# II *Phytophthora* spp. isolated from some economic plants in Thailand

# Summary

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Phytophthora spp. were isolated from the lesions of Phytophthora disease in durian, mandarin orange, pineapple, Hevea rubber and orchid (Vanda and Ascocenda). P. palmivora was obtained from bark, foot and root rot of durian, and black rot of Vanda. P. nicotianae var. parasitica was isolated from root rot of mandarin orange, heart and root rot of pineapple, black rot of orchid and leaf fall (leaf blight) of Hevea rubber. P. botryosa was isolated from leaf fall (lesion on petiole and leaf blight), black stripe and die back of Hevea rubber. But no Phytophthora were detected in black pepper, sesame and others.

*Phytophthora* disease is the most important disease affecting plants of economic importance such as fruit trees, field crops, Hevea rubber and ornamental plants in Thailand. However little information is available on causal organisms of *Phytophthora* disease in such plants. Therefore, more *Phytophthora* spp. have been found on crops through a detailed survey conducted in Thailand. Attempts were made to isolate and identify *Phytophthora* from plants of economic importance.

# I Materials and methods

#### 1 Collection of specimens

The following plants were used in this experiment to isolate *Phytophthora*: Durian; *Durio zibethinus*, mandarin orange; *Citrus reticulata*, *C. sinensis*, pomelo; *C. grandis*, lime; *C. auranti-flora*, pineapple; *Ananas comosus*, Hevea rubber; *Hevea braziliensis*, black pepper; *Piper nigrum*, orchid; *Vanda hybrid*, *V. rothschilidiana*, *Cattleya* sp., *Ascocenda yipsumwath*, mulberry; *Morus alba*, sesame; *Sesamum indicum*, strawberry; *Fragaria chiloensis* var. *ananassa*, and papaya; *Carica papaya*. The specimens of diseased durian consisted of 148 samples collected from 41 sites in 14 provinces. As for *Citrus* spp. there were 72 samples collected from 27 sites in 12 provinces, for pineapple 59 samples collected from 15 sites in 3 provinces, for Hevea rubber 48 samples collected from 17 sites in 7 provinces, for black pepper 30 samples collected from 12 sites in 5 provinces, for orchid 32 samples collected from 8 sites in 3 provinces, etc. For isolation of *Phytophthora*, infested soil was also collected at a 5 - 10 cm depth from the ground surface near the infected plants.

## 2 Isolation

Infected tissues of plants and infested soil were used for the isolation of *Phytophthora*. PB medium (Masago et al. 1970), BNPRA medium (Fujisawa et al. 1975) and  $P_{10}VP$  medium (Tsao et al. 1969) were used for isolation of *Phytophthora* as selective media. The infected tissues were washed in water and cut into small pieces. The specimens were kept for 1 - 2 min. in a 10 % chlorox solution for surface sterilization. Isolation was usually attempted twice for every speci-

men. Baiting, soil dilution and test plant methods were applied for *Phytophthora* isolation from soil. Baiting method which is a modification of Grimm's trap method (Grimm et al. 1973) was applied and about 150 g of infested soil were put into a 250 ml beaker to which sterilized distilled water was added to flood the soil to a depth of 2 - 3 cm. Small pieces of durian leaf (about  $1 \times 1$ mm) removed from mature leaf were floated on the water and incubated at room temperature (22 - 28°C) for 2 days. Durian leaf pieces colonized by fungi were utilized for the isolation of *Phytophthora* using ordinary methods. Soil dilution and test plant methods were used for isolation of fungi from soil (Johnson et al. 1972) with tomato and cucumber plants as test plants.

#### 3 Sporangia formation and microscopic observation

The isolates were grown on oat meal agar for 4 to 7 days at room temperature  $(22 - 28^{\circ}C)$ . The growing mycelia were transferred into mineral solution (Chen et al. 1970). Some isolates were kept for 2 - 3 days at room temperature in the light and some were incubated in the dark. The mineral solution including mycelia and sporangia was incubated at  $18^{\circ}C$  for 30 to 40 min. to induce zoospores swarming. Mycelia, sporangia and chlamydospores were mounted with 0.1 % cotton blue in lactophenol (Waterhouse 1967) and fungus was observed under the microscope.

#### 4 Sex organ formation

Isolates of *Phytophthora* were reacted with two opposite mating types of either of the two species *P. palmivora* (P 253, mating type A<sup>1</sup>, P 255; mating type A<sup>2</sup>) and *P. nicotianae* var. *parasitica* (T 131; mating type A<sup>1</sup>, T 515; mating type A<sup>2</sup>) which were brought from California Culture Collection by Dr. P. H. Tsao on bact-corn meal agar and cleared V-8 agar (Menyonga et al. 1966). The fungi were incubated at 25°C in the dark for 3 weeks.

#### 5 Measurement of fungal growth

Inoculum of *Phytophthora* consisted of an agar disk 4.0 mm in diameter collected at the margin of a colony cultivated for 2 days on PDA at 27°C. Inoculum was transferred to the center of bact PDA and bact corn meal agar and was incubated for 7 days at 5 to 35°C in the dark. Measurement of fungal growth was carried out every day after transfer and growth rate per day was calculated from the measured value. Gradient temperature incubator was also used to determine critical temperature for mycelial growth of the fungus. The minimum and maximum temperatures were set at about 4 to 42°C, respectively. The fungus was incubated in 20 ml of potato dextrose broth for 6 days. After cultivation the fresh weight of fungus was accurately measured.

#### 6 Identification

The identification of *Phytophthora* isolates was performed according to Waterhouse's system (1963, '70)

# II Results

## 1 Symptoms of *Phytophthora* disease used for isolation

The main symptoms observed in durian, citrus, pineapple, Hevea rubber and orchid collected for isolation of *Phytophthora* are as follows:

1) Durian The pathogen attacks mainly foot and roots of durian plants, but sometimes invades trunk, twig and fruit. The surface of bark rot or foot rot lesions becomes dark brown or blackish brown in color and may soften. Sometimes gum appears on infected parts. Infected bark and foot may turn brown or red brown and later the tissues will be worn to rags and die (Fig. 1). Rootlet rot may appear water-soaked and become brown in color and later may change from brown to blackish brown.

2) Citrus *Phytophthora* disease of citrus is known as brown rot of citrus fruit, brown rot gummosis, foot rot and root rot (Klotz 1973). When the pathogen attacks foot and/or roots of citrus, the symptoms which are observed in plant parts above the ground are as follows: (1) Dwarf, yellowing and wilting of leaves. (2) Die back of twigs. (3) Gum exudation. (4) Brown staining and softening of bark of trunk. (5) Cracking of bark. In axial and lateral roots, a light brown color may appear and water softening may affect the epidermis and cortex. Later, there remains only the fibrous tissue of the roots which becomes ragged (Fig. 2). The color of infected rootlets changes from white cream or yellow cream to ligh brown, brown or blackish brown and the cortex of the roots may disappear.

3) Pineapple *Phytophthora* attacks heart, fruit, peduncle and roots of pineapple. Heart rot is the most common disease. If the heart of the plant is infected with *Phytophthora*, the disease may extend to the leaves and the peduncle and the plant will eventually die. At first, water-soaked lesion appears in infected younger leaves, subsequently the leaves soften and become light yellow brown or brown in color. Lastly rot may involve the leaves (Fig. 3). Root rot due to *Phytophthora* has occasionally been observed in yellowing plants or in plants with heart rot lesions.

4) Hevea rubber Phytophthora attacks the petiole, leaf, twig, bud, pod and tapping bark of Hevea rubber. Petioles and/or leaves infected with Phytophthora eventually fall. Petioles show brown to black lesions from which latex is exuded (Fig. 4). Leaf blight appears on vein and/or mesophyll of leaf. The infected parts become brown, gray or black in color and the lesions on the leaves are several millimeters to several centimeters in size. Black stripe is found on tapping bark of Hevea rubber. First, tapping bark softens and becomes brown, dark brown or black in color and infected parts become hollow. Later the pitting expands on tapping parts (Fig. 5). When the symptoms of black stripe appear under the bark, the color becomes brown to dark brown.

5) Orchid Black rot of orchid caused by *Phytophthora* involves buds, leaves and stems of *Vanda, Ascocenda* and others. At the beginning, the infected part shows a light brown to brown color and becomes water-soaked. From that time on, the color changes to black and the plant eventually dies (Fig. 6).

#### 2 Isolation of Phytophthora

## 1) Isolation of Phytophthora from infected tissues of plants

The results of the isolation of *Phytophthora* from the infected tissues of plants are shown in Table 1. Eighty isolates of *Phytophthora* were recovered from the infected tissues of durian, mandarin orange, pineapple, Hevea rubber and orchid plants. Among them, 20 isolates were obtained from 148 specimens of durian tree, and 3 isolates from 72 specimens of mandarin orange. Also 13, 22 and 22 isolates were obtained from pineapple, Hevea rubber and orchid (*Vanda* and *Ascocenda*) plants, respectively. In the infected tissues of durian tree, *Phytophthora* was more frequently isolated from the infected bark of trunk and rootlet than from infected foot and root rot. In mandarin orange, *Phytophthora* was not found in great amount in the infected foot and root. No *Phytophthora* was isolated from foot and root rot of another citrus group such as lime, pomelo and sweet orange. *Phytophthora* was frequently isolated from leaf fall of Hevea rubber (i.e. from lesion on petiole and leaf blight), and black rot of orchid (*Vanda* and *Ascocenda*). In pineapple, *Phytophthora* was isolated from heart rot and root rot lesions but could not be isolated from fruit rot. No *Phytophthora* was isolated from the infected tissues of black pepper, but *Pythium* was frequently isolated from them, and also *Rhizoctonia solani* was isolated from the diseased roots. No pathogenic fungi were isolated from mulberry root rot and collar rot. No *Phytophthora* 

Host plant	Symptoms	en gelde versen.	Number of Phytophtho isolated	ora	/	Numl specin used	per of nens
Durian	Bark rot, foot rot root rot		2	0	/	148	
Citrus group	Foot rot, root rot			3	/	72	
Pineapple	Heart rot, root rot		1	3	/	59	
Hevea rubber	Leaf fall, die back, black stripe		2	2	/	48	
Orchid	Black rot		2	2	/	32	
Black pepper	Root rot			0	/	30	
Sesame	Leaf blight, stem blight			0	/	25	
Mulberry	Foot rot, root rot			0	/	15	
Strawberry	Root rot			0	/	4	
Рарауа	Foot rot			0	/	3	

Table 1. Number of Phytophthora isolated from plants of economical importance

was isolated from diseased tissues of leaf blight, stem blight and black lesion on pods of sesame, root rot of strawberry, and foot rot of papaya.

#### 2) Isolation of Phytophthora from infested soil

The isolation of *Phytophthora* from infested soil was carried out in durian, citrus orchard, pineapple field and others by using baiting, soil dilution and test plant methods. Four isolates of *Phytophthora* were obtained from 40 samples of durian orchard by the baiting method only, at the exclusion of other methods. *Pythium* was isolated from almost every soil sample by the baiting method. In using the test plant method, wilting of tomato and cucumber was observed but *Phytophthora* was not isolated from every plant with wilting. One and six isolates of *Phytophthora* were obtained by the baiting method from the infested soil of mandarin orange and pineapple fields, respectively. But in the soil of black pepper, Hevea rubber, strawberry, sesame and mulberry fields, *Phytophthora* could not be isolated by the methods used.

#### 3 Identification and description of *Phytophthora*

#### 1) Identification of Phytophthora

Phytophthora isolated from infected tissues of durian, mandarin orange, Hevea rubber and orchid were studied. Results are shown in Table 2. All of the isolates obtained from bark, foot and root of durian contained *P. palmivora*. Three isolates of *Phytophthora* from mandarin orange consisted of *P. nicotianae* var. parasitica, while all the samples of *Phytophthora* isolated from heart rot and root rot of pineapple consisted of *P. nicotianae* var. parasitica. P. botryosa was isolated from samples of leaf fall, leaf blight, die back and black stripe lesion in Hevea rubber. One isolate of *P. nicotianae* var. parasitica was obtained from leaf blight of Hevea rubber at Rayong. No *P. palmivora* was found in leaf fall and black stripe of rubber plants in this experiment. From black rot of orchid (Vanda and Ascocenda), 6 isolates of *P. palmivora* and 14 isolates of *P. nicotianae* var. parasitica were identified in Vanda in the same garden. Three isolates from the soil of durian orchard consisted of *P. nicotianae* var. parasitica, *P. nicotianae* var. parasitica were identified in Vanda var. parasitica, *P. nicotianae* var. parasitica var. parasitica var. parasitica var. parasitica and one isolate of *P. nicotianae* var. parasitica, *P. nicotianae* var. parasitica var. parasitica var. parasitica var. parasitica var. parasitica var. parasitica and one isolate of *P. nicotianae* var. parasitica var.

Fungus	Host plant or soil	Symptoms	Number of isolates
Phytophthora	Durian	Bark rot	8
palmivora	do.	Foot rot	4
	do.	Root rot	8
	Vanda	Black rot	7
	Durian orchard soil	<u>-</u> 1997 - 1997	3
P. nicotianae	Mandarin orange	Root rot	3
var. parasitica	Pineapple	Heart rot	9
	do.	Root rot	4
	Hevea rubber	Leaf blight	1
	Vanda	Black rot	12
	Ascocenda	Black rot	2
	Citrus orchard soil	son en	
	Pineapple field soil	a a ser a Reference a ser	konstantia al secondaria. <b>1</b>
P. botryosa	Hevea rubber	Leaf fall (Petiole)	12
	do.	Die back	1
	do.	Leaf blight	4
	do.	Black stripe	4
Phytophthora sp.	Vanda	Black rot	1
	Durian orchard soil	n an	where $1^{(1)}$ is the second sec
	Pineapple field soil	tis falle et di sectori fuelle été. Al Transitione de la Sectoria	5

Table 2. Phytophthora spp. isolated from durian, mandarin orange,<br/>pineapple, Hevar rubber, orchid (Vanda and Ascocenda)<br/>and soil in Thailand

and Phytophthora sp. were isolated from the soil of pineapple fields.

2) Description of selected Phytophthora

Among the isolated *Phytophthora*, three isolates of *P. palmivora*, *P. nicotianae* var. *parasitica* and *P. botryosa* from durian, mandarin orange and Hevea rubber were selected. Their description is as follows:

Isolate D16-21-1 Phytophthora palmivora (Butler) Butler

Specimen: Durian cv. Gopt, 15 years old

Isolation date; June 5, 1975

Symptoms; Rootlet rot

Location: Don Ki Ret, Prachinburi, Soil pH; 6.3

Hyphae 3.6 - 5.7  $\mu$ m wide, 4.5  $\mu$ m av., smooth. Sporangiophores long and slender, irregularly or sympodially branched, 2.3 - 4.5  $\mu$ m wide, 3.3  $\mu$ m, av. Sporangia ovate or elongated elliptical, 35 - 115 x 23 - 46  $\mu$ m av. 52 x 32  $\mu$ m, L:B ratio 1.61, apex distinctly papillate with marked apical thickening up to 4.7  $\mu$ m, cross-walled at the base of sporangium, round at the base, pedicel 2.3 - 4.5  $\mu$ m length, av. 3.3  $\mu$ m, deciduous, terminal, abundantly formed in mineral water after cultivation in oat meal agar (Fig. 7). Chlamydospores terminal, spherical, 25 - 42  $\mu$ m, av. 30 x 30  $\mu$ m, smooth, thin-walled. Sex organs heterothallic, formed with compatible type, i.e. P255 (*P. palmivora*, mating type A<sup>2</sup>, California Culture Collection) or T515 (*P. nicotianae* var. *parasitica*, mating type A<sup>2</sup>). Oogonia spherical, rough, thin-walled, 20 - 28  $\mu$ m diam., av. 25  $\mu$ m, yellow to golden. Antheridia always amphigynous, spherical, subspherical, av. 13 × 13  $\mu$ m. Oospores nearly filling the oogonia, av. 22  $\mu$ m, wall 2.1  $\mu$ m thick, yellow to yellow brown.

Culture thin carpet like, powdery, zonate, no aerial mycelium on PDA. Mycelium sparse, radiate, no aerial mycelium on CMA in room light, 27°C. Min. temp. 10°C, opt. temp. 26 - 28°C, max. temp. 35°C. Growth rate 9.5 mm a day on PDA at 27°C, 7.4 mm on CMA at 27°C.

Isolate C20-2-7 Phytophthora nicotianae B den Haan var. parasitica (Dastur) Waterh.

Specimen: Mandarin orange (Soam Kio Wan), 5 years old

Isolation date; July 11, 1975

Symptoms Root rot

Location; Ban Pha Huan, Nan, light clay, rich humus soil, pH 5.4

Hyphae 4.5 - 6.8  $\mu$ m wide, av. 5.3  $\mu$ m with swellings, hyphae may grow out from swelling point. Sporangiophores long, slender, smooth, irregularly or sympodially branched, 1.8 - 3.4  $\mu$ m wide, av. 2.4  $\mu$ m. Sporangia broadly ovoid, spherical or obpyriform, not noticeably narrowed at the apex, papilla distinct, hyaline, apical thickening up to 4.2  $\mu$ m on av., hemispherical, 27.2 - 52.3 × 20.9 -31.4  $\mu$ m, av. 36.5 × 26.2  $\mu$ m, L:B ratio 1.39, with a short pedicel, abundantly present in mineral water, terminal (Fig. 8). Zoospores spherical to oval, 8 - 11  $\mu$ m in length. Chlamydospores rare in mineral water, terminal, spherical, 22 - 30  $\mu$ m, av. 25  $\mu$ m, thin-walled smooth. Oogonia readily produced when grown with a compatible mating type i.e. P255 (*P. palmivora*, mating type A<sup>2</sup>) or T515 (*P. nicotianae* var. *parasitica*, mating type A<sup>2</sup>), spherical or subglobose, rough wall, 20 -26 × 23 - 28  $\mu$ m, av. 24 × 25  $\mu$ m, yellow to yellow brown. Antheridia all amphigynous, spherical or oval, 10 - 20 × 12 - 18  $\mu$ m, av. 13 × 14  $\mu$ m. Oospores nearly filling the oogonium, thin-walled, yellow to yellow brown.

Culture: carpet like, zonate, medium aerial mycelium on PDA, mycelium sparse, zonate, poor aerial mycelium on CMA at 27°C in room light. Min. temp. for mycelial growth 7.5°C, opt. temp. 27 - 28°C, max. temp. 37°C, growth rate 6.1 mm a day on PDA at 27°C, 5.8 mm on CMA.

Isolate R17-4-1 Phytophthora botryosa Chee

Specimen: Hevea rubber, local clone Isolation date; Oct. 7, 1975

Symptoms Leaf fall (black lesion on petiole)

Location; Wang Wah, Klang, Rayong

Hyphae 4 - 6  $\mu$ m wide, av. 4.8  $\mu$ m, smooth. Sporangiophores long or short, smooth or swollen, sympodially branched, thin-walled, 0.5 - 1.8  $\mu$ m wide, av. 1.3  $\mu$ m. Sporangia produced abundant clumps when kept in mineral water for 3 to 4 days, terminal, oval, ellipsoidal or ovoid, 25 - 44 × 15 - 27  $\mu$ m, av. 30 × 21  $\mu$ m, L:B ratio 1.58, papilla distinct or intermediate, depth av. 2.3  $\mu$ m, width av. 4.7  $\mu$ m, with short pedicel 0.5 - 2.3  $\mu$ m, av. 1.5  $\mu$ m, deciduous, more abundantly found when kept in the dark (Fig. 9). Chlamydospores terminal, spherical, 15 - 20  $\mu$ m, av. 17  $\mu$ m in diam., thin-walled (0.7  $\mu$ m), smooth. Sex organs produced when grown together in interspecific isolate (*P. palmivora*, mating type A<sup>2</sup> or *P. nicotianae* var. *parasitica*, mating type A<sup>2</sup>). Oogonia spherical or subspherical, slightly rough wall, 22 - 31 × 23 - 32  $\mu$ m, av. 26 × 27  $\mu$ m, yellow to golden in color, wall 1.0 - 2.0  $\mu$ m. Antheridia, amphigynous spherical, 7 - 14 × 11 - 15  $\mu$ m, av. 11 × 13  $\mu$ m. Oospores 15 - 21  $\mu$ m in diam. with pairing T515 isolate (*P. nicotianae* var. *parasitica*, mating type A<sup>2</sup>).

Culture: carpet like, flat, moderate aerial mycelium on PDA, sparse radiate, little or no aerial mycelium on CMA in room light. Min. temp. 8.8°C, opt. temp. 26°C, max. temp. 32°C, growth rate

7.2 mm per day on PDA at 27°C, 9.6 mm per day on CMA.



Fig. 1-6. Lesions from which *Phytophthora* was isolated. 1; Root rot of durian, 2; Foot rot and root rot of mandarin orange, 3; Heart rot of pineapple, 4; Leaf fall of Hevea rubber, 5; Black stripe of Hevea rubber, 6; Black rot of orchid (*Venda*).

Fig. 7-9. Sporangia of *Phytophthora* spp. 7; *P. palmivora* isolated from durian root rot, 8; *P. nicotianae* var. *parasitica* isolated from root rot of mandarin orange, 9; *P. botryosa* isolated from leaf fall (petiole) of Hevea rubber. One division of the scale represents 10  $\mu$ m in photographs 7, 8 and 9.

# **III** Discussion

*Phytophthora* was frequently isolated from orchid (*Vanda* and *Ascocenda*), pineapple and Hevea rubber. But *Phytophthora* was not very often isolated from root rot lesions of durian and mandarin orange. It is considered that many kinds of secondary fungi are found in association with the pathogen in the diseased part of the root more often than in the leaf. In citrus root rot, successful isolation depends on the stage of development of the disease. *Pytium, Mucor, Rhizopus, Absidia* and other *Phycomycetes* grew well on PB medium as did *Phytophthora* from orchid, pineapple and leaf fall of Hevea rubber. Therefore, PB medium was inferior to BNPRA and P<sub>10</sub>VP media. No *Phytophthora* could be isolated from the soil by the soil dilution method when grown on PB medium. If other medium had been used, *Phytophthora* could have been isolated.

Only *P. palmivora* was isolated from specimens of durian as previously reported by Phawakul et al. (1969), Thompson (1934), Navaratnam (1966) and Tsao (1974a).

It has been reported that several species of *Phytophthora* such as *P. citrophthora*, *P. nicotianae* var. *parasitica*, *P. cactorum*, *P. syringae*, *P. palmivora* and *P. megasperma* were responsible for disease in the citrus group (USDA 1960, Phytopath. Soc. Japan 1965, Klotz 1973). Kamjaipai (1974) reported that causal organism of foot rot of citrus was very similar to *P. nicotianae* var. *nicotianae* in Thailand, but Tsao (1974a) identified the organism as being *P. nicotianae* var. *parasitica*. In the present experiment, *P. nicotianae* var. *parasitica* was isolated only from root rot of mandarin orange (*C. reticulata*, Soam Kio Wan and Soam Chin in Thai name). Therefore, isolation of *Phytophthora* from citrus groups should be attempted in many more specimens in Thailand.

*P. nicotianae* var. *parasitica, P. cinnamomi, P. palmivora, P. meadii* and *P. nicotianae* var. *nicotanae* have been reported in the USA, Japan and the Philippines to be the pathogenic organisms of heart rot and root rot of pineapple (Sideris et al. 1930, Mehrlich 1936, USDA 1960, Tamori 1974, Quebral et al. 1962). As only *P. nicotianae* var. *parasitica* was isolated from heart rot and root rot of pineapple in the present experiment, it is suggested that *P. nicotianae* var. *parasitica* is the dominant species of *Phytophthora* in pineapple in Thailand.

*P. botryosa* was reported as a causal organism of abnormal leaf fall of Hevea rubber in Malaysia (Chee 1969). The distribution of the fungus was observed in Malaysia and Thailand (Chee 1967, '68, '69, Chowana 1974, Tsao 1974b, '75). In 1969 and 1970, *P. palmivora* was recognized as a causal organism of leaf fall and black stripe of Hevea rubber in Thailand (Chewchin et al. 1970). The results of isolation from Hevea rubber showed that *P. botryosa* was mainly isolated from leaf fall and black stripe and that no *P. palmivora* could be found in any specimens. However, only one isolate of *P. nicotianae* var. *parasitica* was obtained from leaf blight of Hevea rubber at Rayong. This is the first recorded case in Thailand. Judging from the frequency of isolation it can be considered that *P. botryosa* is the dominant species of leaf fall and black stripe of Hevae rubber in Thailand.

*P. nicotianae* var. *parasitica* and *P. palmivora* were isolated from black rot of orchid (*Vanda* and *Ascocenda*) in the ratio of about 2 to 1. It has been reported that *P. palmivora* was the causal organism of black rot of orchid in Thailand (Sutabutra et al. 1974). Kueprakone reported that *P. nicotianae* var. *parasitica* was isolated from *Vanda* in Thailand in 1973. In the present experiment, it could be demonstrated that *P. nicotianae* var. *parasitica* plays an important role in black rot of orchid.

It was reported that *P. palmivora* was the causal organism of foot rot of black pepper in Brazil, Puerto Rico and Malaysia (Alconero et al. 1972), *P. colocasiae* in Malaysia (Thompson 1929) and *P. palmivora* f. *piperis* in East Kalimantan, Indonesia (Hernadi 1972). Somchai (1967) reported that root rot of *Piper nigrum* L. in Thailand was due to *Phytophthora* sp. Tsao (1974a) recovered *Phythium syringe* and *Phytophthora* sp. from black pepper in Thailand. Recently, Tsao et al. (1977) revealed that *Phytophthora* sp. does not fit the description of any of the *Phytophthora* species previously reported as pathogenic agent of *Piper* spp. In the present experiment *Pythium* spp. were isolated from root rot of black pepper, and the fungi were recognized as pathogenic agents for the plants. But no *Phytophthora* was found in black pepper. The results indicate that root rot of black pepper may possibly be produced by *Pythium* and *Phytophthora* sp. but not by *P. palmivora*.

No Phytophthora was recovered from seasame, strawberry, mulberry, etc. in the disease survey. The specimens collected may have had symptoms caused by other fungi. In the reports, the authors referred to Phytophthora in root rot disease of durian, orange and others. But pathogenic fungi such as Pythium, Rhizoctonia, Fusarium, Cephalosporium and others, were frequently isolated from the lesions of the plants. Therefore, it is necessary to elucidate the role of these plant pathogenic fungi in root rot of durian, orange, pineapple, etc.

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#### References

- 1) ALCONERO, R., ALBUQUERQUE, H., ALMEYDA, N. and SANTIAGO, A. G. (1972): *Phytophthora* foot rot of black pepper in Brazil and Puerto Rico. *Phytopathology*, **62**, 144-148.
- 2) CHEE, K. H. (1967): Phytophthora leaf fall and pod rot. Planter's Bull., 90, 99-106.
- 3) and GREENWOOD, J. M. F. (1968): *Phytophthora* leaf fall and pod rot of *Hevea brasiliensis* in Thailand. *FAO*, *Pl. Prot. Bull.*, 16, 1-5.
- 4) (1969): Variability of *Phytophthora* species from *Hevea brasiliensis*. Trans. Br. Mycol. Soc., 52, 425-436.
- 5) CHEN, D. W. and ZENTMEYER, G. A. (1970): Production of sporangia by *Phytophthora* cinnamomi in axenic culture. Mycologia, 62, 397-402.
- CHEWCHIN, N. and SYAMANANDA, R. (1970): Rubber diseases in Thailand. Techn. Bull., 10, 7-15, Dept. Agric., Thailand.
- CHOWANA, C. (1974): Control of black stripe disease of rubber, Conference on Agric. Sci.,
   13, 8, Kasetsart Univ., Bangkok.
- 8) FUJISAWA, T. and MASAGO, H. (1975): Studies on selective medium for *Phytophthora*. Ann. Phytopath. Soc. Japan, 41, 267.
- 9) GRIMM, G. R. and ALEXANDER, A. F. (1973): Citrus leaf pieces as traps for *Phytophthora* parasitica from soil slurries. *Phytopathology*, **63**, 540-541.
- 10) HERNADI, R. (1972): Some notes on foot rot on black pepper in East-Kalimantan, 1st S-E Asia Regional Symp. on Crop Protection (mimeographed).
- 11) JOHNSON, L. F. and CURI, E. A. (1972): Methods for research on the ecology of soil-borne

plant pathogens, Burgess Pub., p. 5-15, Minnesota.

- 12) KAMJAIPAI, W. (1974): Citrus diseases in Thailand, Proc. Nat. Conf. Agric. Biol. Sci. Thirteenth Sess., 120-126, Kasetsart Univ., Bangkok.
- 13) KLOTZ, L. J. (1973). Color handbook of citrus disease, p. 5-15, Univ. Calf., Riverside, Calf.
- 14) KUEPRAKONE, U. et al. (1973): Comparison study of certain plant parasitic Phytophthora spp., Ann. Res. Report, Dept. Agric., Thailand (mimeographed).
- 15) MASAGO, H. and KATSURA, K. (1970): Selective medium for *Phytophthora* isolation and its application. *Ann. Phytopath. Soc.* Japan, **36**, 352.
- 16) MEHRLICH, F. P. (1963) Pathogenicity and variation in *Phytophthora* species causing heart rot of pineapple plants. *Phytopathology*, **26**, 23-43.
- 17) MENYONGA. J. M. and TSAO, P. H. (1966): Production of zoospore suspension of *Phytoph-thora parasitica*. *Phytopathology*, 56, 359-360.
- 18) NAVARATNAM, S. J. (1966). Patch canker of the durian tree. Mal. Agric. J., 45, 291-294.
- 19) PHAWAKUL, K. and CHANGSRI, V. (1969): Root rot of durian, Proc. Sem. Pl. Protection, 60-61, Thailand.
- 20) QUEBRAL, F. C., PORDESIMO, A. N. REYES, T. T. and TAMAYO, B. P. (1962): Heart rot of pineapple in the Philippines. *Philippines Agric.*, **46**, 432-450.
- 21) SIDERIS, C. P. and PAXTON, G. E. (1930): Heart rot on pineapple plants. *Phytopathology*, **20**, 951-958.
- 22) SOMCHAI, K. (1967): Studies on root rot of *Piper nigrum* Linn., B. S. Thesis, Kasetsart Univ., Thailand.
- 23) SUTABUTRA, T. and MONGKOLSOOK, P. (1974): *Phytophthora* black rot of orchids, pl. Path., Kasetsart Univ., Thailand (mimeographed).
- 24) TAMORI, M. (1974): Studies on the genus *Phytophthora* and pineapple heart rot disease found in Okinawa. *Bull. Call. Agric. Univ. Ryukyus*, 21, 1-72.
- 25) THOMPSON, A. (1929): Phytophthora species in Malaya. Mal. Agric. J., 17, 53-100.
- 26) Thompson, A. (1934): A disease of the durian tree. Mal. Agric. J., 22, 369-371.
- 27) TSAO, P. H. and OCANA, G. (1969): Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. *Nature*, **223**, 636-638.
- 28) ——— (1974a): Phytophthora diseases of durian, black pepper and citrus in Thailand, Working Report, FAO (mimeographed).
- 29) (1974b): Isolation and identities of *Phytophthora* species from rubber (*Hevea brasiliensis*) in Thailand, Working Report, FAO (mimeographed).
- 30) ——, CHEW-CHIN, N. and SYAMANANDA, R. (1975): Occurrence of *Phytophthora* palmivora on Hevea rubber in Thailand. Pl. Dis. Reptr., **59**, 955-958.
- 31) ——— and TUMMAKATE, A. (1977): The identity of a *Phytophthora* species from black pepper in Thailand. *Mycologia*, **69**, 631-637.
- 32) WATERHOUSE, G. M. (1963): Key to the species of *Phytophthora* De Bary. CMI Mycol. Paper, No. 92, 1-22.
- 33) ——— (1967): Key to Phythium Pringheim. CMI Mycol. Paper, No.109, 1-15.
- 34) (1970): The genus *Phytophthora* De Bary. CMI Mycol. paper, No.122, 1-59.
- 35) PHYTOPATH. SOC. JAPAN (1965, 1975): Common name of economic plant diseases in Japan.
  1, 158-160, 3, 1-8.
- 36) U. S. D. A. (1960): Index of plant disease in the United States, Agric. Handbook, 165, 1-145.