

### III Cross-inoculation of *Phytophthora* spp. isolated from some economic plants in Thailand

#### Summary

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Cross-inoculation between the selected isolates of *Phytophthora palmivora*, *P. nicotianae* var. *parasitica* and *P. botryosa*, and durian, mandarin orange, pineapple, Hevea rubber, orchid and black pepper was performed. *P. palmivora* isolated from durian attacked durian, mandarin orange and Hevea rubber. *P. palmivora* from orchid (*Vanda*) caused disease in durian, mandarin orange, Hevea rubber and orchid (*Vanda*). *P. nicotianae* var. *parasitica* isolated from mandarin orange attacked all the test plants except black pepper, and the isolates from pineapple caused disease in every test. All the test plants used except black pepper were infected by inoculation of *P. nicotianae* var. *parasitica* from orchid (*Ascoenda*). *P. botryosa* obtained from Hevea rubber caused disease in durian, mandarin orange, Hevea rubber and orchid.

In the previous paper, the authors reported that *Phytophthora palmivora*, *P. nicotianae* var. *parasitica* and *P. botryosa* were isolated from some plants of economic importance in Thailand (Suzui et al. 1979) and demonstrated that the causal organism of *Phytophthora* disease of durian was limited to *P. palmivora*. But *P. palmivora* and *P. nicotianae* var. *parasitica* have a wide range of hosts in the world (Chee 1969, R. A. M. 1968). Therefore, cross-inoculation test was carried out between *Phytophthora palmivora*, *P. nicotianae* var. *parasitica* and *P. botryosa* and some plants of economic importance to clarify the host range of the pathogens and the behavior of *Phytophthora* in Thailand.

#### I Materials and methods

##### 1 Isolates

Twelve *Phytophthora* isolates were selected from among *P. palmivora*, isolated from bark rot of trunk and root rot of durian, and black rot of *Vanda*, *P. nicotianae* var. *parasitica*, isolated from root rot of mandarin orange, heart and root rot of pineapple, and black rot of *Vanda* and *Ascoenda*, and *P. botryosa*, isolated from leaf fall (leaf blight, lesion on petiole) and black stripe of Hevea rubber in Thailand, respectively. The isolates were designated as D16-21-1, D39-32-2, C20-2-7, C27-11-1, P14-32-1, P15-11-1, R12-6-2, R17-2-4, R17-4-1, O 3-1-1, O 4-21-1 and O 6-4-1.

## 2 Test plants used

The test plants used in the experiment were as follows :

Test plant	Variety/Clone/Line	Stage
Durian <i>Durio zibethinus</i>	Local	5-month seedling
Mandarin orange <i>Citrus reticulata</i>	Soam Kio Wan	3-month layering
Pineapple <i>Ananas comosus</i>	Ta dum, Ta dang	Crown before planting
Hevea rubber <i>Hevea brasiliensis</i>	PB 28/59	3-month seedling
Orchid <i>Vanda</i> hybrid	TMA	1-year seedling
Black pepper <i>Piper nigrum</i>	Malaysia	3-month cutting plants

## 3 Zoospore production

The production of sporangia and zoospores was identical to that described in the previous paper (Suzui et al. 1979).

## 4 Inoculation techniques

Soil was steamed at 100°C for 1 hour before planting in pots durian, mandarin orange, pineapple and black pepper. Foot and roots of durian, mandarin orange and black pepper were exposed prior to inoculation. Seventy to one hundred ml of zoospore suspension including sporangia and mycelia (5-10 of sporangia / a field of x 100) were poured on each plant, on the foot and the roots of durian, mandarin orange and black pepper, respectively. After the inoculation, the pots were kept outside in the shade of a bamboo blind. Also immersion-inoculation method was carried out for durian and mandarin orange (Carpenter et al. 1962). The seedlings were incubated in the zoospore suspension for 24 hrs. with air addition at 22 - 23°C for 12 hrs. in the light and 12 hrs. in the dark. After the inoculation, the plants were transplanted into the steamed soil and were kept in the room near a window for several days. Thereafter, the plants were moved outside. The plants were examined for infection 4 weeks after the inoculation.

Pineapple was inoculated by pouring 30 to 50 ml zoospore suspension per plant into the center of the crown. After inoculation, the plants were covered with a polyethylene bag and were incubated at 27 - 28°C under high moisture condition (R.H.; more than 98 %) for 2 weeks, 12 hrs. in the light and 12 hrs. in the dark (Koito-Toron incubator). A sufficient amount of zoospore suspension was sprayed on Hevea rubber and orchid, which were covered with a polyethylene bag. The plants were incubated for a week in the incubator previously described. The pathogens were reisolated from the diseased parts of test plants. When no symptoms appeared after the inoculation, the experiment was repeated 3 times.

## II Results

Results of inoculation of *Phytophthora* isolates (D16-21-1, D39-32-2, C20-2-7, C26-11-1, P14-32-1, P15-11-1, R12-6-2, R17-2-4, R17-4-1, O 3-1-1, O 4-21-1 and O 6-4-1) in durian, mandarin orange, pineapple, Hevea rubber, orchid (*Vanda* hybrid) and black pepper are shown in Table 1. D16-21-1, D39-32-2 and O 3-1-1 isolates (*P. palmivora*) obtained from durian and orchid, C20-2-7, C26-11-1, P14-32-1, P15-11-1, O 4-21-1 and O b-4-1 isolates (*P. nicotianae* var. *parasitica*)

from mandarin orange, pineapple and orchid, and R12-6-2, R17-2-4 and R17-4-1 isolates (*P. botryosa*) from Hevea rubber were able to infect durian roots. Among them, *P. palmivora* isolated from durian and *P. nicotianae* var. *parasitica* from mandarin orange attacked the foot of durian, and they caused the defoliation of durian seedlings. The isolates of D16-21-1 and D39-32-2 from durian were more virulent in durian plants than other isolates. R17-4-1 isolate (*P. botryosa*) from Hevea rubber was able to slightly infect the root of durian but recovery failed when the pathogen was isolated from lesions.

Mandarin orange was infected with *P. nicotianae* var. *parasitica* isolated from mandarin orange, pineapple and orchid (*Ascocenda*), *P. palmivora* from durian and Vanda, and *P. botryosa* from Hevea rubber except for R17-4-1 isolate. But O 4-21-1 isolate (*P. nicotianae* var. *parasitica*) from Vanda, did not cause any lesion in roots of mandarin orange. The symptoms of the mandarin orange consisted only of root rot, and no foot rot was observed in any plot inoculated with *Phytophthora* isolates.

Pineapple was only infected with P14-32-1, P15-11-1, C20-2-7, C26-11-1 and O 6-4-1 isolates of *P. nicotianae* var. *parasitica* obtained from pineapple, mandarin orange and *Ascocenda*. Both P14-32-1 and P15-11-1 isolates obtained from heart and root rot of pineapple caused lesions in pineapple. But O 4-21-1 isolate (*P. nicotianae* var. *parasitica*) from Vanda did not attack the heart of pineapple.

Under natural conditions, leaf fall of Hevea rubber was generally observed as a black lesion on the petiole, and leaf blight was very rare. However leaf blight of rubber was often recognized under artificial inoculation conditions, and lesion of the petiole was found in a low percentage except in the case of R17-2-4 (*P. botryosa*) isolated from black stripe of Hevea rubber. Black lesion on petiole (leaf fall) of Hevea rubber was confirmed by inoculation of R12-6-2, R17-2-4 and R-17-4-1 isolates of *P. botryosa*, D16-21-1, D39-32-2 and O 3-1-1 isolates of *P. palmivora*, C20-2-7, P15-11-1, O 4-21-1 and O 6-4-1 isolates of *P. nicotianae* var. *parasitica*. The isolate R17-2-4 appeared to induce a severe case of leaf fall (petiole and leaf blight) in Hevea rubber. All isolates were able to infect leaf of Hevea rubber. Among them, C26-11-1 and P14-32-1 isolates of *P. nicotianae* var. *parasitica* and O 3-1-1 isolate of *P. palmivora* produced mild lesions on leaf of Hevea rubber.

Although the causal organism of black rot of orchid is assumed to be *P. palmivora*, no lesions appeared on orchid (*Vanda*) despite repeated inoculations of D16-21-1 and D39-32-1 isolates (*P. palmivora*) obtained from durian performed on three different occasions. Meanwhile, P14-32-1 and P15-11-1 isolates (*P. nicotianae* var. *parasitica*) obtained from pineapple and R17-4-1 (*P. botryosa*) from Hevea rubber produced clearcut lesions of black rot on *Vanda* plants. Also O 3-1-1 (*P. palmivora*), O 4-21-1 and O 6-4-1 (*P. nicotianae* var. *parasitica*) isolates collected from orchid, and R12-6-2 and R17-2-4 isolates (*P. botryosa*) from Hevea rubber gave rise to typical lesions in orchid (*Vanda*). But the lesions of black rot were less pronounced in the case of *Vanda* following the inoculation of C20-2-7 and C26-11-1 isolates (*P. nicotianae* var. *parasitica*) from mandarin orange.

P14-32-1, P15-11-1 and O 4-21-1 isolates (*P. nicotianae* var. *parasitica*) obtained from pineapple and *Vanda*, were reisolated from roots of black pepper. But the lesions involving the root were mild. D16-21-1 and D39-32-2 isolates (*P. palmivora*) obtained from durian, C20-2-7 isolate (*P. nicotianae* var. *parasitica*) from mandarin orange, and R17-2-4 and R17-4-1 isolates (*P. botryosa*) from Hevea rubber appeared to cause slight lesions of root rot in black pepper, but re-isolation of inoculum from roots of black pepper failed.

**Table 1. Cross-inoculation of *Phytophthora palmivora*, *P. nicotianae* var. *parasitica* and *P. botryosa* in durian, mandarin orange, pineapple, Hevea rubber, orchid (*Vanda*, *Ascocenda*) and black pepper**

Isolate	Pathogen	Original host plant	Original symptoms	Appearance of symptoms and recovery of inoculum								
				Durian		Mandarin orange		Pineapple	Hevea rubber		Vanda	Black pepper
				Foot rot	Root rot	Foot rot	Root rot	Heart rot	Leaf fall (petiole)	Leaf blight	Black rot	Root rot
D16-21-1	<i>P. palmivora</i>	Durian	Root rot	++*	++*	-	+	-	+	+	-	±
D39-32-2	<i>P. palmivora</i>	Durian	Bark rot	++*	++*	-	±	-	±	+	-	±
C20-2-7	<i>P. nicotianae</i> var. <i>parasitica</i>	Mandarin orange	Root rot	+	+	-	+	+	±	+	±	±
C26-11-1	<i>P. nicotianae</i> var. <i>parasitica</i>	Mandarin orange	Root rot	+	+	-	+	++	-	±	±	-
P14-32-1	<i>P. nicotianae</i> var. <i>parasitica</i>	Pineapple	Root rot	-	+	-	+	++	-	±	++	±
P15-11-1	<i>P. nicotianae</i> var. <i>parasitica</i>	Pineapple	Heart rot	-	+	-	+	++	+	+	++	±
R12-6-2	<i>P. botryosa</i>	Hevea rubber	Leaf blight	-	+	-	+	-	+	+	+	-
R17-2-4	<i>P. botryosa</i>	Hevea rubber	Black stripe	-	+	-	+	-	++	++	+	±
R17-4-1	<i>P. botryosa</i>	Hevea rubber	Leaf fall (petiole)	-	±	-	-	-	+	+	++	±
O 3-1-1	<i>P. palmivora</i>	<i>Vanda</i>	Black rot	-	+	-	+	-	±	±	+	-
O 4-21-1	<i>P. nicotianae</i> var. <i>parasitica</i>	<i>Vanda</i>	Black rot	-	+	-	-	-	+	+	+	±
O 6-4-1	<i>P. nicotianae</i> var. <i>parasitica</i>	<i>Ascocenda</i>	Black rot	-	+	-	±	++	+	+	+	-
Control	-	-	-	-	-	-	-	-	-	-	-	-

Note: ++; Severe symptoms, +; Appearance of symptoms, ±; Mild symptoms, -; No symptoms, \*; Recovery of inoculum

### III Discussion

*P. palmivora* was recognized as an organism responsible for the symptoms of *Phytophthora* disease such as bark rot, foot rot, root rot and fruit rot of durian plants (Thompson 1934, Navaratnam 1966, Phawakul et al. 1969, Tsao 1974). In cross-inoculation, *P. nicotianae* var. *parasitica* and *P. botryosa* in addition to *P. palmivora* attacked durian tree under greenhouse conditions. Isolates of *P. palmivora* obtained from durian could not cause black rot in *Vanda*. Nevertheless *P. palmivora* of orchid attacked durian tree. Turner (1960) reported that distinct strains of *P. palmivora* were found in lesions developed in cocoa pods when 179 isolates from ten cocoa-producing countries were examined. The results of our experiment suggest that these isolates may have a different host range and behavior under natural conditions where there are orchids in the durian orchard. However, some isolates of *P. palmivora* obtained from bark rot of durian at Rayong were able to infect orchid in cross-inoculation test (Kueprakone 1973).

The pouring of inoculum into the soil is a mild inoculation technique for infection of root or foot of durian and mandarin orange. However, the immersion method is more drastic. In the former method, only D16-21-1 and D39-32-2 isolates from durian caused wilting in durian. In the latter method, symptoms of *Phytophthora* disease in durian and mandarin orange appeared as a result of inoculation of *P. palmivora*, *P. nicotianae* var. *parasitica* and *P. botryosa*. But *P. nicotianae* var. *parasitica* was only confirmed in mandarin orange in Thailand and other *Phytophthora* species have not been found in citrus plants under natural conditions.

The results of cross-inoculation indicated that only *P. nicotianae* var. *parasitica* isolated from pineapple, mandarin orange and orchid (*Ascocenda*) caused heart rot in pineapple. Mehrlich (1934) and R. A. M. (1963) reported that *P. palmivora* causes heart and root rot of pineapple. In this experiment, no lesions appeared in pineapple in spite of the inoculation of *P. palmivora* obtained from durian and orchid. But Kueprakone et al. (1973) reported that isolate of *P. palmivora* from durian was able to infect pineapple in inoculation test performed in Thailand.

Leaf blight of Hevea rubber was frequently observed on leaves following spraying of zoospore suspension. But, leaf blight was limited to a few cases under natural conditions. It is suggested that inoculum density, presence of dew on leaves and environmental conditions were different from that of normal conditions. *P. palmivora* isolated from durian and *Vanda*, *P. nicotianae* var. *parasitica* from mandarin orange, pineapple and orchid (*Vanda* and *Ascocenda*), and *P. botryosa* from Hevea rubber, affected the petiole and leaf of Hevea rubber. These results indicate that *P. nicotianae* var. *parasitica* was capable of infecting Hevea rubber under favorable conditions.

*P. palmivora* isolated from durian was unable to infect *Vanda*, but *P. nicotianae* var. *parasitica* obtained from mandarin orange, pineapple, and orchid (*Vanda* and *Ascocenda*), and *P. botryosa* from Hevea rubber caused black rot in *Vanda*. These results suggested that there were *P. palmivora* of different pathogenicity in orchid growing in the durian orchard.

It has been reported that in Brazil, Puerto Rico and Sarawak, the causal organism of foot rot of black pepper was *P. palmivora* (Alconero et al. 1972, Holliday et al. 1963). *P. palmivora* isolated from durian and *Vanda* was unable to infect black pepper, and *P. nicotianae* var. *parasitica* from pineapple and *Vanda* slightly affected the plants.

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