Effect of Soil Moisture Content on the Survival of *Ganoderma* Species and Other Wood-Inhabiting Fungi

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ABSTRACT

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The survival of mycelia of *Ganoderma australe*, *G. boninense*, *G. lucidum*, and *G. weberianum* in colonized wood was measured in soils with different soil matrix potentials. Survival of mycelia of *G. australe* and *G. boninense*, which do not produce chlamydospores, buried in plots subjected to different soil moisture treatments, declined rapidly, and the fungi could not be recovered after 9 to 12 weeks. Survival of mycelia of *G. lucidum* and *G. weberianum*, which produce chlamydospores, rapidly declined from 0 to 15 weeks of incubation but consistently ranged from 35 to 50% after 15 weeks of incubation. In regression analyses for each of the four *Ganoderma* species, there was no difference in the rate of change of mycelial survival over time among different soil moisture treatments. However, when data from only the -0.20 MPa treatment were used, the rates of change of mycelia survival over time of *G. australe* and *G. boninense* significantly differed from those of *G. lucidum* and *G. weberianum*. *G. australe* and *G. boninense* were not recovered from pieces of infested wood subjected to 3 and 1 months of flooding, respectively. In treatments with lower soil moisture, the survival of these two fungi ranged from 80 to 90% over 2 years. In all soil moisture treatments, survival of *G. lucidum* and *G. weberianum* ranged from 80% to more than 90% over 2 years. Similarly, seven species of other wood-inhabiting fungi that do not produce chlamydospores were not recovered from pieces of infested wood subjected to 1 or 5 months of flooding, but chlamydospore-producing species were recovered. These results indicate that, regardless of chlamydospore formation, woody debris in soils harboring wood-decay fungi may be important for long-term survival, and chlamydospores of *Ganoderma* in woody debris enhance the resistance of the fungi to environmental stresses such as flooding. Flooding infested fields may help control those wood-inhabiting fungi such as *G. australe* and *G. boninense* that do not produce chlamydospores.

Additional keywords: disease management

Ganoderma (Basidiomycota: Ganodermatales) is a genus of wood-inhabiting fungi on monocots, dicots, and gymnosperms. Members of the genus are found around the world from tropical to temperate habitats (12). Some species are saprophytic, but several are pathogens that cause decay in roots, butts, and trunks of living trees. *G. australe* (Fr.:Fr.) Pat., *G. boninense* Pat., *G. lucidum* (W. Curtis:Fr.) P. Karst., and *G. weberianum* (Bres. & Henn.) Steyaert are common root pathogens in Taiwan that cause decay and slow decline of numerous orchard and forest tree species (8). Slowly expanding, circular disease patches extending from infection centers can be observed in the field, which indicate that the disease caused by *Ganoderma* species is spread from diseased to healthy trees by root contact (1,2). Infested

root debris may be important as inoculum and for long-term survival. Airborne basidiospores can also initiate new infections on freshly cut stumps or logging debris, with subsequent spread to live trees by root contact (9,11,14).

Abundant chlamydospores are produced in pure culture and in woody debris by some species of *Ganoderma*, such as *G. lucidum* and *G. weberianum*, while others such as *G. australe* and *G. boninense* produce no chlamydospores (6). The role of chlamydospores in the epidemiology of *Ganoderma* species is unknown, and the long-term survival of chlamydospores and mycelia in soil has not been previously documented. In this study, the survival of chlamydospores and mycelia of *Ganoderma* species in artificially infested woody debris was assessed in soils with varying moisture levels. In addition, the survival of some other wood-inhabiting fungi in artificially infested woody debris was studied.

MATERIALS AND METHODS

Fungal isolates, soils, and selective media. All isolates were from fruiting bodies and were identified by the author

(7). For observation of chlamydospores and arthroconidia in culture, the fungi were grown on malt-extract agar (MEA: 2% malt extract, 2% agar) at 25°C for 4 weeks before microscopic examination.

The silty-loam soil used for this study was collected from below the organic horizon of natural forest soil near Taipei, Taiwan. The unsterilized soil was sifted with a mesh (no. 50) and stored at room temperature before use. Initially, it consisted of 36.7% sand, 52.6% silt, and 10.7% clay, with a moisture content of 10% (-0.42 MPa). The concentration of organic matter was not determined. For treatments requiring less than 10% soil moisture, the soil was dried at 40°C until the desired moisture content was reached. For treatments requiring more than 10% soil moisture, distilled water was added to the soil until the desired moisture content was reached. The soil matrix potential was adjusted gravimetrically by comparison with a soil moisture retention curve (5). During the experimental period, the temperature in the greenhouse ranged between 15 and 33°C.

In a preliminary test, a selective medium (4) developed for *Phellinus noxius* (Corner) G.H. Cunningham was determined to be suitable for isolation of the fungi used in this study. The selective medium 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. The antibiotics and fungicides were added after autoclaving.

Survival of *Ganoderma* mycelia in soil.

Soil matrix potentials were adjusted to -0.50 , -0.20 , and -0.025 MPa, and soils were placed in separate plastic containers (15 cm high $\times$ 15 cm diameter). Wetted and autoclaved pieces of cellophane (2 $\times$ 2 cm) were placed on MEA plates and inoculated with *G. lucidum*, *G. boninense*, *G. weberianum*, or *G. australe* cultures for 3 weeks at 25°C. In addition to mycelia, chlamydospores were present on cellophane colonized by *G. weberianum* or *G. lucidum*. Well-colonized cellophane was buried 5 cm below the soil surface of each plastic container, which was then covered with aluminum foil and sealed with tape to maintain constant soil moisture. The colonized pieces of cellophane were carefully removed from the soil after selected incubation periods (3, 6, 9, 12, 15, 18, 26, or 52 weeks), placed on the selective medium, and incubated at 25°C. Three replicate containers were used for each soil moisture

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level. Thirty pieces of cellophane were used for each replicate. Survival of mycelia was calculated as the percentage of colonized pieces of cellophane from which the tested fungi emerged after 2 weeks of incubation. This experiment was performed twice.

Survival in infested wood buried in soil. Wood sections (about 3 to 5 cm in diameter and 10 cm in length) of *Cinnamomum camphora* (L.) Nees et Eberm. were inoculated with *G. lucidum*, *G. boninense*, *G. weberianum*, and *G. australe*. The wood sections were completely colonized after 2 months at 25°C, when the experiment began. The soil matrix potential was adjusted to -0.50, -0.30, or -0.10 MPa, or the soil was flooded with water. Soil of each treatment was placed into a separate plastic container (100 cm high × 40 cm diameter) to a height of 80 cm, and wood sections were buried 15 cm below the soil surface. The caps of the containers were sealed with tape to maintain constant soil moisture. Three replicate containers for each soil moisture treatment were used. Three wood sections were used for isolation of each replicate. When the wood sections were used to test for fungal survival, they were washed with tap water and blotted dry, and 50 wood fragments (about 3 × 6 mm) were cut from each wood section and placed on selective medium at 25°C. Survival in infested wood was calculated as the percentage of wood fragments from which the tested fungi emerged after 2 weeks of incubation. This experiment was conducted twice.

The methods used for testing the survival of the other 16 wood-inhabiting polypore fungi (Table 1) were the same as those for *Ganoderma* species, except that the soil matrix potential was adjusted to -0.50 MPa or the soil was flooded with water.

Statistical analyses. Survival (%) of mycelia and infested wood was arcsine transformed (survival/100)^{1/2}. The transformed data were analyzed by regression

analysis with the PROC GLM procedure of PC-SAS (SAS Institute, Cary, NC) to compare the slope from the empirical regression of fungal survival against time (3). Data were combined from two experimental trials with three replications.

RESULTS

Survival of *Ganoderma* mycelia in soil.

The survival of mycelia buried in soil at -0.025 MPa declined most rapidly for *G. australe* and *G. boninense*, and these fungi could not be recovered at 9 or 12 weeks, respectively (Fig. 1A and B). Their mycelia buried in soil at -0.50 MPa had higher recovery rates than those in the -0.20 and -0.025 MPa soil moisture treatments. Regardless of soil moisture levels, *G. australe* and *G. boninense* were not recovered after 15 weeks. No difference in the rate of change of mycelial survival over time ($P = 0.25$ for *G. australe* and $P = 0.31$ for *G. boninense*) was observed among the different soil matrix potential treatments.

For *G. lucidum* and *G. weberianum*, survival of mycelia in all soil moisture treatments declined from 0 to 15 weeks of incubation (Fig. 1C and D). However, survival of mycelia ranged from 35 to 50% after 18 weeks of incubation. No difference in the rate of change of mycelial survival over time was seen for *G. lucidum* or *G. weberianum* ($P = 0.64$ for *G. lucidum* and $P = 0.58$ for *G. weberianum*) among the different soil matrix potential treatments.

Since there was no difference in the rate of change of mycelial survival over time for each of the four *Ganoderma* species among the different soil matrix potentials, survival of the four *Ganoderma* species was compared only at the -0.20 MPa soil matrix potential. The rates of change of mycelial survival over time of *G. australe* and *G. boninense*, which produced no chlamydospores, significantly differed ($P < 0.05$) from those of *G. lucidum* and *G. weberianum*, which produced chlamydospores.

Survival of *Ganoderma* in infested

wood. *G. australe* and *G. boninense* were not recovered from pieces of infested wood subjected to 3 and 1 months of flooding, respectively. However, in treatments with lower soil moistures, *G. australe* and *G. boninense* survival ranged from 80% to more than 90% over 2 years of study (Fig. 2A and B). Slopes for these survival rates did not significantly differ ($P > 0.05$) among treatments when the flooding treatment was excluded.

For *G. lucidum* or *G. weberianum*, slopes for survival in infested wood with time did not significantly differ among treatments including flooding. In all soil moisture treatments, *G. lucidum* and *G. weberianum* survival ranged from 80% to more than 90% over the 2 years of study (Fig. 2C and D).

Survival of other fungi in infested

wood in soil. Survival of 16 wood-inhabiting fungi in soils with a -0.50 MPa soil matrix potential ranged from 70% to 100% over the 2 years (Table 1). For each species, slopes for survival of these fungi with time did not differ significantly ($P < 0.05$) from a slope of zero. However, when these fungi in infested wood were placed in flooded soils, four of them (*Bjerkandera adusta* (Willd. ex Fr.) P. Karst., *Polyporus grammacephalus* Berk., *Phellinus longisetulosus* Bond. et Herr., and *Trametes versicolor* (L.:Fr.) Pilat) were not recovered after 1 month of treatment, while three of them (*Abortiporus biennis* (Bull. ex Fr.) Sing., *Schizophora flavipora* (Cke.) Ryvarden, and *Trametes hirsuta* (L.:Fr.) Pilat) were not recovered after 5 months (Table 1). These seven species did not produce chlamydospores in culture. Regardless of soil moisture levels, the survival of the nine species that produced chlamydospores ranged from 70 to 100% after 2 years (Table 1). For each of these nine species, slopes for survival with time were not significantly different from a slope of zero ($P > 0.05$).

Table 1. Survival (%) of 16 wood-inhabiting fungi in artificially infested wood sections buried in soils with flooding or a -0.50 MPa soil matrix potential after four incubation periods (months)

Fungus and culture no.	Chlamydospores	Arthroconidia	Flooding				-0.50 MPa			
			1	5	12	24	1	5	12	24
<i>Abortiporus biennis</i> TFRI 274	- ^a	+	20	0	0	0	90	85	80	85
<i>Bjerkandera adusta</i> TFRI 180	-	+	0	0	0	0	80	85	85	75
<i>Coriolus brevis</i> TFRI 552	+	-	90	85	80	85	95	85	90	80
<i>Daedalea dickinsii</i> TFRI 305	+	-	85	90	85	90	85	85	90	75
<i>Fomitopsis cajanderi</i> TFRI 102	+	-	100	85	75	80	100	90	90	85
<i>Inonotus rickii</i> TFRI 712	+	-	85	90	70	85	90	80	85	75
<i>Perenniporia formosana</i> TFRI 375	+	-	100	100	85	90	85	90	80	80
<i>Perenniporia latissima</i> TFRI 281	+	-	100	95	100	85	100	85	80	80
<i>Phaeolus schweinitzii</i> TFRI 434	+	-	100	85	95	90	95	100	80	75
<i>Polyporus arcularius</i> TFRI 597	+	-	95	85	85	80	95	85	70	85
<i>Polyporus grammacephalus</i> TFRI 326	-	+	0	0	0	0	85	100	80	80
<i>Rigidoporus ulmarius</i> TFRI 763	+	-	80	80	75	70	80	75	70	70
<i>Phellinus longisetulosus</i> TFRI 201	+	+	0	0	0	0	90	95	80	70
<i>Schizophora flavipora</i> TFRI 401	-	-	25	0	0	0	85	80	75	90
<i>Trametes hirsuta</i> TFRI 211	-	-	20	0	0	0	95	85	90	70
<i>Trametes versicolor</i> TFRI 31	-	-	0	0	0	0	95	90	70	80

^a Presence (+) or absence (-) in culture.

DISCUSSION

Woody debris infested with *G. australe*, *G. boninense*, *G. lucidum*, and *G. weberianum* survived significantly longer than mycelium when buried in soil. Mycelia of *G. lucidum* and *G. weberianum*, which produced chlamydospores, survived over 52 weeks of incubation in soil, but mycelia of *G. australe* and *G. boninense*, which do not produce chlamydospores, survived only up to 12 weeks of incubation. In addition, *G. lucidum* and *G. weberianum* in woody material survived in flooded soil, but *G. australe* and *G. boninense* did not. Chlamydospores appear to be important to survival, and woody material infested with *Ganoderma* species, regardless of chlamydospore production, appears to be more important than mycelia per se for long-term survival of these fungi in soil.

Survival of 16 other wood-inhabiting fungi in soil was similar to that of the four *Ganoderma* species. Chlamydospores of these wood-inhabiting fungi may be important for long-term survival, and these structures may provide resistance to severe

environmental stresses such as soil flooding. These results are consistent with those reported for woody debris infested with *Phellinus noxius* (5). Arthroconidia are produced by some basidiomycetous wood-inhabiting fungi (13), but arthroconidia of *P. noxius* do not appear to be important survival structures (5). In our study, the four species of wood-inhabiting fungi that produced arthroconidia but not chlamydospores did not survive in flooded soil.

Submersion of wood in water has been used to prevent postharvest colonization by wood-decay fungi (10). The benefit might be due to the activities of anaerobic microorganisms. In this study, when wood sections colonized by test fungi that produced chlamydospores were submerged in water, the fungi survived for over 2 years. However, when wood sections colonized by test fungi that did not produce chlamydospores were submerged in water, the fungi died within 3 months. When wood sections colonized by *P. noxius*, which does not contain chlamydospores, were submerged in water, the fungus died within 1 month

(5). Thus, for disease management of root and butt rot caused by wood-inhabiting fungi that do not produce chlamydospores, flooding infested fields might eliminate the inoculum source in infested wood. However, the pieces of infested wood that were tested in this study were relatively small, and field studies are required to test this hypothesis.

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Fig. 1. Survival of mycelia of four *Ganoderma* species buried in soil with various soil moisture treatments for different incubation times. Each value is the mean of three replicates averaged over two experimental trials. Survival of mycelia was calculated as the percentage of colonized cellophane pieces from which the fungi grew after 2 weeks of incubation. A, *G. australe*, B, *G. boninense*, C, *G. lucidum*, D, *G. weberianum*.

Fig. 2. Survival of four *Ganoderma* species in artificially infested wood sections buried in soils with various soil moisture treatments for different incubation times. Each value is the mean of three replicates averaged over two experimental trials. Survival in infested wood was calculated as the percentage of wood fragments from which the fungi grew after 2 weeks of incubation. A, *G. australe*, B, *G. boninense*, C, *G. lucidum*, D, *G. weberianum*.

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