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Decline of Some Forest Trees Associated with Brown Root Rot Caused by *Phellinus noxius*

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ABSTRACT

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Foliage wilting, leaf discoloration, brown mycelial mat growing on roots and basal stems and death of trees were found on some forest trees including *Cinnamomum camphora, Cycas taiwaniana, Delonix regia, Eucalyptus camaldulensis, E. citriodora, E. grandis, Liquidambar formosana* and *Murraya paniculata* var. *paniculata* in the eastern and western coasts of central and southern Taiwan. All diseased trees observed were associated with brown root rot. The fungus isolated from the diseased tissues produced resupinate, brownish polypore fruiting bodies on sawdust medium which was identified as *Phellinus noxius*. The disease was reproduced by inoculation of the seedlings with *P. noxius* grown in the wheat-oat medium. Inoculated seedlings included *C. camphora, C. taiwaniana, D. regia, E. camaldulensis, E. citriodora, E. grandis, L. formosana* and *M. paniculata* var. *paniculata*. *P. noxius* was reisolated from diseasd tissues of inoculated roots and basal stems. Brown root rot reported previously in *C. camphora* and *D. regia* was considered to be also caused by *P. noxius*, while the pathogenicity of *P. noxius* to those hosts has not been established.

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

INTRODUCTION

In early summer of 1991, wilting and yellowing of the foliage followed by death of trees including *Cinnamomum camphora* (Linn.) Nees *et* Eberm., *Cycas taiwaniana* Carr., *Delonix regia* (Boj.) Raf., *Hibiscus rosa-sinensis* Linn. and *Murraya paniculata* var. *paniculata* (Linn.) Jack. was found in the second growth forest at Hualien county. The roots and basal stems of diseased trees were always encrusted by various sizes of brown mycelial mats which eventually turned black. Subsequently, the same disease was widely observed on various forest trees in the eastern and western coasts of central and southern Taiwan during the warm seasons. The relation between decline of these trees and brown root rot caused by *Phellinus noxius* (Corner) Cunnigham is reported in this paper. A brief account of this study has been published (4).

MATERIALS AND METHODS

Isolation of pathogen

Diseased roots and stems were obtained from declining or dead trees. The exposed diseased tissues were cut out with a sterilized blade. Pieces (*ca.* 2 × 2 × 1 mm) of newly exposed diseased tissues were cut and transferred to malt extract agar (MEA; 2% malt-extract, 2% Bacto agar) in petri plates and incubated at 25 C. The fungus growing from the diseased tissues was transferred to 2% water agar. Single hyphal tips cut from the margin of colonies growing on water agar were cultured and maintained on MEA.

Pathogenicity test

Isolates B8, B16 and B18 obtained from *C. camphora, Eucalyptus citriodora* Hook, and *Liquidambar formosana* Hance, respectively, were grown in a wheat-oat medium (20 ml whole wheat grains, 20 ml whole oat grains and 20 ml distilled water) in a 200 ml flask for one month at 25 C (8). About 10 ml of wheat-oat culture was placed on roots of test plants which were exposed, surface-sterilized with 70% alcohol, and wrapped with a sheet of parafilm. Two to three-year-old seedlings of *C. camphora, C. taiwaniana, D. regia, Eucalyptus camaldulensis* Dehn., *E. citriodora, Eucalyptus grandis* Hill ex Maiden, *L. formosana* and *M. paniculata* var. *paniculata* were used for the pathogenicity tests. Isolate B8 was inoculated on *C. camphora, C. taiwaniana, D. regia*, and *M. paniculata* var. *paniculata*; isolate B16 was inoculated on *E. camaldulensis, E. citriodora* and *E. grandis*; and isolate B18 was inoculated on *L. formosana*. After inoculation, seedlings were

replanted in the same pots. Seedlings inoculated with sterile wheat-oat medium were used as controls. Ten seedlings were tested for each treatment and the experiment was repeated once.

Production of basidiocarps

All isolates obtained from the diseased plants were grown on sawdust medium consisting of saw dust (40 kg), rice bran (10 kg), sucrose (50 g), NH_4NO_3 (10 g), citric acid (5 g) and about 15% water in plastic bags (*ca.* 20 cm long, 10 cm diam.). After incubation at 30 C for one month, the plastic bag was removed and the colonized medium was placed on moist sand in the greenhouse to induce formation of fruiting bodies.

Characterization of pure culture

The methods of Stalpers (14) were used to study characteristics of the fungus in pure culture. The fungus was grown on MEA or PDA (potato dextrose agar) at 25 C. The culture code of the fungus was also followed Stalpers (14). The method of Bavendamm (3) was employed to test secretion of extracellular oxidases. The fungus was cultured on MEA, MEA + 0.5% tannic acid or MEA + 0.5% gallic acid and inculated at 25 C for one week.

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 advancing margin of a colony were placed on one side of MEA in petri plates (9 cm diam.) and incubated at 10, 15, 20, 25, 30 or 35 C in the dark. Five plates were used for each treatment and the experiment was repeated once.

RESULTS

Symptoms and hosts

The initial symptoms of diseased trees were willting and yellowing of the foliage followed by death of trees (Fig. 1). Root and basal stem rot were observed on all diseased trees examined. A dark brown encrusting mycelial mat usually appeared at the base of the trunk (Fig. 2). The roots of diseased trees were often encrusted with a mass of sands and stones binded with the brown mycelium, which eventually turned black. The infected wood was brown and subsequently became white and soft with a network of brown lines irregularly distributed in the decayed tissues (Fig. 3). A black zone was present between the decayed wood and the sound wood. In the late stage of decay, the wood became light, dry and spongy, and a brown network of lines appeared on the surface of the wood under the bark (Fig. 4). Well developed fruiting bodies were rarely observed on diseased trees in the fields.

The disease has been observed on various forest trees including *C. camphora, C. taiwaniana, D. regia, E. camaldulensis, E. citriodora, E. grandis, L. formosana*, and *M. paniculata* var. *paniculata* on the eastern and western coasts of central and southern Taiwan. This disease usually occurred in warm seasons and at lower elevation areas (<800 m).

Isolation and pathogenicity tests

Phellinus noxius was consistently isolated from diseased tissues of declining and dead trees. The same species of fungus was also isolated from the brown mycelial mats collected from the surface of diseased roots and basal stems.

Two months after inoculation, seedlings of *C. camphora, E. citriodora*, and *L. formosana* killed by inoculated fungus ranged from 40–60%, 60–70% and 80–90%, respectively, in two experiments (Table 1). Since the diseased trees of *C. camphora, C. taiwaniana, D. regia* and *M. paniculata* var. *paniculata* were observed at the same location of Hualien county, isolate B8 which was isolated from *C. camphora* at that location was also used to inoculate seedlings of *C. taiwaniana, D. regia* and *M. paniculata* var. *paniculata*. Two months after inoculation, seedlings of *C. taiwaniana, D. regia* and *M. paniculata* var. *paniculata* killed by the inoculated fungus ranged from 60–70%, 90–100% and 40–50%, respectively, in two experiments (Table 2). Since the diseased trees of *E. camaldulensis, E. citriodora* and *E. grandis* were observed at the same area of Taitung county, isolate B16 which was isolated from *E. citriodora* at that area was also used for inoculation of seedlings of *E. camaldulensis* and *E. grandis*. Two months after inoculation, seedlings of *E. camaldulensis* and *E. grandis* killed by the inoculated fungus ranged from 60–70% and 50–60%, respectively, in two experiments (Table 2). Wilting and yellowing of the foliage was first observed on the inoculated seedlings which eventually died. The brown mycelial mat was present on the surface of basal stems and roots of inoculated seedlings. Formation

TABLE 1. Pathogenicity of isolated *Phellinus noxius* on their hosts

Plant species inoculated	Isolates used	Disease incidence (%)[1]	
		Exp. I	Exp. II
Cinnamomum camphora	B8	40	60
Eucalyptus citriodora	B16	60	70
Liquidambar formosana	B18	90	80

1. Percentage of trees killed was based on a total of 10 trees tested.

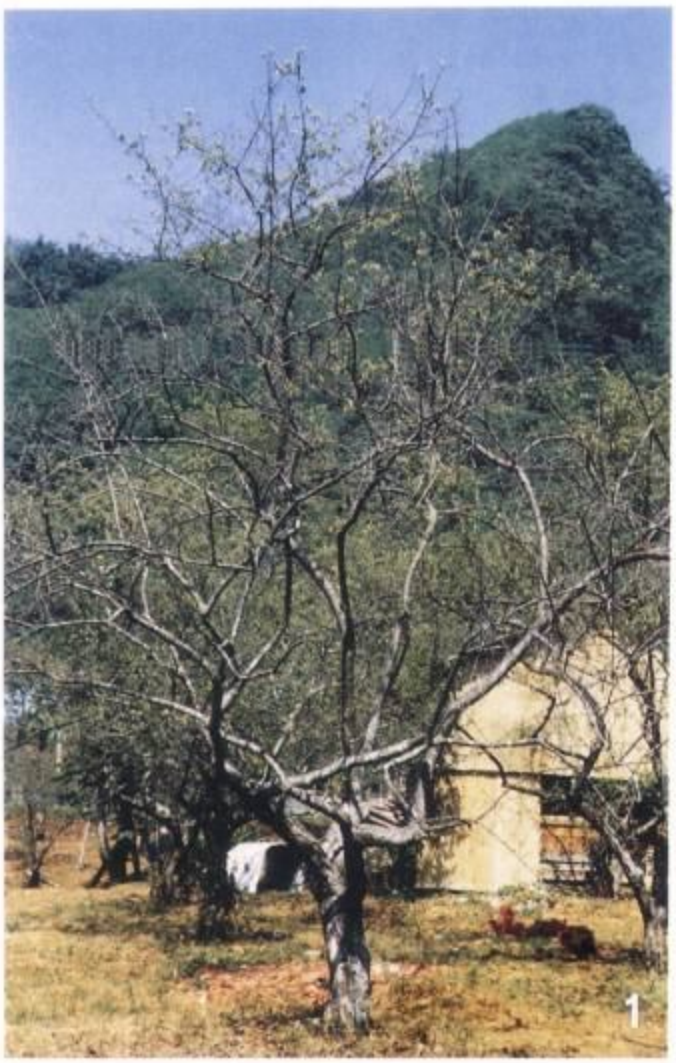

Fig. 1. Appearance of a *Cinnumomum camphora* with symptoms of decline.
Fig. 2. Outer surface of an infected stem of *Delonix regia.*
Fig. 3. White rot and brown lines on the cross section of diseased stem of *Liquidambar formosama.*
Fig. 4. A network of brown lines on infected root tissue of *Delonix regia.*

TABLE 2. Pathogenicity of isolated *Phellinus noxius* on other tree species

Plant species inoculated	Isolates used	Disease incidence (%)[1]	
		Exp. I	Exp. II
Cycas taiwaniana	B8	60	70
Delonix regia	B8	100	90
Murraya paniculata var. *paniculata*	B8	50	40
Eucalyptus camaldulensis	B16	60	70
Eucalyptus grandis	B16	60	50

1. Percentage of trees killed was based on a total of 10 tees tested.

of fruiting bodies was often observed on inoculated seedlings of *D. regia* and *L. formosana*. The pathogen, *P. noxius*, was reisolated from all diseased tissues of inoculated seedlings. All control plants remained healthy during the experiments.

Morphology of fruiting bodies

Since it was difficult to obtain well developed fruiting bodies of *P. noxius* from naturally infected trees, fruiting bodies used for identification were formed on sawdust medium. The fungus produced brown resupinate fruiting bodies about 0.4–2.5 cm thick on sawdust medium after 1–2 months. Fruiting bodies permanently turned black when 5% KOH solution was applied to the surface of basidiocarps. They were grayish brown to pale brown on pore surface. The hyphal system was dimitic including generative hyphae and skeletal hyphae. Generative hyphae measuring 2–4 μm in diameter were hyaline to yellow, and without clamps. Skeletal hyphae were yellow brown to bay with a diameter of 3–6 μm. Contextural setal hyphae were dark ferruginous and up to 450 μm long and 13 μm in diameter (Fig. 5). Basidiospores were smooth, hyaline, and broadly ellipsoid to subglobose, 3–4 × 4–6 μm (Fig. 6). The characteristics of fruiting bodies which fit the description of *P. noxius* in the family Hymenochaetaceae (5) are comparable to those described in Ann & Ko's paper (1).

Characterization of pure culture

On PDA and MEA at 25 C the fungus grew more than 70 mm in diam. in 14 days. Hyphae of the advancing zone appeared raised to appressed. The colonies were white to straw yellow first and became amber brown to sayall brown later. Aerial mycelia were cottony and farinaceous. Arthrospores (Fig. 7) and trichocysts (Fig. 8) were present in the culture and the diseased tissues, while clamps were absent on generative hyphae. The fungus produced extracellular oxidases indicating that *P. noxius* belongs to the group of white rotting fungi. It also produced laccase and peroxidase but not tyrosinase. The culture code of 1, 3, 5, 12, 13, (14), (18), (19), 21, 22, (25), 28, 30, (31), 34, 35, 38, 46, 48, 52, 53, (54), 61, 67, 84, 89, 90 fits the description of *P. noxius* (14) except that arthrospores were not observed by Stalpers (14), and are comparable to those described in Ann & Ko's paper (1).

The fungus isolated from *C. camphora* (B8), *E. citriodora* (B16) and *L. formosana* (B18) was used for testing growth response to temperature. These three isolates showed similar growth response to temperature, with the optimum temperatures of 25–35 C and a minimum temperature of 15 C. All test isolates did not grow at 10 C. These results are comparable to those of Ann & Ko (1).

DISCUSSION

P. noxius is widely distributed in the tropical regions (6, 11). It causes brown root rot of numerous agricultural and forest trees including tea (*Camellia sinensis* (L.) Ktze), rubber (*Hevea brasibiensis* Muell.), oil palm, *Delonix regia*, and *Cordia alliodora* resulting in decline of trees (7,10,11). It has been reported that *P. noxius* caused severe decline of longan and many other fruit trees in the central and southern mountain areas of Taiwan (1,9). This study shows that decline of eight species of trees in Taiwan is also caused by brown root rot incited by *P. noxius*. In addition, the same disease has been observed on four other species of trees including *Araucaria cunninghamii* Sweet, *Elaeocarpus serratus* Linn., *Cordia* sp., and *Hibiscus rosa-sinensis* Linn. (unpublished data). The disease mainly occurred in the eastern and western coasts of central and southern Taiwan at lower altitudes (<800 m). Although most of hosts in the present study are not economically important trees, plantations of *C. camphora* exceed 5,000 ha in Taiwan. Moreover, plantations of *E. camaldulensis* and *E. grandis* used for pulp wood are increasing in Taiwan. Since *P. noxius* has caused a very serious problem in longan plantations, the incidence of brown root rot in *C. camphora*, *E. camldulensis* and *E. grandis* should not be regarded lightly.

Although arthrospores have not been observed by Stalpers (14) and Bakshi *et. al.* (2), they were present at an oil palm (*Elaeis guineensis* jacf.) isolate of *P. noxius* (CBS 170.32) obtained from Centraalbureau voor Schimmelcultures, Barrn, Netherlands observed by the author. The substrate for living of *P. noxius* was only on angiosperms (14). In the present paper, *P. noxius* is able to infect *Cycas taiwaniana* which belongs to gymnosperms.

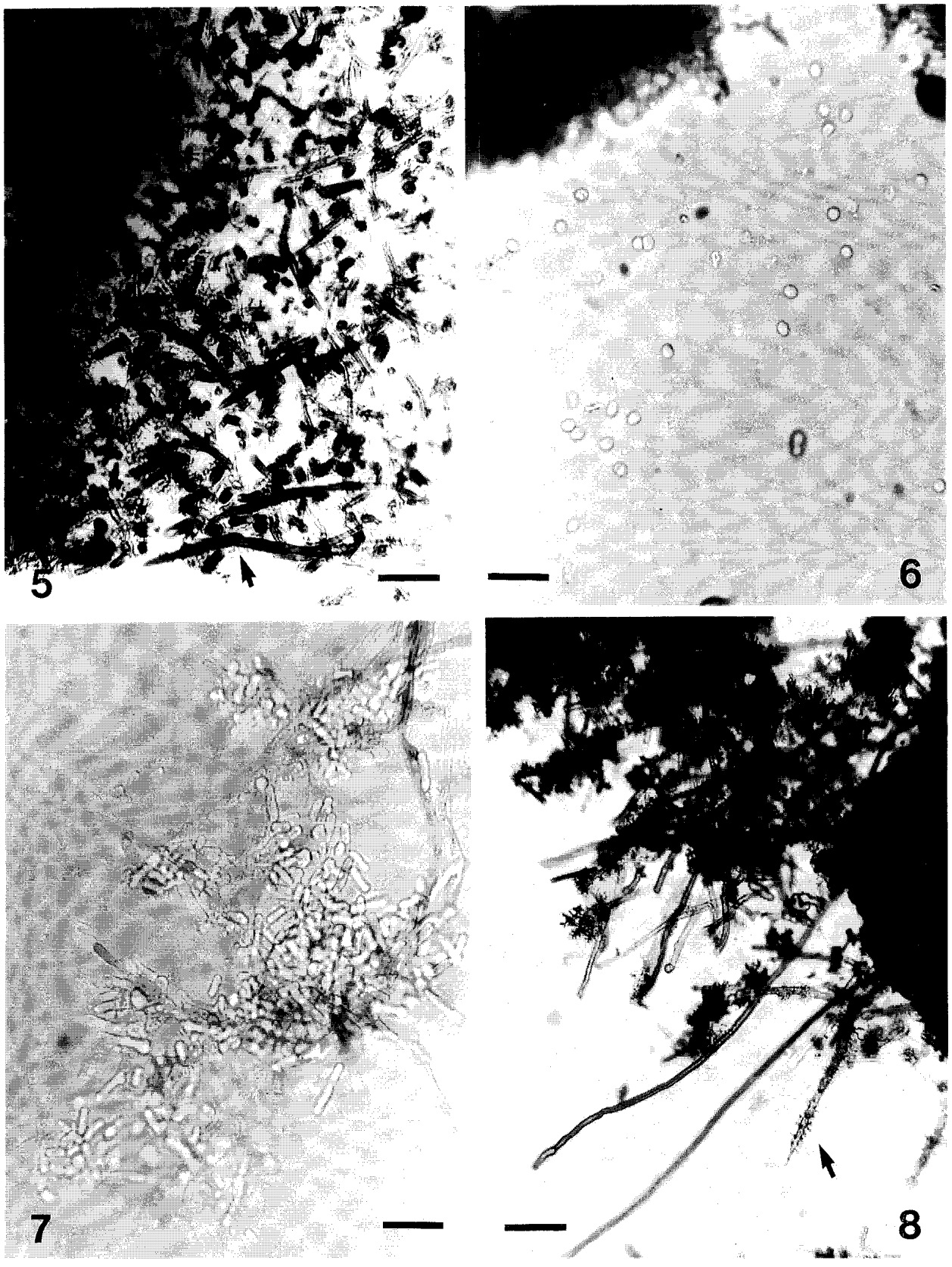

Fig. 5. Setal hyphae (arrow) of *Phellinus noxius* in context of fruiting body. Bar = 30 μm.
Fig. 6. Basidiospores of *Phellinus noxius* Bar = 15 μm.
Fig. 7. Arthrospores of *Phellinus noxius*. Bar = 15 μm.
Fig. 8. Trichocysts (arrow) of *Phellinus noxius*. Bar = 30 μm.

It was reported that *Phellinus lamaensis* (Murr.) Heim. (= *Fomes lamaensis* (Murr.) Sacc. & Trott.) caused brown root rot of *C. camphora* in Taiwan in 1928 by Sawada (12). However, only symptoms were described and the pathogen was determined mainly based on symptoms and signs on diseased trees. He did not observe any fruiting bodies of *P. lamaensis* at that time. Also, the pathogenicity of *P. lamaensis* to *C. camphora* still has not been proven. During the survey of brown root rot disease in this study, *P. lamaensis* has never been observed on declining forest trees. Moreover, Sawada (13) treated *P. noxius* (= *Fomes noxius* Corner.) as a synonym of *P. lamaensis* (= *Fomes lamaoensis*). It is, therefore, considered that previously reported brown root rot of *C. camphora* was also incited by *P. noxius*. A brown root rot of *D. regia* (7) caused by *P. noxius* has been reported in the Mariana Islands, but the causal relationship between *P. noxius* and brown root rot of the host still has not been established.

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摘　　要

張東柱．1992．林木立枯與 *Phellinus noxius* 引起的褐根病之相關性．植病會刊 1:90-95．（台北市　台灣省林業試驗所森林保護系）

在本省東岸與西岸中南部多種林木發現葉片萎凋、變色，根部及莖基部褐根腐，繼而全株死亡。罹病的樹木包括樟樹 (*Cinnamomum camphora*)，台灣蘇鐵 (*Cycas taiwaniana*)、鳳凰木 (*Delonix regia*)、赤桉 (*Eucalyptus camaldulensis*)、檸檬桉 (*E. citriodora*)、玫瑰桉 (*E. grandis*)、楓香 (*Liquidambar formosana*)、月橘 (*Murraya paniculata* var. *paniculata*) 等 8 種。從罹病的組織均可分離到 *Phellinus noxius*，此眞菌可在鋸木屑太空包上形成完整的子實體。但在自然界不易發現完整的子實體。將培養在燕麥—小麥培養基上的 *Phellinus noxius* 接種於樟樹，台灣蘇鐵、鳳凰木、赤桉、檸檬桉、玫瑰桉、楓香與月橘苗之根部，可以引起接種植株死亡，且在人工接種罹病組織上可再分離得 *P. noxius*。雖文獻上曾記載 *P. noxius* 可引起樟樹及鳳凰木之褐根病，但其病原性並未被證實。

關鍵字：*Phellinus noxius*、褐根病、樹木立枯。