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Decline of Longan Trees: Association with Brown Root Rot Caused by *Phellinus noxius*

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ABSTRACT

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Leaf discoloration, unthrifty appearance and death of longan trees have become a serious problem in the mountain areas of central and southern Taiwan. All declining trees surveyed were associated with brown root rot. The fungus isolated from the diseased tissues produced fruiting bodies of *Phellinus noxius* on sawdust medium similar to those produced on the basal stems of the declining trees. Upon inoculation of roots with *P. noxius* grown in the wheat-oat medium, healthy longan seedlings were killed. *P. noxius* was reisolated from diseased tissues of artificially inoculated roots. Previously reported brown root rot of longan was considered to be also caused by *P. noxius* misidentified as *Phellinus lamaensis*.

Key words: *Dimocarpus longana*, *Phellinus noxius*, *Phellinus lamaensis*, brown root rot, tree decline.

INTRODUCTION

Longan (*Dimocarpus longana* Lour. = *Euphoria longana* Lamarek) is an important economic fruit crop in Taiwan. Currently, there are about 20,000 ha of longan orchards mainly distributed in the mountain areas of central and southern counties. Slow decline with discoloration of leaves followed by death of longan tress has been noticed since the early 1970s. Occasionally the whole canopy turned brown and the affected trees died within few months. Hard, flat and dark brown fruiting bodies were frequently found on basal stem of diseased trees. Currently, it is the most serious problem in longan plantations. We report here the isolation of a root pathogen and its relation to longan decline. A brief account of this work has been published (1).

MATERIALS AND METHODS

Isolations

Diseased roots were taken from declining longan trees. Pieces (*ca.* 5 × 2 × 1 mm) of infected tissues cut from the advancing margins were surface-sterilized with 0.5% NaClO for 3 min, placed on potato-dextrose agar (PDA) in petri plates and incubated at 24 C. The fungal mycelia growing from the diseased tissues were transferred to 2% water agar. Single hyphal tips obtained from the fungus growing on water agar were cultured and maintained on PDA.

Pathogenicity tests

The fungus was grown in a wheat-oat medium (10 ml whole wheat grains, 10 ml whole oat grains and 10 ml distilled water) for one month at 24 C (6). Roots of longan seedlings individually planted in pots (25 cm diam) for two to three years were exposed, surface-sterilized with 70% alcohol and scraped gently to remove the epidermis. About 5 ml of wheat-oat culture was placed on the scraped portion of the root, wrapped with a sheet of clear plastic and secured at both ends. After inoculation, seedlings were replanted in the same pots. Roots similarly inoculated with sterile wheat-oat medium were used as controls. Five seedlings were used for each treatment.

Production of fruiting bodies

Commercial sawdust medium (7) consisting of saw dust, rice bran, sucrose, and $CaCO_3$ in plastic bags (*ca.* 20 ml long, 10 cm diam) was used for formation of fruiting bodies. Each plastic bag was inoculated with 5 pieces of the inoculum. After one month, the plastic sheet was removed and the clonized medium was placed on moist sands.

Growth of fungi

To determine the effect of temperature on growth of

the test organisms, agar discs (5 mm diam) cut with a sterile cork borer from the periphery of a colony were each placed on PDA in a growth rate tube (30 cm long, 1.5 cm diam) (9). Inoculated tubes were incubated at 10, 12, 15, 18, 21, 24, 27, 30, 33, 36, or 38 C in darkness. Colonies were measured daily until the fungus reached the end of the tubes or 20 days after inoculation. Three tubes were used for each treatment and the experiment was done twice.

Electrophoresis of soluble proteins

To compare the electrophoretic pattern of soluble proteins, test organisms were grown in 50 ml of 5% V-8 broth (5% V-8 juice and 0.02% $CaCO_3$) in 250 ml flasks at 24 C for 5–7 days. Mycelia were washed three times with 0.05 M phosphate buffer at pH 7.0, dried on nylon nets and frozen overnight at −20 C. The frozen mycelia were ground in a mortar containing sea sand and phosphate buffer. The mixture was centrifuged at 27,000 *g* at 4 C for 30 min, and soluble proteins in the clear supernatant were separated in sodium dodecyl sulfate (SDS)-ploy acrylamide gel in a Buchler's polyslab gel electrophoresis system (5.10).

RESULTS

Symptoms

The initial symptoms of longan decline were discoloration of leaves followed by gradual defoliation and death of trees (Fig. 1). Occasionally the whole canopy turned brown within a short period of time and the affected trees died suddenly (Fig. 2). Extensive root rot was found on all diseased trees examined (Fig. 3). Infected roots turned brown initially and became white and soft subsequently with a conspicuous network of brown lines permeated the wood tissues (Fig. 4). The outer surface of the infected bark appeared rough because of being covered with a layer of adhering soil particles (Fig. 5). The inner surface of the infected bark was covered with white mycelial mats (Fig. 6). Eventually the diseased trees died when the infection reached the basal stems and the transportation of water and nutrients was cut off completely.

During the rainy periods, hard and flat fruiting bodies were occasionally found on the surface of basal stems of diseased trees. They were whitish at the beginning and their centers turned yellowish and brown gradually (Fig. 7). Eventually all fruiting bodies became dark grey or brownish grey (Fig. 8).

Isolation and pathogenicity test

A fungus was consistently isolated from diseased roots of declining longan trees collected from counties of Taichung, Nantow, Yunlin, Chiayi, Tainan, and Kaohsiung. Similar fungus was isolated from fruiting bodies collected from the basal stem of a diseased tree.

Six months after inoculation, longan seedlings killed by the inoculated fungus were 40% in the first test and 60% in the second test. Tissues of dead roots became soft with brown zone lines. The same fungus was reisolated from all diseased roots of artificially infected seedlings. All control seedlings remained healthy during the experiments.

Identification of the pathogen

The pathogen produced whitish colony on PDA during the early stage of growth. Subsequently the colony turned brown with irregular dark brown zone lines or patches permeated the culture (Fig. 9). Clamp connection was absent, but arthrospores (Fig. 10) and trichocysts (Fig. 11) were commonly present in the culture. About 30% of arthrospores germinated on PDA after incubation at 24 C for 24 hr.

When grown on sawdust medium for 3–4 mo, the pathogen produced hard and unevenly flat basidiocarps about 0.5–2.0 cm thick similar to those produced in nature (Fig. 12). They were yellowish brown with white margin at the beginning, and became brown and then dark grey. Occasionally basidia and basidiospores were found in hymenial pores (Fig. 13). Basidiospores were smooth, hyaline, and ovoid to broadly ellipsoid, measuring 3.8–6.3 × 1.5–5.0 μm (*av.* 5.4 × 3.3 μm) (Fig. 14). Contextual setal hyphae were reddish brown, up to 650 μm long and 12 μm in diameter (Fig. 15). The fungus was identified as *Phellinus noxius* (Corner) G. H. Cunningham (= *Fomes noxius* Corner) of the Hymenochaetaceae, Aphyllophorales by Dr. T. T. Chang of the Taiwan Forestry Research Institute, Taipei.

The pathogen (PN-3) isolated from longan was compared with an oil palm (*Elaeis guineensis* Jacf.) isolate of *P. noxius* (CBS 170.32) obtained from Centraalbureau voor Schimmelcultures, Baarn, Netherlands. The electrophoretic pattern of soluble proteins of PN-3 was identical with that of CBS 170.32 (Fig. 16). These two isolates also showed similar growth response to temperature, with the optimum temperatures of 24–33 C and minimum temperatures of 10–12 C (Fig. 17). CBS 170.32 did not grow, while PN-3 grew poorly at 36 C.

DISCUSSION

P. noxius is widely distributed in the tropical regions (8). It caused brown root rot of a number of important agricultural crops including oil palm, rubber (*Hevea brasiliensis* Muell.), and tea (*Camellia sinensis* (L.) O. Ktze) (3,8). Our results show that decline of longan trees in Taiwan is also caused by brown root rot incited by *P. noxius*. The disease has become a very serious problem in recent years, destroying several longan orchards in the central and southern mountain areas. Moreover, a number of fruit crops in Taiwan appears to be susceptible

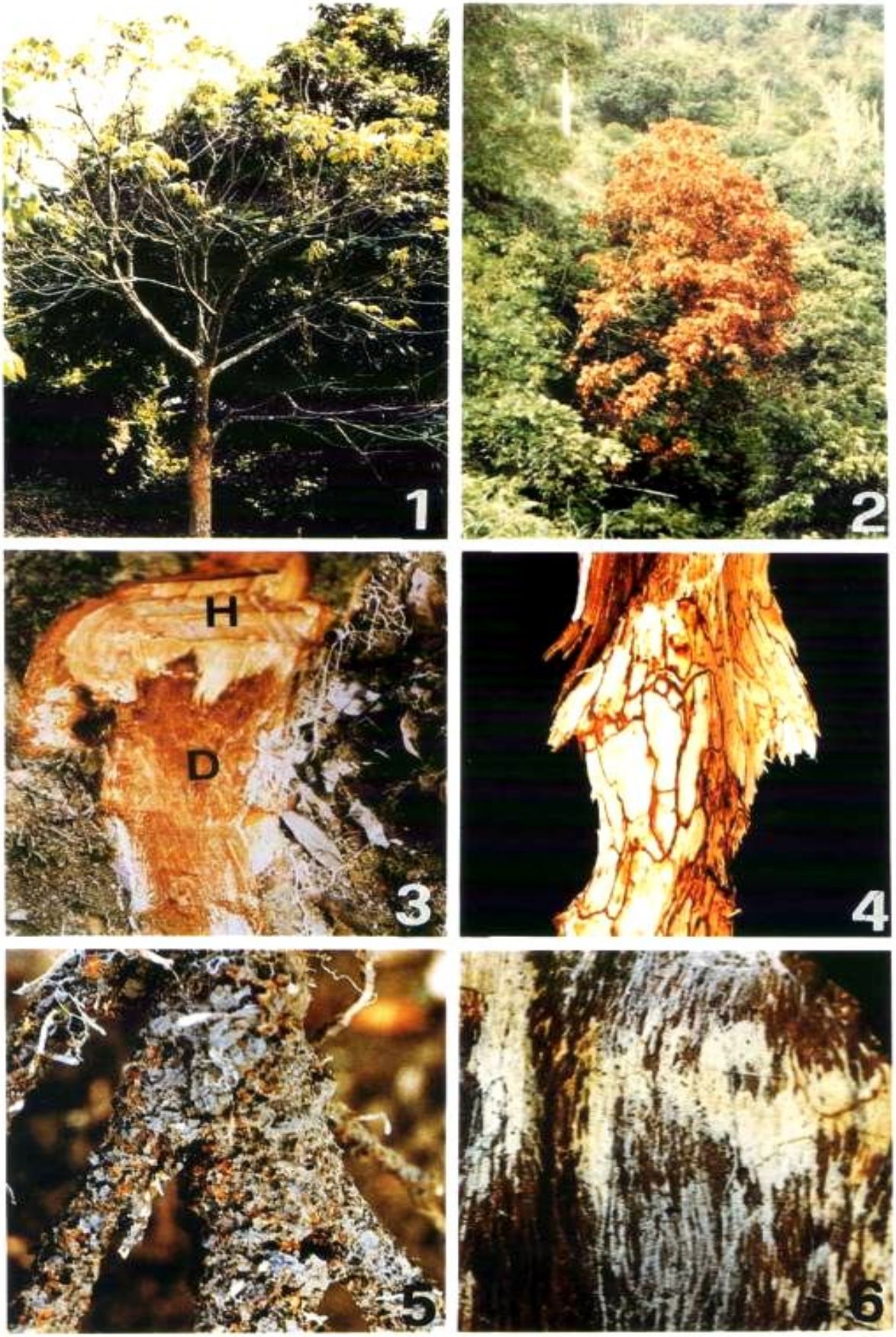

Fig. 1. Appearance of a longan tree with symptom of slow decline.
Fig. 2. Appearance of a longan tree with symptom of quick decline.
Fig. 3. A tap root showing brown rot.
Fig. 4. Brown lines on an infected root tissue.
Fig. 5. Outer surface of an infected root tissue.
Fig. 6. Mycelial mates on inner surface of an infected bark.

Fig. 7. Young fruiting bodies of *Phellinus noxius* produced on basal stem of a declining longan tree.
Fig. 8. Old fruiting bodies of *Phellinus noxius* produced on basal stem of a declining longan tree.

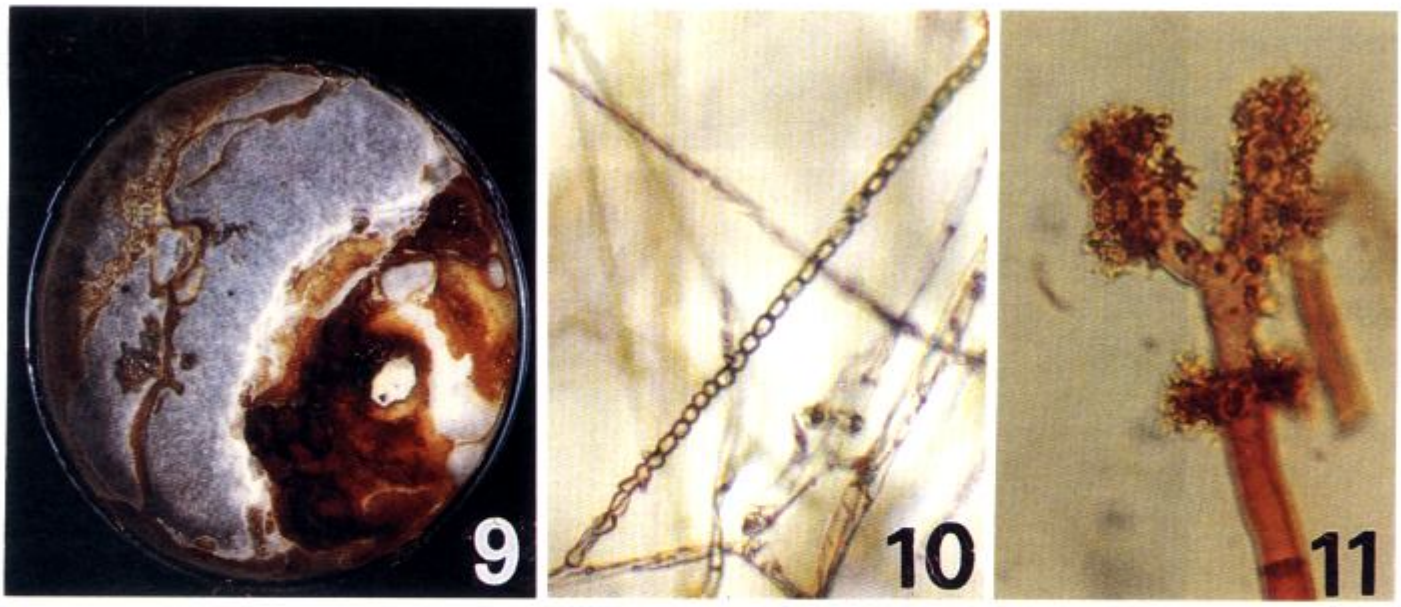

Fig. 9. Culture of *Phellinus noxius* on potato dextrose agar.
Fig. 10. Arthrospores of *Phellinus noxius* (170 ×).
Fig. 11. Trichocyst of *Phellinus noxius* (480 ×).

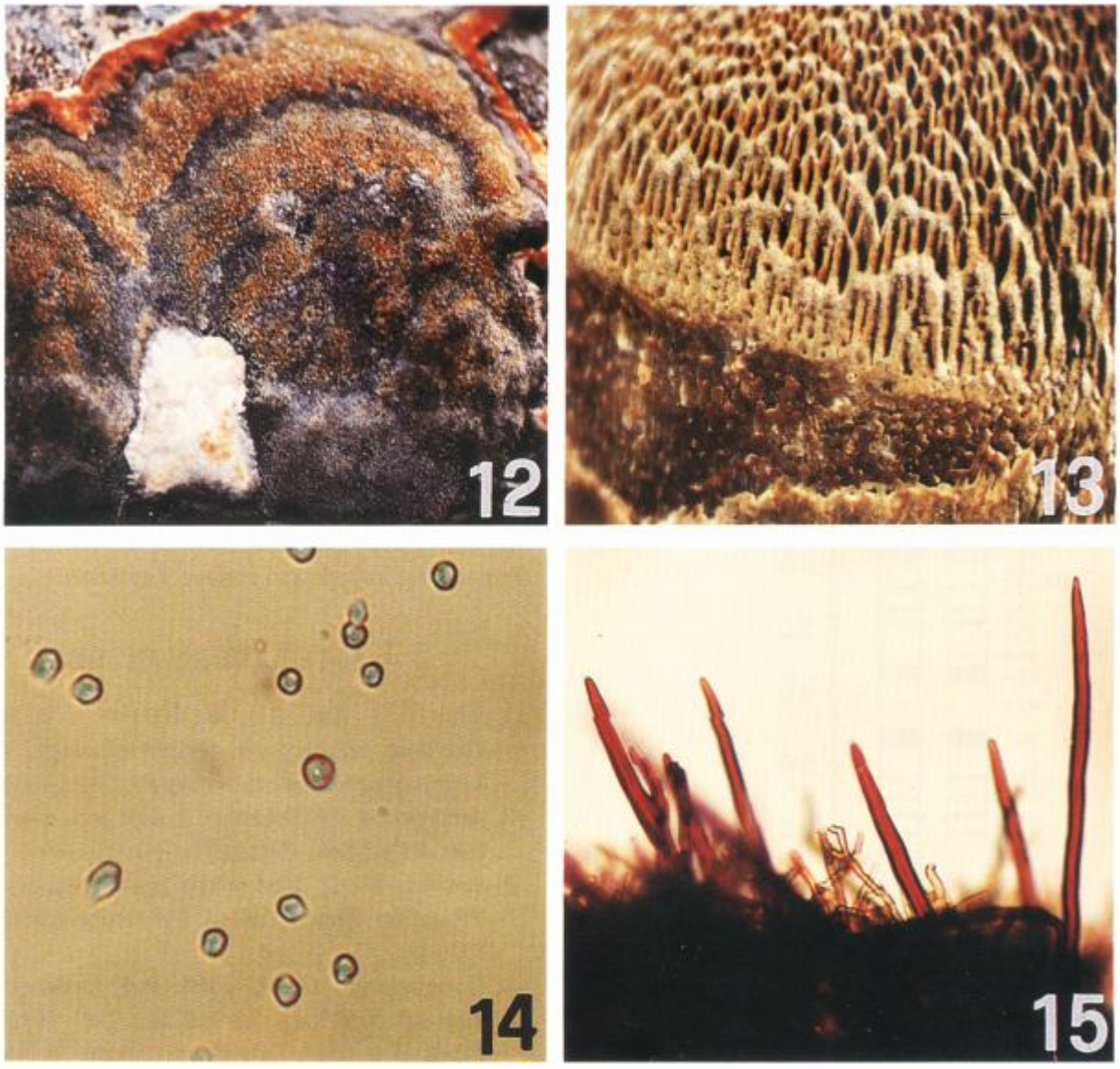

Fig. 12. Fruiting body of *Phellinus noxius* produced on sawdust medium.
Fig. 13. Hymenial pores of *Phellinus noxius* (10 ×).
Fig. 14. Basidiospores of *Phellinus noxius* stained with cotton blue (660 ×).
Fig. 15. Setal hyphae of *Phellinus noxius* (120 ×).

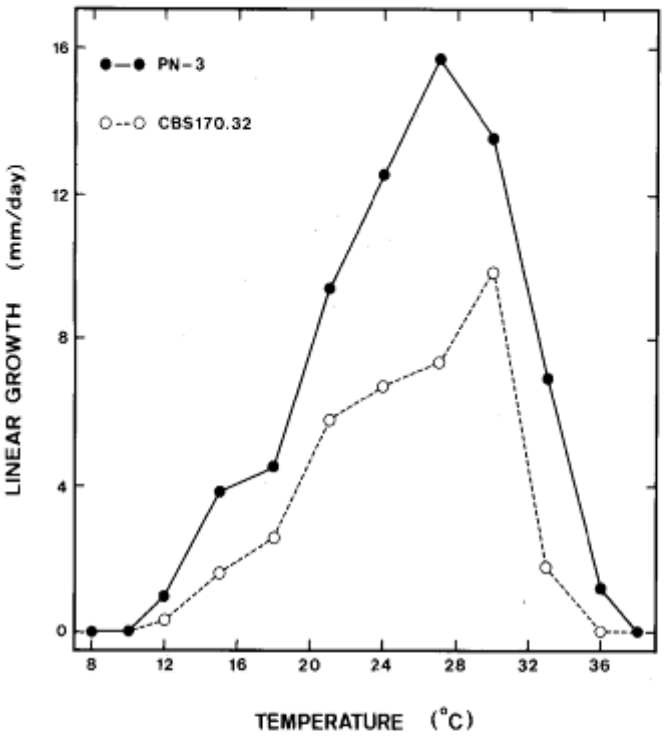

Fig. 16. Growth rate of isolates of PN-3 and CBS 170.32 of *Phellinus noxius* on potato dextrose agar.

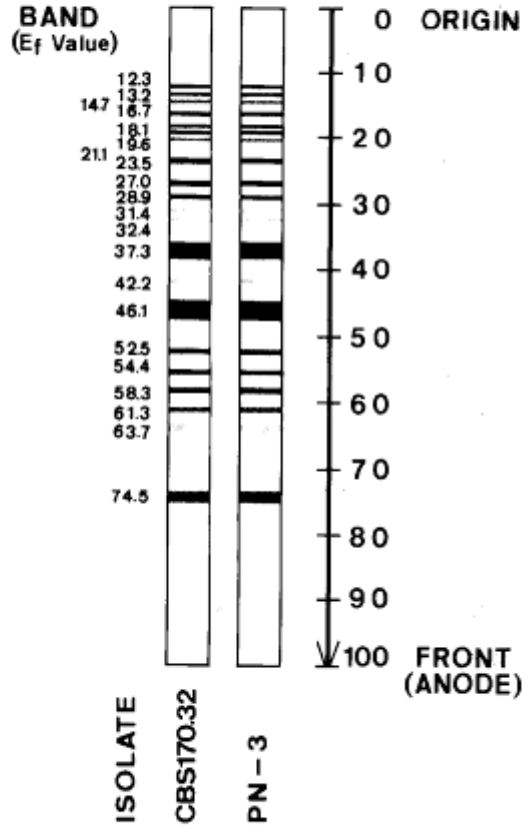

Fig. 17. Diagrammatic presentation of electrophoretic pattern of soluble proteins of isolates of PN-3 and CBS 170.32 of *Phellinus noxius*.

to this disease because *P. noxius* has also been isolated from declining trees of litchi (*Litchi chinensis* Sonn.), loquat (*Eriobotrya japonica* (Thunb.) Lindl.), Japanese apricot (*Prunus mume* Siels. & Zucc.), pear (*Pyrus pyrifolia* (Burm.) Nakai), persimmon (*Diospyros kaki* Thunb.), star fruit (*Averrhoa carambola* L.), and wax apple (*Syzygium samarangense* (Buume) Merrill & Perry) (Ann and Ko, unpublished data). Determination of the relation between *P. noxius* and decline of these fruit crops are currently in progress.

Phellinus lamaensis (Murr.) Heim. (= *Fomes lamaensis* (Murr.) Sacc. & Trott.) was reported to be the cause of brown root rot of longan in Taiwan in 1965 (2). However, only symptoms were described. The causal relationship between *P. lamaensis* and brown root rot of longan still has not been established. *P. noxius* and *P. lamaensis* are closely related species. The main distinctive characters are scanty hymenial setae and wide setal hyphae (up to 14 μm diam.) in *P. noxius*, and abundant hymenial setae and narrow setal hyphae (up to 7 μm diam only) in *P. lamaensis* (4,8). All declining longan trees surveyed at various counties during this study were associated with *P. noxius*. *P. lamaensis* has never been observed on declining longan trees. It is, therefore, considered possible that previously reported brown root rot of longan was also caused by *P. noxius* misidentified as *P. lamaensis*.

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摘　　要

安寶貞[1]、柯文雄[2]. 1992. 龍眼立枯病與 *Phellinus noxius* 引起的褐根病之相關性. 植病會刊 1:19-25. (1. 嘉義市　台灣省農業試驗所嘉義分所，2. 美國夏威夷大學植病系)

生長衰退、葉片變色、繼而整株果樹枯死，目前已成爲本省中南部山區龍眼的嚴重病害。所有調查之立枯病龍眼植株均罹患褐根病。從罹病龍眼的病組織上均分離得一種眞菌 *Phellinus noxius*，它在鋸木屑太空包上會形成子實體，與其在自然界罹病株的樹幹基部上形成之子實體相同。將培養於燕麥—小麥培養基上之 *P. noxius* 菌種接種於健康龍眼苗之根部，會引起植株死亡，而 *P. noxius* 均可從人工接種的罹病組織中再分離得到。在本省，曾記載之龍眼褐根病亦應由 *P. noxius* 引起，而被誤認爲 *Phellinus lamaensis*。

關鍵字：龍眼、*Phellinus noxius*、*Phellinus lamaensis*、褐根病、果樹立枯病。