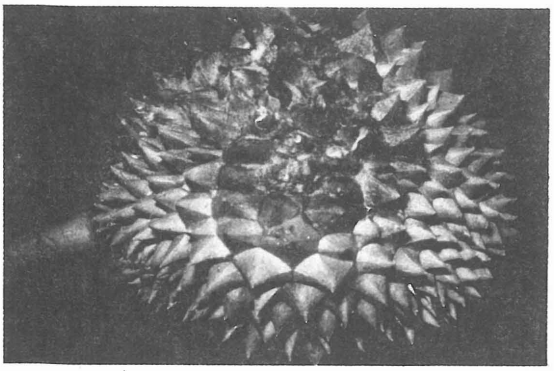

Fig. 3: Durian fruit naturally infected by ***Phytophthora palmivora.*** *Note the white bloom of mycelia and sporangia of the pathogen on the necrotic lesion (arrowed).*

DISCUSSION

The above studies clearly demonstrated that the *Phytophthora* isolates from durian fruit belonged to the *P. palmivora* (Butler) Butler group. The cultural and morphological characteristics of the isolates subscribed to those of *P. palmivora* as described by Newhook *et al.* (1978); Brasier and Griffin, (1979); and Waterhouse *et al.* (1983). The failure of both isolates to grow at the temperature of around 36°C further distinguish them from *P. nicotianae* var. *nicotianae* and *P. nicotianae var. parasitica* which could grow well at temperatures above 35°C (Newhook *et al.*, 1978; Waterhouse *et al.*, 1983; Weste, 1983). The ability of the two *Phytophthora* isolates to produce chlamydospores in abundance in agar culture also distinguish them from other *Phytophthora* species recorded in Malaysia like *P. meadii, P. hevea* and *P. botryosa* which do not or rarely produce chlamydospores in culture (Waterhouse, 1974).

The caducous nature of their sporangia with short pedicels of < 3 μm, their stellate and striate colonies and their inability to form oospores by *Trichoderma* induction are similar to the traits described for Morphological Form 1 (MF1)in the *P. palmivora* group (Newhook *et al.*, 1978; Brasier and Griffin, 1979; Waterhouse *et al.*, 1983). To avoid taxonomical confusion, Brasier and Griffin (1979), reiterated that the MF1 designation should be discarded and all MF1 isolated be regarded as typical *P. palmivora* species.

The formation of oospores by the isolate only when crossed with A2 mating type of *P. palmivora* tester and from cocoa confirmed that the isolates are heterothallic or cross-inducing (Ko, 1978), and of the A1 compatibility type.

The *Phytophthora* isolates were capable of infecting both subterranean and aerial plant parts similar to some aerial-soil parasites like *P. cactorum* and *P. parasitica* (Ko, 1980). Although *P. palmivora* has an extensive host range (Chee, 1969), some specificity do exist among isolates from various hosts as are exemplified by the durian isolates which were avirulent on cocoa.

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REFERENCES

AL-HEDIATHY, S.S.A. and P.H. TSAO. (1979): Sporangium pedicel length in *Phytophthora* species and the consideration of its uniformity in determining sporangium caducity. *Trans. Brit. Mycol. Soc.* **72(1):** 1 – 13.

BRASIER, C.M. (1975): Stimulation of sex organ formation in *Phytophthora* by antagonistic species of *Trichoderma*. 1. The effect invitro. *New Phytologist* **74:** 183 – 194.

BRASIER, C.M. and M.J. GRIFFIN. (1979): Taxonomy of *Phytophthora palmivora* on cocoa. *Trans. Brit. Mycol. Soc.* **72:** 111 – 143.

CHEE, K.H. (1969): Hosts of *Phytophthora palmivora*. *Rev. Appl. Mycol.* **48:** 337 – 344.

KO, W.H. (1978): Heterothallic *Phytophthora:* Evidence for hormonal regulation of sexual reproduction. *J. Gen. Microbial.* **107:** 15 – 18.

KO, W.H. (1980): Sexuality, evolution and origin of *Phytophthora*. *Plant. Prot. Bull. Taiwan* **22:** 141 – 151.

LEE, B.S. and G. VARGHESE. (1974): Studies of the genus *Phytophthora* in Malaysia. Reproduction and sexuality. Mal. Agri. Res. **3:** 137 – 149.

MILLER, P.M. (1955): V-8 juice agar as a general medium for fungi and bacteria. *Phytopathol.* **45:** 461 – 462.

NAVARATNAM, S.J. (1966): Patch canker of the durian tree. *Mal. Agri. J.* **45(3):** 291 – 294.

NEWHOOK, F.J., G.M. WATERHOUSE and D.J. STAMPS. (1978): Tabular keys to the species of *Phytophthora* de Bary. *CMI. Mycol. Paper No.* 143, 20 pp.

OCANA, G. and P.H. TSAO. (1966): A selective agar medium for the direct isolation and enumeration of *Phytophthora* in soil. *Abstr. Phytopathol.* **56:** 893.

TAI, L.H. (1970): Studies of *Phytophthora palmivora*, the causal organism of patch canker disease of durian. Cawangan Perkembangan, Jabatan Pertanian, K. Lumpur. 7 pp.

THOMPSON, A. (1934): A disease of the durian tree. *Mal. Agric. J.* **22:** 367 – 371.

WATERHOUSE, G.M. (1974): Other *Phytophthora* species recorded in cacao. *In:* P.H. Gregory (ed.) *Phytophthora* disease of cocoa. London: Longman, pp 71 – 79.

WATERHOUSE, G.M., F.J. NEWHOOK and D.J. STAMPS. (1983): Present criteria for classification of *Phytophthora*. *In:* D.C. Erwin, S. Barknicki-Gracia, and P.H. Tsao (eds.) Am. Phytopathol. Soc. St. Paul, Minnesota. pp 139 – 147.

WESTE, G. (1983): Population dynamics and survival of *Phytophthora*. *In:* D.C. Erwin, S. Barknicki-Gracia, and P.H. Tsao (eds.), *Phytophthora — its biology, taxonomy, ecology* and *pathology*. Am. Phytopathol. Soc. St. Paul, Minnesota, pp 237 – 257.

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