Survival of *Phellinus noxius* in Soil and in the Roots of Dead Host Plants

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ABSTRACT

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The survival of *Phellinus noxius* arthroconidia, basidiospores, and mycelia and of *P. noxius*-colonized wood was measured in soils with different soil matrix potentials. Survival of arthroconidia declined more slowly in soils with –0.50 and –0.42 MPa of soil matrix potential compared with soils with –0.15 and –0.025 MPa. However, arthroconidia were rarely recovered from the treatments after 3 months. Basidiospores were not recovered after 3, 3.5, 4, and 4.5 months in the –0.025, –0.15, –0.42, and –0.50 MPa soil matrix potential treatments, respectively. Mycelia that were buried in the soil with –0.025 MPa were not recovered after 4 weeks, whereas mycelia in soils with –0.50, –0.42, and –0.15 MPa were not recovered after 10 weeks. *P. noxius* was not recovered from pieces of artificially infested wood subjected to 1 month of flooding. However, in treatments with lower soil moisture, *P. noxius* survival ranged from 80% to more than 90% over 2 years. Colonies of *P. noxius* were not recovered from the rhizosphere of soils around the infested roots of three host species, *Calophyllum inophyllum*, *Casuarina equisetifolia*, and *Cinnamomum camphora*. However, *P. noxius* was recovered from naturally infected roots of these hosts 1 to 10 years after they were killed. These results indicated that woody debris in soils harboring *P. noxius* played an important role in the long-term survival of the fungus. Flooding infested fields may help control *P. noxius* in the field.

Additional keywords: brown root rot disease, disease management.

Phellinus noxius (Corner) Cunningham is widely distributed in tropical regions (10,13,14,15,17,20), causing a brown root rot and a decline of numerous orchard and forest tree species. 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 (2,5,6). Several slowly expanding, circular disease patches extending from infection centers were observed in the field indicating that the disease caused by *P. noxius* is spread from diseased to healthy trees by root contact (3,4,10,17,20). Infested root debris may play an important role as primary inoculum and for the long-term survival of the fungus. Airborne basidiospores can also initiate new infections on freshly cut stumps or infest logging debris with subsequent spread to live trees by root contact (3,4,10,15,17,20). Although fruiting bodies of *P. noxius* are rarely seen in the fields (5,6,10,13,14), abundant arthroconidia have been produced in pure culture (5). Alston (1) studied the role of basidiospores in the infection process and concluded that spores can germinate on a cut surface of a tree. However, the role of arthroconidia is still unknown, and the long-term survival of basidiospores, arthroconidia, and mycelia in the soil has not been previously documented.

P. noxius can easily be isolated from infected wood, but it was very difficult to isolate from soil. Recently, a selective medium was developed by the author to quantitatively recover the fungus from infected woody tissue and infested soils (7). In this study, the survival of *P. noxius* basidiospores, arthroconidia, and mycelia was assessed in soils of varying moisture levels. In addition, the survival of *P. noxius* in artificially infested woody debris, naturally infected roots, and their rhizosphere soils was studied.

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MATERIALS AND METHODS

Soils, selective medium, and the fungal isolate. The silt loam soil used for this study was collected from a natural forest. Initially, it consisted of 36.7% sand, 52.6% silt, and 10.7% clay, and had about 10% moisture content (–0.42 MPa). For treatments requiring less than 10% soil moisture, the soil was dried at 40°C until the desired moisture was reached. For treatments requiring greater than 10% soil moisture, distilled water was added to the soil until the desired moisture level was achieved. Soil matrix potential was adjusted gravimetrically by comparison with a soil moisture retention curve. The selective medium (7) consisted of 20 g of malt extract, 20 g of agar, 10 mg of benomyl, 10 mg of dicloran, 100 mg of ampicillin, and 500 mg of gallic acid per liter. For isolation of the fungus from soil, an additional 1 g of tergitol NP-7 per liter was required. *P. noxius* (isolate B-8) used in the study was obtained from diseased roots of *Cinnamomum camphora* (Linn.) Nees et Eberm. collected in Hualien county, Taiwan (5). The temperature range was between 15 and 30°C during the experimental period in the greenhouse.

Survival of arthroconidia. An arthroconidial suspension was prepared by adding 10 ml of sterile distilled water to a 2-week-old *P. noxius* culture growing on MEA (2% malt-extract and 2% agar) medium. The concentration of the arthroconidial suspension was determined with the microliter syringe method (12). The suspension was added to the soil, which contained about 3.6×10^5 arthroconidia/g of dried soil, and was mixed as uniformly as possible in sealed plastic bags. Soil matrix potential was adjusted to –0.50, –0.42, –0.15, and –0.025 MPa for the four treatments.

Soil suspensions were prepared by grinding 5 g of soil (dry weight) with sterile distilled water at a 50-ml final volume in an Omni mixer (Omni International, Inc., Waterbury, CT) operated at 3,000 rpm for 2 min. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) (11) were plated on the selective medium amended with 1 g of tergitol NP-7 per liter. Five petri dishes were used for each dilution and treatment. Three plastic bags (replicates) were conducted for each treatment. After incubation for 2 weeks, colony numbers were counted and the dilution with the most colonies in each treatment was used to represent the recovery frequency. This experiment was conducted twice.

Survival of basidiospores. Basidiospores were collected by suspending the basidiocarps about 2 cm above a weighing paper. The basidiospore suspension was prepared by transferring the basidiospores into a 50-ml beaker and adding 10 ml of sterile distilled water; the suspension was then added to the soil treatments to a final concentration of 3.6×10^5 basidiospores/g of dried soil. The soil treatments, the methods, and the number of replicates for recovery of basidiospores from soil were the same as those described above for arthroconidia. This experiment was conducted twice.

Survival of mycelia. The soil matrix potential was adjusted to –0.50, –0.42, –0.15, and –0.025 MPa, respectively, and soils were placed in separate plastic containers (15 cm high by 15 cm in diameter). Pieces of cellophane (2 by 2 cm) colonized by *P. noxius* mycelia were placed on MEA medium for 2 weeks at 25°C. Well-colonized cellophanes were buried 5 cm below the soil surface and each plastic container was covered with aluminum foil. To test mycelial survival, pieces of cellophane were carefully removed from the soil, placed on the selective medium, and incubated at 25°C. Three replicate containers were used for each soil moisture. Thirty pieces of cellophane were used in each replicate. Survival of mycelia was calculated as the percentage of colonized cellophanes from which *P. noxius* emerged after 2 weeks of incubation. This experiment was performed twice.

Survival in infested wood. Wood sections (about 3 to 5 cm in diameter and 10 cm in length) of *Cinnamomum camphora* were artificially infested with *P. noxius* 2 months before the start of the experiment. *P. noxius* completely colonized the wood sections in 2 months at 25°C. Soil matrix potential was adjusted to –0.50, –0.30, or –0.025 MPa, or the soil was flooded with water. Eighty-cm-high columns of each soil type were placed in separate plastic container (100 cm high by 40 cm in diameter) and wood sections were buried 15 cm below the soil surface. The cap was sealed with plastic tape to maintain constant soil moisture. One container for each soil moisture treatment was used. Three wood sections (replicates) were used for isolations for each treatment. In another treatment, three wood sections infested with *P. noxius* were placed on the surface of the soil. When wood sections were used to test *P. noxius* survival, they were washed with tap water and blotted dry. Fifty wood fragments (about 3 by 3 by 6 mm) were cut from each wood section and placed on the selective medium at 25°C. Survival in infested wood was calculated as the percentage of wood fragments from which *P. noxius* emerged after 2 weeks of incubation. This experiment was conducted twice.

Isolation from soils. Rhizosphere soil was collected from naturally infected roots of *Calophyllum inophyllum* Linn., *Casuarina equisetifolia* Linn., and *Cinnamomum camphora* to determine if *P. noxius* was present in field soils, not in conjunction with any woody debris. Four soil samples were collected from the rhizosphere of each of four trees of the three tree species in Hualien, Chiayi, and Yunlin counties, Taiwan. The same methods described in the section on the survival of arthroconidia were used for detection of *P. noxius* in the soil.

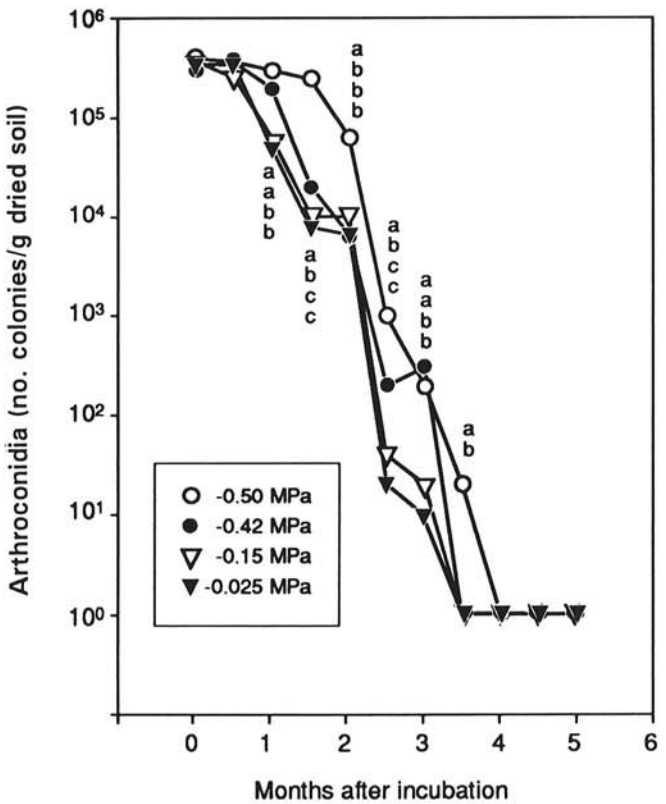

Fig. 1. Survival of *Phellinus noxius* arthroconidia mixed in soils with varying soil moisture treatments at different incubation times. Each point is the mean of six replicates. Means at each incubation time followed by the same letter are not significant according to the least significant difference test ($P < 0.01$). The multivariate analysis of variance showed that trends among soil matrix potential treatments with time were significant at $P = 0.01$.

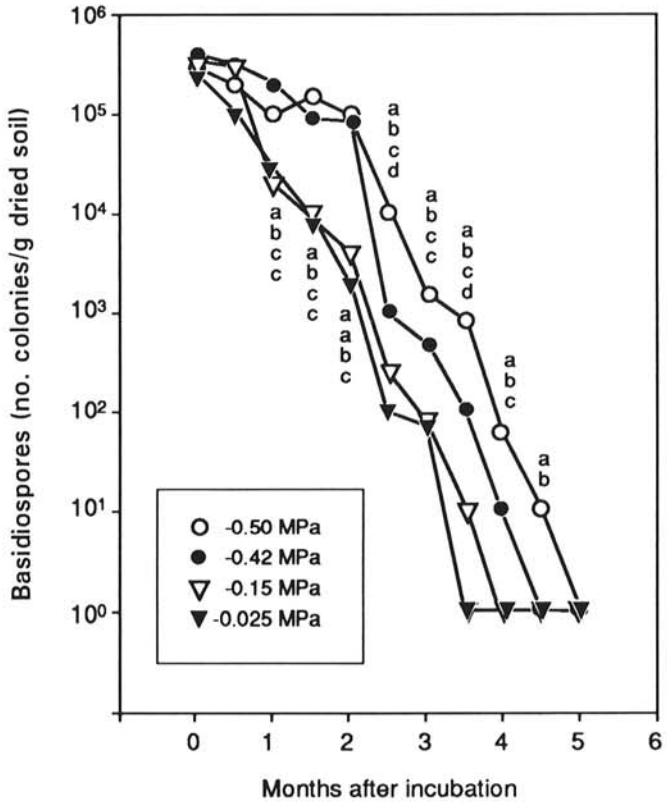

Fig. 2. Survival of *Phellinus noxius* basidiospores mixed in soils with varying soil moisture treatments at different incubation times. Each point is the mean of six replicates. Means at each incubation time followed by the same letter are not significant according to the least significant difference test ($P < 0.01$). The multivariate analysis of variance showed that trends among soil matrix potential treatments with time were significant at $P = 0.01$.

Isolation from infected roots. Naturally infected root sections, about 10 cm below the soil surface, were collected from dead trees of *Calophyllum inophyllum*, *Casuarina equisetifolia*, and *Cinnamomum camphora* at different times after they were killed by *P. noxius* in the field. Four sections were cut from each tree. Fifty root fragments (about 3 by 3 by 6 mm) were cut from each root section and placed on the selective medium at 25°C. Survival in infected roots was calculated as the percentage of root fragments from which *P. noxius* emerged after 2 weeks of incubation.

Statistical analyses. Since variances between two repeated trials in all experiments were not significant ($P = 0.05$) by SAS analysis of variance (ANOVA) (SAS Institute, Cary, NC), the data from each of the two repeated trials were pooled. SAS repeated-measures analysis (SAS Institute) was used for statistical analyses. Propagules of arthroconidia and basidiospores were transformed to log10, whereas survival (%) of mycelia and infested wood was transformed to arsin[square root(survival/100)] before ANOVA. The significance of response for soil matrix potential at each incubation time was analyzed by the least significant difference (LSD) test if the ANOVA F test was significant. The multivariate ANOVA (Wilks' lambda F test) was used to analyze significant trends for soil matrix potential responses.

RESULTS

Greenhouse test. Soil matrix potential had significant effects ($P = 0.01$) on the survival of arthroconidia with time. There were significant differences ($P = 0.01$) among soil matrix potential treatments at six individual incubation times (Fig. 1). The number of colonies derived from arthroconidia in the –0.50 and –0.042 MPa soil matrix potential treatments declined more slowly with time than in the –0.15 and –0.025 MPa treatments. However, no arthroconidia survived for 3.5 months in any treatment.

The decline in basidiospore survival in the soil moisture treatments was similar to that of arthroconidia. Trends for survival of basidiospores with time were significant among soil matrix potential treatments ($P = 0.01$), and there were significant differences ($P = 0.01$) among soil matrix potential treatments at eight individual incubation times (Fig. 2). Basidiospores declined more slowly with time in the –0.50 and –0.42 MPa soil moisture treatments than in the –0.15 and –0.025 MPa treatments. However, basidiospores were not recovered after 3, 3.5, 4, and 4.5 months in the –0.025, –0.15, –0.42, and –0.50 MPa treatments, respectively.

Trends for survival of mycelia with time were significant among soil matrix potential treatments ($P = 0.01$), and there were significant differences ($P = 0.01$) among soil matrix potential treatments at five individual incubation times (Fig. 3). Mycelia buried in the –0.025 MPa soil moisture treatment declined most rapidly and could not be recovered after 4 weeks. Mycelia buried in the –0.50 MPa soil moisture treatment had relatively higher recovery rates at the same incubation times compared with those in the –0.42 and –0.15 MPa soil moisture treatments. However, mycelia were not recovered after 10 weeks in these three treatments.

P. noxius was not recovered from pieces of artificially infested wood subjected to 1 month of flooding. However, in treatments with lower soil moisture, *P. noxius* survival ranged from 80% to

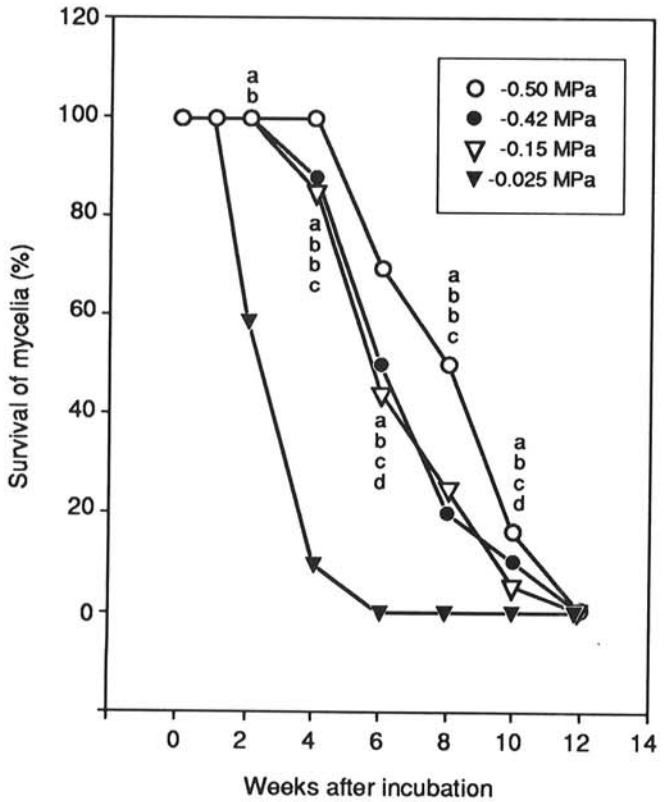

Fig. 3. Survival of *Phellinus noxius* mycelia buried in soils with varying soil moisture treatments at different incubation times. Each point is the mean of six replicates. Survival of mycelia was calculated as the percentage of colonized cellophanes from which *P. noxius* emerged after 2 weeks of incubation. Means at each incubation time followed by the same letter are not significant according to the least significant difference test ($P < 0.01$). The multivariate analysis of variance showed that trends among soil matrix potential treatments with time were significant at $P = 0.01$.

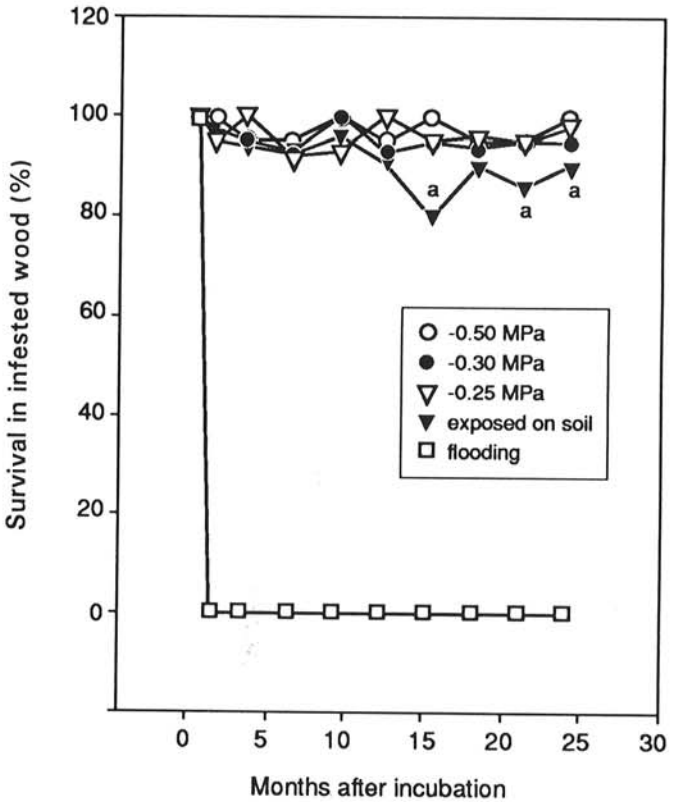

Fig. 4. Survival of *Phellinus noxius* in artificially infested wood sections buried in soils with varying soil moisture treatments at different incubation times. Each point is the mean of six replicates. Survival in infested wood was calculated as the percentage of wood fragments from which *P. noxius* emerged after 2 weeks of incubation. Trends among all treatments with time were not significant ($P = 0.05$) when the flooding treatment was excluded, but significant ($P = 0.01$) when the flooding treatment was included by the multivariate analysis of variance. The least significant difference test among all treatments at different incubation times when the flooding treatment was excluded showed that the infested wood exposed on soil treatment at only three incubation times (a) were significantly different ($P = 0.01$) from other treatments.

more than 90% over 2 years (Fig. 4). Trends for survival of infested wood with time were not significant ($P = 0.05$) among all treatments when the flooding treatment was excluded, but significant ($P = 0.01$) when the flooding treatment was included. The LSD test among all treatments at different incubation times when the flooding treatment was excluded showed that the infested wood exposed to soil treatment at three incubation times, 15, 21, and 24 months, were significantly different ($P = 0.05$) from other treatments.

Field test. Colonies of *P. noxius* were not recovered from any of the soil samples collected from the rhizospheres of three host plants, *Calophyllum inophyllum*, *Casuarina equisetifolia*, and *Cinnamomum camphora*. *P. noxius* was recovered in naturally infected root sections of five trees of *Calophyllum inophyllum* from 1 to 5 years after they were killed by the fungus (Fig. 5). Similar results were obtained from eight trees of *Casuarina equisetifolia* from 1 to 10 years after the trees were killed and from five trees of *Cinnamomum camphora* from 1 to 6 years after they were killed by the fungus (Fig. 5). It appeared reasonable that survival of *P. noxius* in naturally infected roots declined rather slowly with time.

DISCUSSION

Woody debris infested with *P. noxius,* but not arthroconidia, basidiospores, or mycelia in the soil, played an important role in the long-term survival of this fungus. Although the longest survival time of *P. noxius* in dead roots in this study was 10 years after the host plant was killed, it appeared that the fungus could survive longer based on approximately 50% survival over 10 years. The survival pattern of *P. noxius* on different hosts for a long period of time might show differences, but only 10-year-old dead trees were available in the study. It was difficult to find roots that were dead for longer than 10 years because the disease has not been studied carefully until recently. After the death of its host, *Phellinus weirii* (Murr.) Gilb., which causes laminated root rot of Douglas fir in the western United States and Canada, continues to live saprophytically in the lower portion of the trunk and the root system for 50 years or more (9,18). In order to survive over the long-term, the hyaline conidia and mycelia of most pathogenic, root-infecting fungi in soils usually need to form chlamydospores (8). Chlamydospores were not found in pure culture of *P. noxius* (4). When arthroconidia or basidiospores from infested soils and infected wood sections were smeared on slides and observed under a light microscope, chlamydospores were not found (T. T. Chang, *unpublished data*). Based on these results, it was proposed that *P. noxius* does not form chlamydospores as the long-term survival structure. However, *P. noxius* was able to live in woody debris in soils until the wood was completely rotted.

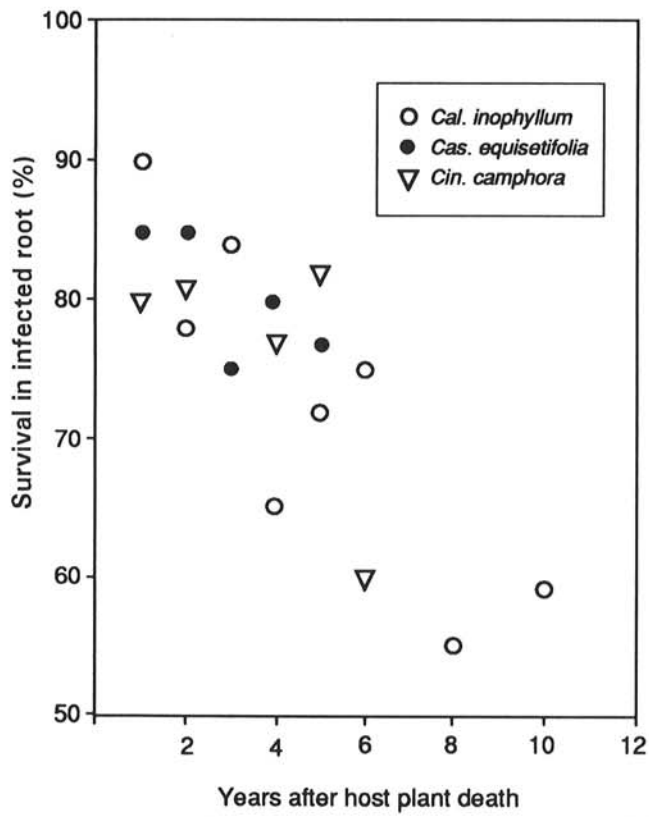

Fig. 5. Survival of *Phellinus noxius* in naturally infected roots in the field after the death of the host plant. Each point represents survival of *P. noxius* in one dead tree. Survival in infected root was calculated as the percentage of root fragments from which *P. noxius* emerged after 2 weeks of incubation.

Viability of basidiospores, arthroconidia, and mycelia declined quickly in the soils, compared with infested woody debris. These propagules were never recovered 5 months after being added to the soils. Although it was possible that low populations of basidiospores and arthroconidia in soils after 5 months were not recoverable by the methods used in the study, these propagules were apparently not good structures for long-term survival. Basidiospores and arthroconidia, which are airborne spores, played an important role in the long distance dissemination of *P. noxius*. Infection of trees, presumably by airborne basidiospores, has been reported on cacao (15,19) and rubber trees (4,15).

Submerging wood in water has been used to prevent postharvest colonization by wood-decaying fungi (16). In this study, when *P. noxius*-colonized wood sections were submerged in water, the fungus died within 1 month. It might have been because of the activities of anaerobic microorganisms, but further study is required to determine its mechanism. This result was promising and may lead to more effective field control practices. Flooding infested fields for more than 1 month might eliminate the inoculum source in infected wood and roots. However, field studies are required to test this hypothesis. During a disease survey, *P. noxius* occurred more frequently in fields with sandy soils, probably because sandy soils drain better and are less likely to become submerged for any significant length of time, even during high rainfall. On the other hand, clay soils drain poorly; therefore, these soils are more likely to be submerged, a situation that is detrimental to the survival of *P. noxius*.

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