Potential Use of Native Fungi for Value-Added Spalting in Chile

Felipe Galleguillos Vicente A. Hernández Victor Hernández
Goetz Palfner Fabian Figueroa Sara C. Robinson

Abstract

Chile is host to several types of temperate forests, many of which are plantations of moderate- to low-value woods. In an effort to explore potential methods of adding value to radiata pine and southern beech, these woods were inoculated with native Chilean fungi to determine if spalting could be induced under a reasonable time frame. Results showed that pine spalted more readily than beech, both internally and externally. *Ophiostoma* sp. and *Phialocephala* sp. performed the best in terms of spalting. In addition, a new red-staining fungus was discovered: *Eurotium* sp. Both wood species did show some level of spalting, and all the captured fungi produced some visual effects. It is concluded that two of the major plantation trees of Chile, radiata pine and southern beech, are suitable for controlled spalting and that native Chilean fungi can be used for this process. These results open a new method for increasing the value of Chilean plantation timber and can be done entirely on a local scale, without reliance on materials or organisms from North America.

Many of the forests in Chile are temperate, with a similar biodiversity to North American Pacific Northwest forests. Past logging practices in Chile have altered the dominant forest types to plantations, primarily of radiata pine (*Pinus radiata* D. Don), eucalyptus (*Eucalyptus* spp.), and southern beech (*Northofagus* spp.). Much of the lumber from these forests is not used for decorative work, instead being chipped for pulp or exported for a variety of low-cost purposes.

A new method for adding value to otherwise low-value timber species in North America is inducing spalting, a process that adds color to wood and thereby also additional value through fungal inoculation (Robinson et al. 2013a). Recent patents have even been filed to protect some of the developed processes, including inoculating the wood with mycelium of spalting species in a controlled chamber to produce spalted veneers (Beakler 2007, 2012, 2013). Spalted wood has a long history of use in decorative arts and crafts, an example of which is the green-stained wood of *Chlorociboria* species in designs of intarsia during the Renaissance in Italy in the 15th century (Blanchette et al. 1992).

Although spalted wood has been utilized for centuries, it is only recently that the term has been defined and the process understood and induced under laboratory conditions. The term *spalting* was defined by Robinson et al. (2007) and includes all types of wood pigmentation produced internally in wood by fungi. Primarily, fungal melanins (Langfelder et al. 2003, Eisenman and Casadevall 2012) and xylindein from *Chlorociboria* species (Robinson and Laks 2010b) are the most well known for their production of black zone lines or green stain, respectively. Superficial pigments produced by molds such as *Penicillium* are not included in this definition, although in specific cases they may be considered (Robinson and Laks 2010a). Interestingly, although the basic mechanisms of spalting have been understood for many years in terms of wood decay, the research to control and improve the amount of produced pigment has been carried out only in recent years, and it has been undertaken by only a small group of researchers (Robinson 2012).

The pigmentation mechanisms of spalting have been usually divided into three categories: (1) changes produced on wood by structural modification of lignin caused by white rot fungi (bleaching), (2) the production of zone lines owing to inter- or intrafungal antagonism or changes in environmental conditions, and (3) the production of colored pigments capable of penetrating the wood (Robinson 2012).

The authors are, respectively, Research Assistant, Biotechnol. Center (fgalleguillos@udec.cl [corresponding author]), Research Associate, Biotechnol. Center (vhernandezc@udec.cl), Assistant Professor, Assistant Professor, and Graduate Student, Dept. of Botany (vhernand@udec.cl, gpalfner@udec.cl, ffigueroa@udec.cl), Univ. de Concepcion, Concepcion, Chile; and Assistant Professor, Dept. of Wood Sci. and Engineering, Oregon State Univ., Corvallis (sara.robinson@oregonstate.edu). This paper was received for publication in September 2014. Article no. 14-00093.

Forest Prod. J. 66(3/4):250–256.
doi:10.13073/FPJ-D-14-00093

The fungi utilized in spalting studies can be classified according to the mechanisms used to alter the wood. Pigmentation occurs in two ways: (1) when pigments attached to the cell wall of the fungus accumulate in high enough amounts to cause a visible color change and (2) when pigments are secreted extracellularly and move into the wood (Bell and Wheeler 1986). The Ascomycete fungi in the *Chlorociboria* genus have been heavily utilized for producing a blue-green color in wood. These fungi secrete xylindein, which produces a penetrating green pigment (Blackburn et al. 1965, Edwards and Kale 1965, Giles et al. 1990, Saikawa et al. 2000). Other fungi used are Ascomycetes of the genus *Ophiostoma,* which have the capacity to synthesize melanin inside the hyphae and grow through the sapwood, producing colors that range from black to blue (Seifert 1993), and the fungus *Scytalidium cuboideum* (Sacc. & Ellis) Sigler & Kang (formerly known as *Arthrographis cuboidea*), which produces colors between rose and red and occasionally blue (Golinski et al. 1995).

The induction of antagonism lines (zone lines) has been achieved with monocultures by means of the species *Xylaria polymorpha* (Pers.) Grev. (Ascomycete) and *Trametes versicolor* (L.) Lloyd (Basidiomycete) and with the antagonistic reactions between *T. versicolor* and *Bjerkandera adusta* (Willd.) P. Karst on sugar maple (*Acer saccharum* Marsh; Robinson et al. 2007). No structural differences have been found between the zone lines produced naturally and those produced under controlled, laboratory conditions (Qin et al. 2011). Among the group of wood-decaying fungi that cause bleaching, *T. versicolor* and *B. adusta* (Robinson et al. 2007, 2009b; Robinson 2012) have been utilized on different types of substrates, achieving unique designs and colorations.

The wood species thus far investigated for induced spalting have been mainly high-quality woods, such as sugar maple (*A. saccharum* Marsh), basswood (*Tilia americana* L.), yellow birch (*Betula alleghaniensis* Britt.), and American elm (*Ulmus americana* L.; Robinson et al. 2007, 2009a, 2011a; Tudor et al. 2012). Minimal research has been conducted on wood of medium and low economic value, such as tree of heaven (*Ailanthus altissima* (Mill) Swingle), Norway maple (*Acer platanoides* L.), silver maple (*Acer saccharinum* L.), trembling aspen (*Populus tremuloides* Michx.), and horse chestnut (*Aesculus hippocastanum* L.), aimed at generating added value through spalting (Robinson et al. 2011b). Little research has been conducted on conifers, with the sole study done by Robinson et al. (2013b), resulting in additional coloration on already blue-stained wood. Because spalting is a process in an initial stage of development and because of the noticeable variation related to strains and substrates, active researchers in this field have recommended continued experimentation on other wood species.

With the noted low diversity of tree species commonly found in Chilean plantation forests and the relatively low value of the wood harvested from such forests, it is of interest to determine (1) if these types of forests are host to fungi capable of spalting and (2) if radiata pine and southern beech are well suited to laboratory-induced spalting using native Chilean fungi, considering the huge variability in fungal preference for wood species in terms of spalting. The results of this research will help determine if Chile hosts compatible wood and fungal species that can be studied and eventually utilized for commercial-scale spalting. Discovery and cultivation of successful fungi on Chile's low-value wood species could have a large economic impact on the forestry sector in this country in terms of both native uses and exports.

Methods

Fungi collection

Fungi for this study were collected in two ways: isolated from the natural environment and from already established culture collections of native fungi. For natural isolations, fungi were cultured from Lucanidae and Tenebrionidae beetles (sample size of 8 from the former and 10 from the latter). The beetles were found on *Nothofagus macrocarpa* (A.DC.) F. M Vázquez & R. A. Rodr. and *Nothofagus dombeyii* (Mirb. Oerst.) from trees in the Maule Region, Altos de Lircay National Reserve, Talca, Chile. Collected beetles were maintained in 1.5-mL Eppendorf tubes and processed the same day of collection.

To culture the fungi, the beetles were kept at room temperature (21°C) and rinsed with sterile water for 30 seconds using forceps to remove large particulates. They were then rolled across 2 percent malt extract agar with sterile forceps. The medium was supplemented with 200 mg of streptomycin in 1 liter of distilled water to control bacterial growth. The growth of the fungi was carried out by incubation on plates for 7 days in a dark drawer at 21°C.

Monocultures were achieved through standard isolation procedures where continual reculturing occurred until only one species remained. The resulting pure strains were genetically sequenced. Isolates were selected for use in continuing experimentation based on their capacity to produce intra- and extracellular colored extracts on the media during the incubation time of 3 weeks.

Identification of isolated fungi

The extraction of the DNA was carried out from the mycelium using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). The amplification of the region corresponding to the internal transcribed spacer (ITS) was carried out using the primers ITS1 to ITS4 described by White et al. (1990). The amplification of the polymerase chain reaction (PCR) was followed by the following thermocycling pattern: 94°C for 5 minutes (1 cycle), 94°C for 40 seconds, 60°C for 40 seconds, 72°C for 1 minute (30 cycles), and 72°C for 10 minutes (1 cycle).

The PCR products were sent directly for sequencing to Macrogen (Seoul, Korea), where the identity and coverage were evaluated with the sequences on the database GenBank using the algorithm BLAST (Altschul et al. 1997).

The isolated species sequenced and selected for the inoculation assays were *T. versicolor*, *B. adusta*, *Phialocephala* sp., *Eurotium* sp., and *Ophiostoma* sp. A second *Ophiostoma* isolate (hereafter referred to as O2) was cultured from a colection at the Laboratory of Natural Products Chemistry (Table 1). All fungi were kept on 2 percent malt extract agar and stored at 4°C before being inoculated onto the wood. In order to keep the initial characteristic of fresh fungi, the cultures were transferred to a new plate every 3 months. *Ophiostoma* was selected from a culture collection owing to its known blue-staining effects on wood. It was hoped this species would serve as a sort of ''control'' for the other species tested in that if the

Table 1.—Isolation source, host tree species, and similarity (BLAST) of the fungi used in the study.

Source of isolation (type of beetle)	Host tree species	Type of fungus	% similarity
Tenebrionidae	*Nothofagus dombeyii*	*Trametes versicolor*	100
Tenebrionidae	*Nothofagus obliqua*	*Bjerkandera adusta*	99
Lucanidae	*N. obliqua*	*Phialocephala* sp.	99
Tenebrionidae	*N. obliqua*	*Eurotium* sp.	98
Tenebrionidae	*N. dombeyii*	*Ophiostoma* sp.	92
Culture collection[a]	—	*Ophiostoma* sp.	—

[a] Strain provided by the Laboratory of Natural Products Chemistry, University of Concepción.

Ophiostoma did not produce blue stain, it would be apparent that there was an issue with testing conditions, not the fungi.

Wood

Twelve clear, unstained sapwood blocks of *P. radiata* were cut into smaller blocks measuring 4 by 1.5 by 1 cm and three blocks were cut to 10 by 4.5 by 4 cm; six *Nothofagus obliqua* blocks were cut down to 14.5 by 4.5 by 4 cm. *Nothofagus* tests were limited due to the wood's dark color and the improbability that any developed staining would be visible. Instead, a mixed culture was applied on *Nothofagus* in an attempt to produce enough color for a significant color change. Blocks were sterilized at 121°C for 20 minutes and conditioned for 7 hours at 40°C until a consistent humidity in the chamber was reached (Kottermann model 2716). The blocks were weighed before and after conditioning and placed in sterile plastic boxes with sterile vermiculite (40 g with 60 mL of water), according to the modified protocol by Robinson et al. (2009b).

Inoculation and incubation

The selected fungi were inoculated in both monoculture and coculture (mixed cultures) as described above (Table 2). Strips of agar with actively growing mycelia (1.5 by 1.5 cm) were placed on the transverse face, and mixed cultures were placed on one transverse face with one or two fungi and on the opposite end with a different fungus on *P. radiata* and *N. obliqua* wood. Specimens were incubated under laboratory conditions—25°C ± 2°C and an ambient relative humidity of 80 ± 3 percent—in the dark for a period of 8 and 12 weeks on *P. radiata* and *N. obliqua* wood, respectively, according to a modification of the protocol by Robinson et al. (2011b). Experiments were performed in triplicate and with wood without fungus as a negative control.

Table 2.—Inoculation (monoculture and mixed culture) and incubation time on wood.[a]

Wood	Fungus	Incubation time (wk)
Pinus radiata	P.s.	8
	E.s.	8
	O.p.	8
	T.v./B.a.	8
	O.p.	8
Nothofagus obliqua	P.s./O.p./Es.	12
	P.s./O.p./T.v.	12

[a] P.s. = *Phialocephala* sp.; E.s. = *Eurotium* sp.; O.p. = *Ophiostoma* sp.; T.v. = *Trametes versicolor*; B.a. = *Bjerkandera adusta*.

Data analysis

After incubation, blocks were removed from the plates and cleaned with a sponge in order to remove the mycelia and vermiculite debris from the surface. They were then dried at 40°C overnight in an incubation chamber (Kottermann model 2716) and weighed in order to determine weight loss. The stain was measured on both the external and the internal faces of the wood to visually measure the change of color against the respective negative controls with photos using the program ImageJ 2.0 (Robinson et al. 2011b). The internal faces were evaluated by cutting each block in half and then scanning and color reading one internal face. Results were expressed as mean percentage of color change in relation to the negative control. Data were analyzed using a 2-way analysis of variance (ANOVA) with fungus and wood species as the independent variables and external and internal coloration as dependent variables. The Tukey highly significant difference test was run to determine the location of the significant difference. The statistical procedures were perfomed using the Statistica version 7.0. Before analysis, data were verified to a normal distribution.

Results

There were 31 fungal strains in total, isolated from the beetle species. Table 1 shows the fungi used to spalt as well as their isolation source and similarity. In general, spalting was more prevalent on pine than on beech.

External spalting

External spalting was highest on pine, with the exception of the combination of *T. versicolor* and *B. adusta*, which did not differ significantly in amount from the *Eurotium* pairing on beech (40% and 51%, respectively). *Ophiostoma* on pine produced significantly more external spalting than any of the aforementioned fungi or fungal pairings ($P < 0.0001$), but its amount was not significantly different from *Phialocephala* (80.3%), the O2 isolate (70.3%), or *Eurotium* (70%). Visually, bleaching occurred from *T. versicolor* and *B. adusta* on the outer surface of the pine. Zone lines were present only in the mixed cultures on beech (Fig. 1a). *Eurotium* produced a red stain both externally and internally in the wood (Figs. 2a and 2b), and *Phialocephala* produced a heavy black stain externally and internally.

Internal spalting

Results of the 2-way ANOVA found that *Ophiostoma* (71%) and *Phialocephala* (71%) produced significantly more color ($P < 0.0001$) on pine than any other fungus. *Eurotium* (49.3%) produced the next significant amount on pine, followed by the O2 isolate (30.3%). The combinatory

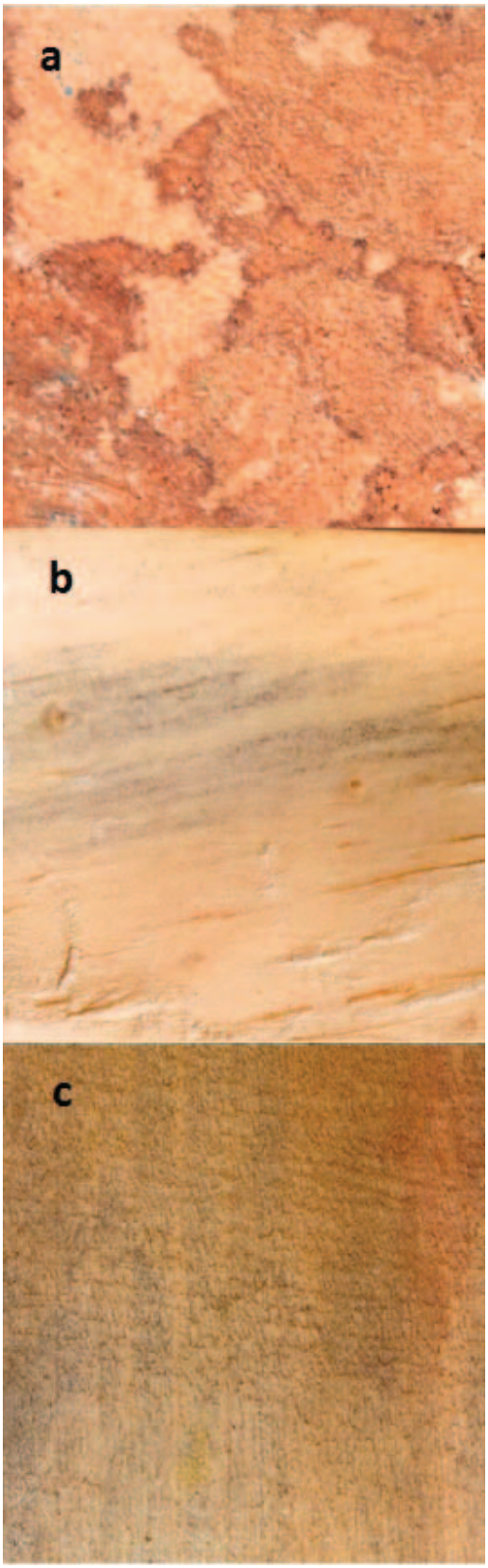

Figure 1.—Coloration produced on Pinus radiata *and* Nothofagus obliqua*: (a) zone lines caused by the mix of fungi* Trametes versicolor, Ophiostoma *sp., and* Phialocephala *sp. on* N. obliqua*; (b) blue stain caused by* Ophiostoma *sp. on* P. radiata*; and (c) coloration produced on* N. obliqua *with the mix of strains* Ophiostoma *sp.,* Eurotium *sp., and* Phialocephala *sp. (Color version is available online.)*

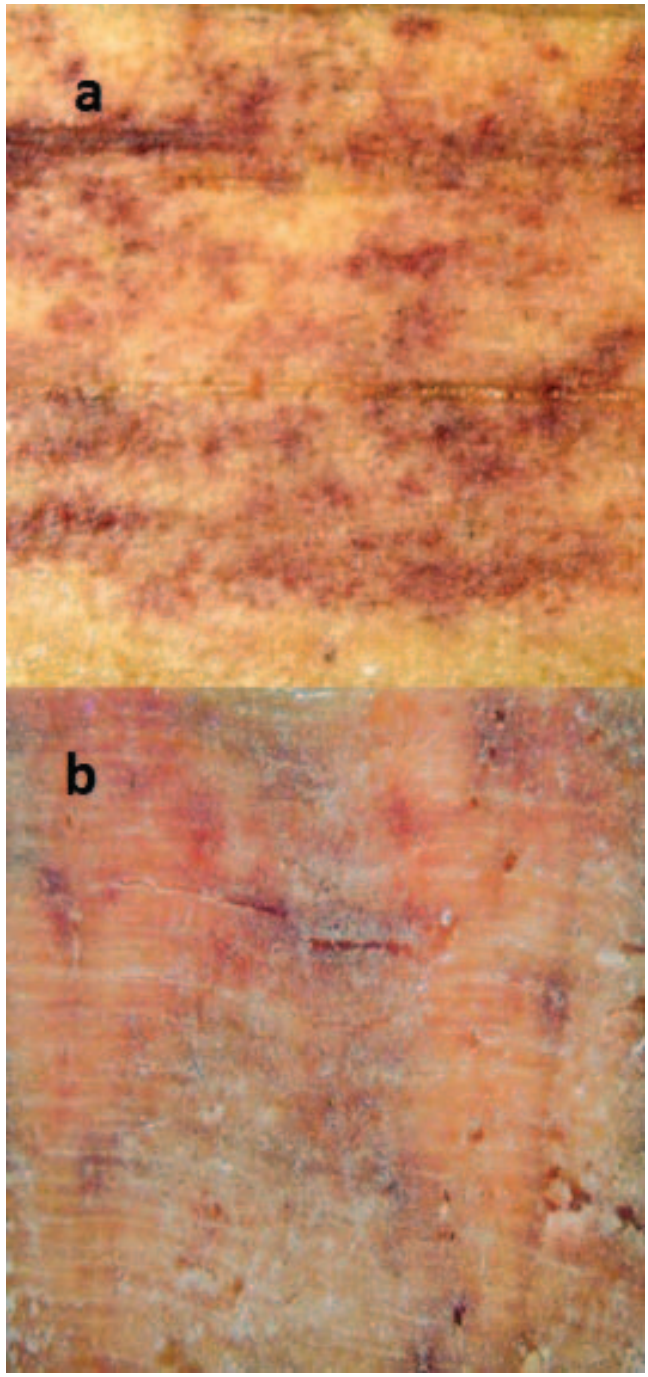

Figure 2.—Coloration produced on Pinus radiata*: external (a) and internal (b) coloration achieved by* Eurotium *sp. (Color version is available online.)*

pairings on beech produced the least amount of spalting (20.7% for each), while the combination of *T. versicolor* and *B. adusta* did not produce any internal spalting.

Discussion and Conclusions

The threshold established for what constitutes ''successful'' spalting varies depending on spalting type, with higher percentages generally desired for pigments and lower percentages being allowed for zone lines (Robinson et al. 2014). All fungi that were tested on wood blocks achieved external spalting well above 30 percent, and only the

combination of *T. versicolor* and *B. adusta* failed to produce any internal spalting on radiata pine. It is interesting that this pairing failed to produce internal spalting, as it is one of the pairings identified as ideal in other articles (Robinson et al. 2007, Robinson 2012). The likely cause of this discrepancy is wood species: both *T. versicolor* and *B. adusta* are white rot fungi, common to hardwoods, and previous spalting work has looked at these two fungal species only on hardwoods.

In terms of native fungi that were isolated from the beetles, *T. versicolor* and *B. adusta* are commonly used spalting fungi (Robinson et al. 2007, 2009b; Qin et al. 2011) and strains of *Ophiostoma* sp. are known to cause blue stain (Popa et al. 2012). Several new species of spalting fungi were also found. (1) The ascomycete *Phialocephala* spp. has been described as a dark septate endophyte and wood-decaying fungus (Saikkonen 2007, Tian et al. 2010) with an extensive biodiversity and abundance in woody plants (Saikkonen 2007). It is reported to have a beneficial interaction with *Nothofagus* (Salgado et al. 2013) with occasional pathogenic effects on its plant hosts (Tellenbach et al. 2011). (2) The ascomycete *Eurotium* spp. is found in grain products, fruits, hypersaline waters, and forest soil (Butinar et al. 2005, Fraga et al. 2010). Some species are known to produce bioactive compounds but are not directly pathogenic (Wu et al. 2014).

Eurotium spp. are able to produce pigments related to anthraquinones, which are synthesized mainly in the mycelium (Anke et al. 1980, Hamasaki et al. 1980, Smetanina et al. 2007, Wu et al. 2014). The isolate used in this study produced a red pigment similar to the red/pink pigments produced by *Scytalidilium cuboideum*, a fungal species heavily used in spalting (Robinson et al. 2011b). Both of these pigments are able to stain deeply and spread inside the sapwood (Fig. 2b), with *Eurotium* sp. doing so quite well on *P. radiata*. This fungus could likely be used in a manner similar to *S. cuboideum* as an ''overstain'' for blue-stained pine (Robinson et al. 2013b) to turn the blue color into a purple and red combination, thereby increasing its marketability (Robinson et al. 2013b).

In terms of blue stain, the *Ophiostoma* isolated created a deep blue color on *P. radiata* (Fig. 1). The ease with which the fungus was able to pigment the wood might be related to the natural adaptation of *Ophiostoma* fungi to colonize *Pinus,* but also to anatomical features, such as homogeneity of parenchymatic tissues, where the presence of tracheids and intercellular spaces favor a greater growth of the mycelial hyphae (Lee et al. 2002, Hudgins et al. 2005) and appearance of deep stain. Another fungus that was isolated, *Phialocephala* sp., is able of form melanin in its structures (Grunig et al. 2011). In our tests, the melanin was able to enter inside the sapwood, producing an intense black coloration in both woods (Figs. 1b and 1c).

For the wood species *N. obliqua*, less overall spalting was produced by the fungi. In general, the pigments that worked the best on this wood came from the black/blue colors, characteristic of the fungi *Ophiostoma* sp. and *Phialocephala* sp. In the first triple inoculation test, the red pigment of *Eurotium* did not get produced. This selective staining may be owing to the development of a system of secondary metabolites (Suryanarayanan 2013) because endophytes produce a variety of antifungal compounds, including phenolics, terpenoids, and alkaloids (Kumar and Kaushik 2012). It is known that some strains of *Ophiostoma* have not been completely inhibited by highly aggressive antagonists (Zulpa et al. 2003, Díaz et al. 2013), which is likely why our *Ophiostoma* was able to grow in the presence of the other fungi (Fig. 3).

In the second mixed culture on *N. obliqua,* an inhibition of the growth of *T. versicolor* was achieved. A study on beech (*Fagus sylvatica* L.) by Dowson et al. (1988) utilized an inoculated pairing with basidiomycete decomposers and showed a complete replacement, with only one living strain at the end of the test. In our mixed culture, antagonism between interfungal mycelia could have some correlation with the form and specialization of mycelial outgrowth for resource capture and space (Rayner and Webber 1983, Boddy 2000). No weight loss in the wood was observed. Unfortunately, bleaching and zone lines were detected only in the initial inoculation zone.

In the present study, spalting potential of native Chilean fungi on plantation wood was confirmed under controlled conditions in both monocultures and mixed cultures inoculated onto *P. radiata* and *N. obliqua* wood. These new combinations of fungi on previously untested wood species provides an additional method of adding value via

Figure 3.—Colonization and growth of fungal structures after mixed cultures of Ophiostoma *sp.,* Eurotium *sp., and* Phialocephala *sp. were applied on* Nothofagus obliqua. *(Color version is available online.)*

spalting and will be particularly relevant for obtaining products with new designs for the wood industry, especially furniture. This research represents the pilot initiative for spalting research in Chile, a country that relies heavily on its crop-forest industry but that has the capacity to move into the decorative woods market given the opportunity. Future research will focus on identifying additional fungi capable of spalting and methods that can optimize and scale the process of laboratory spalting.

Acknowledgments

We thank the scholarship MECESUP Program of the Chilean Government and FONDEF IDEA grant CA12-10142 for supporting this work.

Literature Cited

Altschul, S., T. Madden, A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. Lipman. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res*. 268:3389–3402.

Anke, H., I. Kolthoum, and H. Laatsch. 1980. Metabolic products of microorganisms. 192. The anthraquinones of the *Aspergillus glaucus* group. II. Biological activity. *Arch. Microbiol*.126:231–236.

Beakler, B. W. (Inventor); Armstrong World Industries, Inc. (Assignee). 2007. Spalted wood veneers, spalted engineered wood flooring and method of making. US patent 8,287,971.

Beakler, B. W. (Inventor); Armstrong World Industries, Inc. (Assignee). 2012. Spalted wood veneers and spalted engineered wood floring. US patent 8,399,075.

Beakler, B. W. (Inventor); Armstrong World Industries, Inc. (Assignee). 2013. Method of producing spalted wood veneers and method of producing spalted wood products. Application no. 20,130,153,114.

Bell, A. A. and M. H. Wheeler. 1986. Biosynthesis and functions of fungal melanins. *Annu. Rev. Phytopathol*. 24(1):411–451.

Blackburn, G. M., D. E. Ekong, A. H. Neilson, and L. Todd. 1965. Xylindein. *Chimia* 19:208–212.

Blanchette, R. A., A. M. Wilmering, and M. Baumeister. 1992. The use of green-stained wood caused by the fungus Chlorociboria in intarsia masterpieces from the 15th century. *Holzforschung* 46:225–232.

Boddy, L. 2000. Interspecific combative interactions between wood-decaying basidiomycetes. *FEMS Microbiol Ecol*. 31:185–194.

Butinar, L., P. Zalar, J. C. Frisvad, and N. Gunde-Cimerman. 2005. The genus *Eurotium*—Members of indigenous fungal community in hypersaline waters of salterns. *FEMS Microbiol. Ecol*. 51:155–166.

Díaz, G., A. I. Córcoles, A. D. Asencio, and M. P. Torres. 2013. In vitro antagonism of *Trichoderma* and naturally occurring fungi from elms against *Ophiostoma novo-ulmi*. *Forest Pathol*. 43:51–58.

Dowson, C. G., A. D. M. Rayner, and L. Boddy. 1988. The form and outcome of mycelial interactions involving cord-forming decomposer basidiomycetes in homogeneous and heterogeneous environments. *New Phytol*. 109:423–432.

Doyle, J. and L. Doyle. 1990. Isolation of plant of plant DNA from fresh tissue. *Focus* 12:13–15.

Edwards, R. and N. Kale. 1965. The structure of xylindein. *Tetrahedron* 21:2095–2107.

Eisenman, H. and A. Casadevall. 2012. Synthesis and assembly of fungal melanin. *Appl. Microbiol. Biotechnol*. 93:931–940.

Fraga, M. E., M. G. Pereira, D. J. Barbosa, and P. Maruzanete. 2010. Diversity of isolated Trichocomaceae from soil in two forest ecosystems. *Cienc Flores* 20(1):167–175.

Giles, R. G. F., I. R. Green, and V. I. Hugo. 1990. Model studies toward xylindein precursors. *S. Afr. J. Chem*. 43:28–33.

Golinski, P., T. P. Krick, R. A. Blanchette, and C. J. Mirocha. 1995. Chemical characterization of a red pigment (5,8-dihydroxy-2,7-dimethoxy-1,4-naphthalenedione) produced by *Arthrographis cuboidea* in pink stained wood. *Holzforschung* 49:407–410.

Grunig, C. R., V. Queloz, and T. N. Sieber. 2011. Structure of diversity in dark septate endophytes: From species to genes. *In:* Endophytes of Forest Trees: Biology and Applications. A. M. Pirttila and A. C. Frank (Eds.). Springer, Zurich. pp. 3–30.

Hamasaki, T., M. Fukunaga, Y. Kimura, and Y. Hatsuda. 1980. Isolation and structures of two new metabolites from *Aspergillus ruber*. *Agr. Biol. Chem. Tokyo* 44:1685–1687.

Hudgins, J. W., G. McDonald, P. Zambino, N. Klopfenstein, and V. Franceschi. 2005. Anatomical and cellular responses of *Pinus monticola* stem tissues to invasion by *Cronartium ribicola*. *Forest Pathol*. 35:423–443.

Kumar, S. and N. Kaushik. 2012. Metabolites of endophytic fungi as novel source of biofungicide: A review. *Phytochem. Rev*. 11:507–522.

Langfelder, K., M. Streibel, B. Jahn, G. Haase, and A. A. Brakhage. 2003. Biosynthesis of fungal melanins and their importance for human pathogenic fungi. *Fungal Genet. Biol*. 38:143–158.

Lee, S., S. H. Kim, and C. Breuil. 2002. The use of the green fluorescent protein as a biomarker for sapstain fungi. *Forest Pathol*. 32:153–161.

Popa, V., E. Deziel, R. Lavalle, E. Bauce, and C. Guertin. 2012. The complex bark beetle symbiotic relationship with microorganisms: A potential practical approach for biological control in forestry. *Pest Manag. Sci*. 68:963–975.

Qin, L., M. Guo, J. Qiu, and C. Liu. 2011. Study on the formation of wood zone line pattern induced by fungi. *Adv. Mater. Res*. 190:197–198.

Rayner, A. D. M. and J. F. Webber. 1983. Interespecific mycelial interactions—An overview. *In:* The Ecology and Physiology of the Fungal Mycelium. D. H. Jennings and A. D. M. Rayner (Eds.). British Mycological Society Symposia no. 8. pp. 383–417.

Robinson, S. C. 2012. Developing fungal pigments for ''painting'' vascular plants. *Appl. Microbiol. Biotechnol*. 93:1389–1394.

Robinson, S. C. and P. E. Laks. 2010a. Culture age and wood species affect zone line production of *Xylaria polymorpha*. *Open Mycol. J*. 4:18–21.

Robinson, S. C. and P. E. Laks. 2010b. Wood species affects laboratory colonization rates of *Chlorociboria* sp. *Int. Biodeterior. Biodegrad*. 64:305–308.

Robinson, S. C., P. E. Laks, and D. L. Richter. 2011a. Stimulation spalting in sugar maple using sub-lethal doses of copper. *Eur. J. Wood Wood Prod*. 69:527–532.

Robinson, S. C., P. E. Laks, and J. Turnquist. 2009a. A method for digital color analysis of spalted wood using Scion Image software. *Materials* 2:62–75.

Robinson, S. C., D. L. Richter, and P. E. Laks. 2007. Colonization of sugar maple by spalting fungi. *Forest Prod. J*. 57(4):24–32.

Robinson, S. C., D. L. Richter, and P. E. Laks. 2009b. Effect of substrate on laboratory spalting of sugar maple. *Holzforchung* 63:491–495.

Robinson, S. C., D. Tudor, and P. A. Cooper. 2011b. Wood preference of spalting in urban hardwood species. *Int. Biodeterior. Biodegrad*. 65:1145–1149.

Robinson, S. C., D. Tudor, S. Hipson, H. Snider, S. Ng, E. Korshikov, and P. A. Cooper. 2013a. Methods of inoculating *Acer* spp., *Populus tremuloides*, and *Fagus grandifolia* logs for commercial spalting applications. *J. Wood. Sci*. 59:351–357.

Robinson, S. C., D. Tudor, G. MacDonald, Y. Mansourian, and P. A Cooper. 2013b. Repurposing mountain pine beetle blue wood for art through additional fungal colonization. *Int. Biodeterior. Biodegrad*. 85:372–374.

Robinson, S. C., G. Weber, E. Hinsch, S. Vega Gutierrez, L. Pittis, and S. Freitas. 2014. Utilizing extracted fungal pigments for wood spalting—A comparison of induced fungal pigmentation to fungal dyeing. *J. Coatings Techol. Res*. Article ID 759073. DOI:10.1155/2014/759073

Saikawa, Y., T. Watanabe, K. Hashimoto, and M. Nakata. 2000. Absolute configuration and tautomeric structure of xylindein, a blue-green pigment of *Chlorociboria* species. *Phytochemistry* 55:237–240.

Saikkonen, K. 2007. Forest structure and fungal endophytes. *Fungal Biol. Rev*. 21:67–74.

Salgado, M. E. S., C. Barroetavena, and M. Rajchenberg. 2013. Occurrence of dark septate endophytes in *Nothofagus* seedlings from Patagonia, Argentina. *South. Forests* 75(2):97–101.

Seifert, K. A. 1993. Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*. *In: Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology, and Pathogenicity. M. J. Wingfield, K. A. Seifert, and J. F. Webber (Eds.). American Phytopathological Society, St. Paul, Minnesota. pp. 141–151.

Smetanina, O. F., A. I. Kalinovskii, Y. V. Khudyakova, N. N. Slinkina,

M. V. Pivkin, and T. A. Kuznetsova. 2007. Metabolites from the marine fungus *Eurotium repens*. *Chem. Nat. Compd*. 43(4):395–398.

Suryanarayanan, T. 2013. Endophyte research: Going beyond isolation and metabolite documentation. *Fungal Ecol*. 6:561–568.

Tellenbach, C., C. R. Grunig, and T. N. Sieber. 2011. Negative effects on survival and perfomance on Norway spruce seedlings colonized by dark septate root endophytes are primarily isolate-dependent. *Environ. Microbiol*. 13:2508–2517.

Tian, B. T., Q. G. Huang, Y. Xu, C. X. Wang, R. R. Lv, and J. Z. Huang. 2010. Microbial community structure and diversity in a native forest wood-decomposed hollow-stump ecosystem. *World J. Microbiol. Biotechnol*. 26:233–240.

Tudor, D., S. C. Robinson, and P. A. Cooper. 2012. The influence of pH on pigment formation by lignicolous fungi. *Int. Biodeterior. Biodegrad*. 80:22–28.

White, T., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* PCR Protocols: A Guide to Methods and Application. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (Eds.). Academic Press, San Diego, California. pp. 315–322.

Wu, M. D., M. J. Cheng, S. Y. Hsieh, and G. F. Yuan. 2014. Chemical constituents of the fungus of *Eurotium chevalieri* BCRC 07F0022. *Chem. Nat. Compd*. 49(6):1175–1176.

Zulpa, G., M. C. Zacarro, F. Boccazzi, J. L. Parada, and M. Storni. 2003. Bioactivity of intra and extracellular substances from cyanobacteria and lactic acid bacteria on ‘‘wood blue stain’’ fungi. *Biol. Control* 27:345–348.