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BETEL DECLINE IN TAIWAN[1]

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Abstract

Betel decline widely existed in plantations was recognized recently. *A Phytophthora* sp. was frequently isolated from soil samples and decaying underground stems and roots and was thought to be associated with the decline of betel. The characteristics and taxonomic status of the fungus were studied and discussed.

Introduction

Betel (*Piper betle* L.), a vine, is planted in several locations in Taiwan, mainly in Tsao-tun (Nan-tou), Yeong-ching (Chang-hua), and Chia-li (Tainan). Betel leaves, barries and underground stems are used as masticatory together with areca nut. It was noticed for the first time by the senior author in the summer of 1977 that there were always several sections of betel stands along the highway from Tsao-tun to Kuo-shing on the way to Pu-li appeared pale and thinner (Fig. 1, A and B). The leaves were pale green to yellowish green, and in the severe cases the whole plants became dried out and dead. It was speculated that this disease has already been there for a long period of time. However, no one has paid attention to it because the betel is not an economically important crop in Taiwan.

Symptoms

In the aerial parts, at the beginning the branches and leaves of betel shrivelled, lost their original deep green appearance, and finally became pale and chlorotic green. The whole canopy became thinner because the leaves and branches glaccid and slightly droop. In advance stage of the disease development the branches and leaves turned yellow and withered, and eventually driedout. The disease development and symptom apperance are exactly similar as those described by Turner (1969a) on betel foot rot occurred in Sarawak and by

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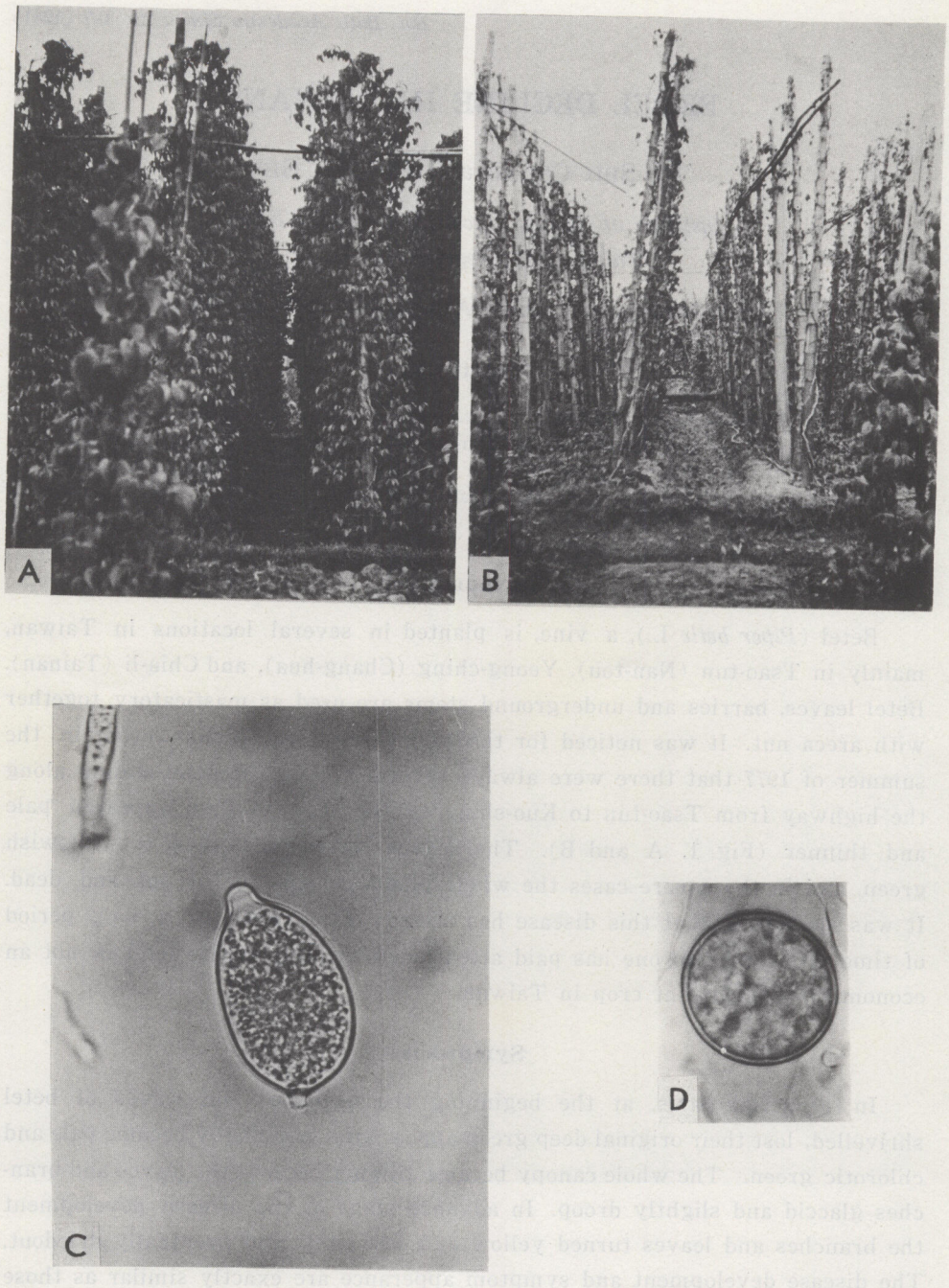

Fig. 1. Betel decline and associated *Phytophthora* sp. A. healfhy stands, B. diseased stands, C. sporangium, and D. chlamydospore.

Holliday and Mowatt (1963) on black pepper foot rot also occurred in Sarawak. No necrotic lesion was found on leaves so far as the writers have examined at Tsao-turn and Chia-li. In the underground parts, no intensive examinations were conducted by the writers, however, in advance stage of the disease development the underground stems and adventitous roots were decayed and disintegrated. The processes of infection and symptom development of underground parts are remained to be investigated.

Associate Pathogenic Fungus

When we noticed the existence of betel decline, we were engaging in the survey of the distribution and the kinds of species of the genus *Phytophthora* occurring in Taiwan. Instantly *Phytophthora* sp. was suspected to be in connection with the decline of betel.

Isolation

Eggplant fruits were used as bait to isolate *Phytophthora* spp. The fruits were surface sterilized by 75% ethanol. A fruit (about 30 cm) were made four holes, 4 cm apart, by a cork-borer (0.5 cm in dia.) which was first flamed. Soil samples were collected from the vicinity of the underground parts of diseased stems and roots. A small amount of soil was put into each hole and then two drops of sterile water were added before detached tissue was plugged back into the original place (hole). Each inoculated site then was sealed with transparent tape to prevent drying out. The inoculated fruits were kept in PVC bag and were incubated at room temperatures (25° to 28°C). Two to 3 days after incubation brown rot lesions appeared in some of the inoculation sites. The rot lesions enlarged fairly rapidly. To ensure the purity of isolation cultures the marginal areas of the lesion was surface sterillized with ethanol and stripped the peel. A piece of inner tissue ramified with aseptate, irregular hyphae, was cut and tranferred onto V-8 juice agar plate. Plates inoculated with fresh rot tissue were then incubated at 28°C. Two to 3 days after incubation typical *Phytophthora* hyphae emerged and developed into a colony. Small piece of mycelial agar was subcultured onto V-8 slopes for storage. For further identification and experiments single sporangium culture was conducted. Isolations were also made by culturing pieces of necrotic underground stems and roots on selective agar (V-8 juice 50 ml; $CaCO_3$ 2g; mycostatin 50 mg; PCNB 1 mg; ampicillin 100 mg; agar 18 g; and H_2O 950 ml) plates. Hyphae with characteristics of *Phytophthora* spp. emerged. Purification was conducted by placing a pieces of agar with hyphae inside a van Tieghem cell which was placed in a 9 cm Petri-plate before agar was poured. In such a way the fungal hyphae were separated from the contaminated bacteria by growing through the bottom end of van Tieghem cell and emerged to the agar surface

outside of the cell. Repeated the procedure for 2 to 3 times, pure culture of the isolated fungus was obtained and subculture was made onto V-8 agar slants for storage.

Morphology

Macroscopically, the colony appeared fairly uniform without distinct patches and zonation as was usually observed on *P. parasitica*, however, it was not as smooth and uniform as *P. palmivora*. Fairly amount of aerial hyphae developed, the texture of colony was quite dense, and the margin of colony is not definite. Microscopically, hyphae were very irregular, the width ranged from 4.2 to 12.6 μm, and intensively branching originated from point areas of main hyphae and finally became bush-type branches. Sporangia varied in shape, from ovoid to pyriform (broad lemon shape), with distinct papilla (Fig. 1, C), usually terminated, however, in some cases intercalary, 30 to 36 μm in length and 19 to 33 μm in breadth, and the length/breadth ratio ranged from 1.2 to 1.8 and was mainly 1.5, usually with a short pedicel when detached (Fig. 1, C). Rare sporangia formed on V-8 juice agar, and other solid agar media tested including cormeal agar, carrot agar and potato dextrose agar, but produced abundantly when submerged in Petri's solution or soil extract solution. The mature oogonia and oospore moderate thick-walled, usually smooth and golden brown, 22 to 28 μm in diameter for oogonia and 21 to 27 μm for oospores; antheridia amphigynous, 14 to 19 μm in diameter. These isolates formed sparse fusion organs (oogonia, oospores and antheridia) only after mated with an isolate UCR-97 (A1 mating type) of *P. cinnamomi*, on V-8 juice agar and incubated at 23°C in darkness for two weeks. The evidence of formation of fusion organs only after mating with compatible isolate indicated that the fungus is heterothallic. Chlamydospores are spherical, thin-walled, terminate and intercalary, hyaline, 19 to 37 μm in diameter. Abundant chlamydospores produced rapidly when growing in liquid cultures particularly in the cucumber broth. In old cultures, one month or longer, the thickness of chlamydospors wall reached about 1 μm (Fig. 1, D). Chlamydospores germinated by producing sporangia in diluted V-8 juice 12 hr after incubation at 25°C. One chlamydospore often produced more than one sporangium, and in one case, seven sporangia were produced. However, in most of the case chlamydospores germinated by germ tubes.

Mycelial growth response to temperature

Tucker (1931) reported that all isolations of *P. parasitica* developed on corn meal agar at 35°C, while none of *P. palmivora* did. To compare the present fungus (isolate Pb-2 was used as test fungus) with *P. parasitica* (isolates tested including Ro-1, PPA2 and P991) and *P. palmivora* (isolate

tested was Pau-1) in their response to temperature, an agar block (4 mm in dia.) with mycelia of each test isolate was inoculated onto the center of a 9 cm Petri-plate containing 20 ml V-8 juice agar, A set in 3 replications of each isolate was incubated at 28°C, and another set was incubated at 35°C in darkness. The growth of colonies was measured after four days incubation. The results shown in Table 1 revealed that the mycelial growth of *Phytophthora* sp. (Pb-2) *isolated* from betel possesses high optimal temperature ranging from 28° to 35°C. Most of the isolates of *P. parasitica* tested performed the similar results regarding the temperature response; however *P. palmivora* grew slowly and exhibited no growth at 35°C.

Table 1. *Linear mycelial growth of betel isolate Pb-2 in comparison with isolates Ro-1, PPA2 and P991 of* ***Phytophthora parasitica*** *and isolate Pau-1 of* ***P. palmivora*** *on V-8 juice agar at 28°C and 35°C*

Species	Isolate	Colony diameter (mm) 4 days after incubation	
		28°C	35°C
Phytophthora sp.	Pb-2	65	64
P. parasitica	Ro-1	64	68
	PPA2	65	62
	P991	56	55
P. palmivora	Pau-1	62	13

Discussion

Betel (*Piper betle* L.) decline (or foot rot) occurs throughout the betel fields at Tsao-tun, Yeong-ching and Chia-li. We do not know how long the disease has been existing in Taiwan because there is no record kept in the leterature. We frequently isolated a *Phytophthora* sp. from soils around the decayed underground stems and roots. No inoculation test has been conducted to prove exact relationship between the betel decline and this *Phytophthora* sp. However, the writers incline to speculate that the betal decline is caused by this fungus. In India betel foot rot cause a *Phytophthora* sp. (*P. parasitica* var. *piperina*) was reported (McRae, 1934; Dastur, 1935, in Waterhouse, 1970). Turner (1969a) also investigated the foot rot of betel in Sarawak and proved that a *Phytophthora* sp. was the cause of this disease. He followed Waterhouse's opinion and named the fungus as an atypical strain of *P. palmivora*, The symptoms and their development observed in Taiwan are similar to those of the foot rot of betel described by Turner except that no leaf lesions were found in Taiwan (Turner, 1969b). The way of cultivation of betel

in Taiwan is similar to that of black pepper (*Piper nigrum*) in Sarawak, and the symptoms of foot rot of black pepper caused by *P. palmivora*, also an atypical strain (Holliday and Mowatt, 1963), which is almost identical to those on the betel decline in Taiwan.

The characteristics and behavior of this fungus have been carefully examined by the authors. The characteristics, such as irregular hyphae, bush-type branching of hyphae, rare or even no production of sporangia on solid agar media tested, and higher optimal temperatures for mycelial growth, make our isolate very similar to *P. parasitica*. Dastur (1935, in Waterhouse, 1970), named a *Phytophora* sp. which caused foot rot on betel as a strain of *P. parasitica*. Muller (1936, in Turner, 1969a; and Holliday and Mowat, 1963) however, considered that the isolate of *Phytophthora* sp. which caused foot rot of pepper in Dutch East Indies was *P. palmivora*. In our isolate, the morphology of sporangium which is typical pear-shape with prominant papilla and the length to breadth ratio of sporangia, i. e., 1.5 in averge and moreover, a short pedicel always on a detached sporangium which make it similar to the group I of *P. palmivora* (Zentmyer *et al.* 1977) Based upon the characteristics mentioned above we realize that our fungus, in many respects, is very close to those isolates from betel foot rot occurred in Sarawak and India on black pepper and betel. However, we also realize that this fungus is not exactly similar to either *P. palmivora* or *P. parasitica*. Before more evidences are obtained the writers tentatively follow the previous workers' opinion designate our fungus also as an atypical strain of *P. palmivora*.

The behavior of chlamydospore formation of this fungus was similar to that of *P. parasitica* reported by Tsao (1971) that low temperatures enhanced its chlamydospore formation in liquid cultures. We found that cucumber broth is the best medium tested in the present study to produce chlamydospores. Chlamydospore germination occurred in 5% V-8 juice at 25°C, either by germ types or producing sporagia, frequently more than one sporangia were formed. No chlamydospore germination occurred in distilled water, glucose and sucrose solution, and this demonstrated that chlamydospore germination is nuterient-dependent exogenously other than simple carbon sources as Mirecetich *et al* (1968) demonstrated on *P. cinnamomi* chlamydospore germination. The thickness of chlamydospore wall of our fungus is an intermediate in between those of *P. palmivora* and *P. parasitica* based on *Kadooka and* Ko (1973). A proportion of chlamydospore of the former fungus were thick-walled formed in papaya fruit and fruit juice, while the latter only thin-walled chlamydospores under the same conditions. Our fungus not only formed thin-walled chlamydospores but also formed intermediate one in cucumber broth and papaya fruit juice.

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臺灣枸醬萎凋病

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枸醬萎凋病很普遍發生於本省，特別是南投縣草屯鎮雙冬地區的枸醬園之枸醬受害最鉅，從病株腐敗的地下莖，根部以及附近土壤分離往往得到一種疫病菌。本疫病菌之特徵和分類在本文中有詳細說明和討論，筆者等認爲本疫病菌是 *Phytophthora palmivora* 之一非典型株。