Decline of Nine Tree Species Associated with Brown Root Rot Caused by *Phellinus noxius* in Taiwan

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ABSTRACT

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A decline disease characterized by brown root rot, wilting of foliage, leaf discoloration, a brown mycelial mat growing on roots and stem bases of affected trees, and ultimate death of some trees was found on several tree species growing in the eastern and western coastal regions of central and southern Taiwan. These tree species were mainly growing in landscape plantings rather than in the forest. Species affected by the disease included *Acacia confusa, Bauhinia variegata, Calophyllum inophyllum, Casuarina equisetifolia, Ficus microcarpa, Koelreuteria henryi, Podocarpus macrophyllus* var. *macrophyllus, Salix babylonica,* and *Swietenia mahagoni.* A fungus isolated from diseased tissue produced resupinate, brownish polyporus fruiting bodies on sawdust medium, and was identified as *Phellinus noxius*. Seedlings of the various host plants found naturally infected in the field were inoculated with *P. noxius* grown on camphor twig medium. Seedings of all inoculated species were infected, and the fungus was reisolated from diseased tissues. This is the first report of *P. noxius* on these hosts. In cross pathogenicity tests, isolates of *P. noxius* from twelve tree species were used to inoculate each of the host species except for the original source of the inoculum. All isolates caused infection on all eleven hosts, indicating that the pathogen does not exhibit host specificity. However, *A. confusa* and *S. babylonica* showed lower mortality than other hosts.

Phellinus noxius (Corner) G. H. Cunningham is widely distributed in tropical regions (8), causing a brown root rot and decline of numerous orchard and forest tree species (4,6–8). In recent years, the disease has become one of the most serious problems of fruit and forest trees in central and southern Taiwan at altitudes less than 800 m (1,2). Recently, wilting and yellowing of the foliage followed by death of some trees were found on shade and windbreak trees in central and southern Taiwan. The roots and stem bases of affected trees were always encrusted with thick, brownish black mycelial mats, characteristic of *P. noxius*. This paper reports results of studies to confirm *P. noxius* as the cause of the root disease observed and on cross inoculation studies to determine the relative susceptibility of different hosts.

MATERIALS AND METHODS

Isolation and production of basidiocarps. Diseased roots and stem bases were obtained from declining or dead trees of several tree species in the field. These were washed with tap water and blotted dry. Pieces (approximately 3 × 3 × 3 mm) of diseased tissues were cut and placed in petri plates on a selective medium developed in a previous study (3). The fungus growing from the diseased tissues was transferred to and maintained on malt-extract agar (MEA: 20 g of malt extract per liter, 20 g of Bacto agar per liter). All isolates obtained from the diseased plants were grown on a hardwood 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% (wt/wt) water in plastic bags (approximately 20 cm long, 10 cm in diameter). After incubation at 30°C for 1 month, the plastic bag was removed and the colonized medium was placed on moist sand in the greenhouse to induce formation of fruiting bodies. Isolates B18 (CCRC 35248) and B28 (CCRC 35250) are deposited at the Culture Collection and Research Center, Hsinchu, Taiwan, while others are deposited at the Forest Pathology Laboratory, Taiwan Forestry Research Institute, Taipei, Taiwan.

Pathogenicity tests. Information regarding the sources of the isolates of *P. noxius* used is presented in Table 1. All isolates were grown on sterilized segments of camphor tree (*Cinnamomum camphora* (L.) J. Presl.) twigs (less than 1 cm in diameter and 5 cm long) in a 250-ml flask for 1 month at 25°C. Two- to three-year-old seedlings or rooted cuttings of each host plant were inoculated by placing five pieces of infected twigs on the roots and covered with soil. Seedlings or rooted cuttings inoculated with sterile twigs were used as controls. Ten plants of each species were inoculated and the experiment repeated once. Each host species was inoculated with the isolate obtained from that host species. Disease incidence was defined as percentage of trees killed based on total trees tested.

Cross pathogenicity tests. In order to determine the variation in host susceptibility and virulence of isolates from various hosts, seedlings or rooted cuttings of twelve tree species were inoculated in all possible combinations with an isolate from each of the hosts, with the exception that an individual host species was not inoculated with the isolate from that host species. The study included the nine hosts and isolates listed in Table 1, as well as an additional three hosts and isolates, *C. camphora* (isolate B8), *Eucalyptus citriodora* Hook. (isolate B16), and *Liquidambar formosana* Hance (isolate B18), which had been studied previously (2). Preparation of inoculum and inoculation procedure were as described above. Five plants were used for each host-isolate combination and the experiment repeated once. SAS logistic regression analysis of survival on host species and pathogen isolates was used for statistical analyses.

Table 1. Source of isolates of *Phellinus noxius* and pathogenicity of these isolates on their respective source hosts

Host	Isolate	Locality collected (county)	Disease incidence (%)[a]	
			Experiment 1	Experiment 2
Acacia confusa Merr.	B28	Hualien	40	30
Calophyllum inophyllum L.	B29	Hualien	70	80
Bauhinia variegata L.	B30	Taitung	70	80
Salix babylonica L.	B33	Yunlin	50	40
Podocarpus macrophyllus var. *macrophyllus* (Thunb.) D. Don	B34	Yunlin	60	90
Casuarina equisetifolia L.	B36	Chiayi	80	70
Swietenia mahagoni L. Jacq.	B39	Chiayi	70	100
Koelreuteria henryi Dumm.	B40	Taichung	60	70
Ficus microcarpa L.	B45	Changhus	60	70

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

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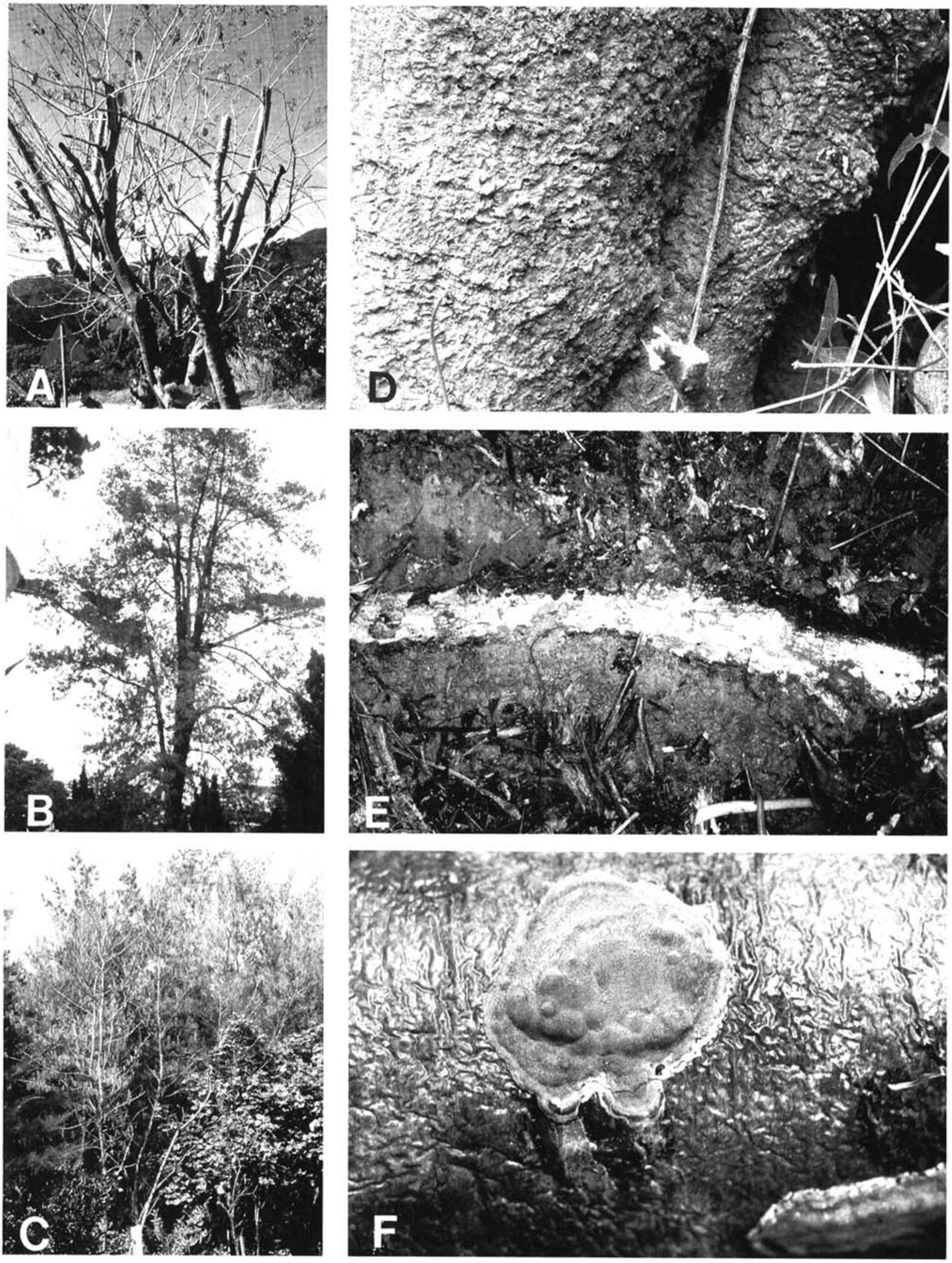

Fig. 1. (**A**) Appearance of *Bauhinia variegata* with symptoms of decline. (**B**) Appearance of a 50-year-old tree of *Podocarpus macrophyllus* var. *macrophyllus* with symptoms of decline. (**C**) Appearance of *Casuarina equisetifolia* with symptoms of decline at a windbreak plantation. (**D**) Outer surface of an infected basal stem of *Acacia confusa*. (**E**) A network of brown lines on infected root tissue of *Bauhinia variegata*. (**F**) A fruiting body of *Phellinus noxius* on sawdust medium.

RESULTS

Hosts and symptoms. The disease was observed on nine species of shade and windbreak trees in the eastern and western coasts of central and southern Taiwan, including: *Acacia confusa, Bauhinia variegata, Calophyllum inophyllum, Casuarina equisetifolia, Ficus microcarpa, Koelreuteria henryi, Podocarpus macrophyllus* var. *macrophyllus, Salix babylonica,* and *Swietenia mahagoni*. The disease was present on trees of all ages and was commonly observed in warm seasons at lower elevation areas (lower than 800 m), although it could be found year around.

Infected trees usually exhibited wilting and yellowing of the foliage, and most infected trees eventually died (Fig. 1A–C). A dark brown mycelial mat was formed on the surface of the roots and up the base of the stem, but usually no farther than 1 m above the ground (Fig. 1D). Masses of sand and stone usually adhered to the mycelium on the root and stem surface. The infected wood was at first discolored, but subsequently became white and soft with a network of brown lines irregularly distributed in the decaying tissues (Fig. 1E). Black zone lines were present between the decayed and sound wood. In the late stages of decay, the wood became light, dry and spongy. Well-developed fruiting bodies were rarely observed on diseased trees in the field.

Isolation and identification of the pathogen. A fungus was consistently isolated from diseased tissues of declining and dead roots and stem bases. Although fruiting bodies were rarely seen in the field, they could be produced on a sawdust medium. The brown, resupinate, fruiting bodies, 0.4 to 2.5 cm thick (Fig. 1F), permanently turned black when 5% KOH solution was applied to the surface. They were grayish brown to pale brown on the surface. The hyphal system was dimitic including generative hyphae and skeletal hyphae. Generative hyphae measuring 2 to 4 μm in diameter were hyaline to yellow, and without clamps. Skeletal hyphae were yellowish brown to bay with a diameter of 3 to 6 μm. Contextural setal hyphae were dark ferruginous and up to 450 μm long and 13 μm in diameter. Basidiospores were smooth, hyaline, and broadly ellipsoid to subglobose, 3 to 4 × 4 to 6 μm. The characteristics of the fruiting bodies fit the description of *P. noxious* (8) and are comparable to those described in a previous study (1).

Pathogenicity tests. Three months after inoculation, the percentage of inoculated seedlings of each species killed in the two experiments varied from 30 to 40 to 70 to 100 (Table 1). Fewer seedlings of *A. confusa* and *S. babylonica* were killed than those of the other hosts. Infected seedlings exhibited wilting and yellowing of the foliage before they died. The typical brown mycelial mat was present on the surface of the lower stem and roots of inoculated seedlings. Fruiting bodies were often observed on inoculated plants of *C. inophyllum, B. variegata,* and *C. equisetifolia.* The fungus was reisolated from diseased tissues of inoculated plants. All control plants remained healthy during the experiments.

Cross pathogenicity tests. Three months after inoculation, all isolates of *P. noxious* used were able to cause disease in all 11 tree species. However, disease incidence was quite variable, ranging form 20 to 90% of the trees killed (Table 2). *Acacia confusa* and *S. babylonica* exhibited lower mortality than other host plants. Survival of *A. confusa* and *S. babylonica* was significantly different ($P = 0.01$) from that of others with the logistic regression analysis. But, there were no significant differences ($P = 0.05$) among pathogen isolates. Similar symptoms and signs were present in the inoculated plants. *Phellinus noxius* was reisolated from diseased tissues of inoculated plants.

DISCUSSION

This study showed that decline of nine tree species in Taiwan is caused by brown root rot incited by *P. noxius*. The disease was found primarily in the eastern and western coasts of central and southern Taiwan at altitudes lower than 800 m. The problem was especially severe in windbreak plantations of *C. equisetifolia,* one of the most important windbreak species in Taiwan. Fortunately the disease was not severe on *A. confusa,* which is an economically important plantation and shade tree. However, the disease was found to occur commonly on the other hosts listed, all of which are used as shade trees in Taiwan.

Cross pathogenicity tests demonstrated that *P. noxius* had a wide host range and apparently did not have host specificity for these species. Twelve isolates obtained from different hosts were able to cause the disease on all of those hosts. However, it has been previously reported that *P. noxius* isolated from *Hevea brasiliensis* (Willd. ex Adr. Juss) Müll. Arg. and *Cedrela odorata* Linn. in west Africa showed variation in virulence to *H. brasiliensis* (5). This may be due to inoculation procedures or other factors. Until more solid evidence is obtained, the occurrence of pathogenic races of the fungus cannot be excluded.

Phellinus noxius has previously been reported to occur only on angiospermous hosts (9). In this study, however, the fungus was recovered from *P. macrophyllus* var. *macrophyllus,* which is a gymnosperm. An isolate (B34) of *P. noxius* from this species infected seedlings of the hardwood hosts tested, and was itself infected by isolates from the hardwood hosts. These results indicate that the host range of *P. noxius* is not restricted to hardwoods.

The incidence of disease with all isolates tested was significantly lower on *A. confusa* and *S. babylonica* than on the remaining hosts, indicating that these two

Table 2. Pathogenicity of isolates of *Phellinus noxius* on a variety of hosts

Plant species	Isolate used and disease incidence (%)[a]												
	B8	B16	B18	B28	B29	B30	B33	B34	B36	B39	B40	B45	Average
Acacia confusa	40	30	20	. . .[b]	40	30	30	50	20	40	30	50	34[c]
Calophyllum inophyllum	80	70	60	60	. . .	70	50	70	60	40	80	70	65
Cinnamomum camphora	. . .	50	60	70	70	80	60	90	60	70	60	50	66
Casuarina equiselifolia	60	60	50	70	90	60	60	50	. . .	70	60	70	64
Bauhinia variegata	70	80	90	80	60	. . .	80	60	60	70	70	50	70
Eucalyptus citriodora	50	. . .	80	70	70	60	50	90	60	60	70	70	66
Ficus microcarpa	50	70	90	70	60	80	50	70	60	60	70	. . .	66
Koelreuteria henryi	60	70	60	90	60	60	70	80	50	50	. . .	50	64
Liquidambar formosana	70	60	. . .	70	60	80	90	90	60	70	80	70	73
Podocarpus macrophyllus var. *macrophyllus*	80	90	50	60	60	70	70	. . .	70	80	50	80	69
Salix babylonica	40	20	30	20	30	40	-	50	50	20	30	20	32[c]
Swietenia mahagoni	70	90	60	50	70	70	90	50	80	. . .	70	80	70
Average	66	63	59	65	61	64	64	68	57	57	61	60	

[a] Percentage of trees killed was based on a total of 10 trees tested in two experiments.

[b] Data were presented in Table 1.

[c] *Acacia confusa* and *S. babylonica* were significantly different ($P = 0.01$) from other hosts, and there was no significant difference ($P = 0.05$) among pathogen isolates in accordance with logistic regression of survival on host species and pathogen isolates.

species may have some level of resistance to the disease. These results also show that *P. noxius* apparently does not have host specificity; however, different degrees of resistance to the fungus might be present in different tree species. As has been observed in the fields, some trees such as *Melaleuca leucadendra* (L.) L. and *Alstonia scholaris* R. Br. were found to be free of disease when they were growing in close proximity to diseased trees of other species (T.-T. Chang, *unpublished data*). However, further study is required to determine whether resistant tree species exist.

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