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Colonization of Conifers by Ophiostoma Fungi

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Every year fungi inhabiting on coniferous wood cause considerable economic losses to the Forest Products industry. To protect wood and forest resources from these fungi, it is necessary to develop efficient control strategies. For establishing useful control methods, information on the interactions of the microorganisms and their wood host is required. However, so far, our knowledge on the biology of the wood-inhibiting fungi is very limited and fragment.

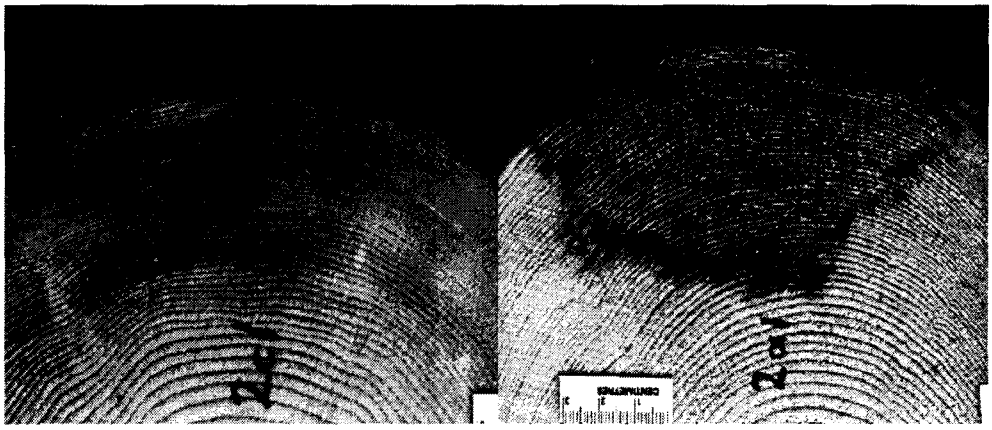
To better understand the growth of sapstaining fungi on wood, we examined the changes in wood nutrients. For the examination, soluble sugars, starch, and lipids were analyzed from non-infected (control) and infected wood samples. Logs and sawnwood were infected with 14 fungal species belonging to *Ophiostoma, Leptographium*, and *Ceratocystis* genera. Both in infected billets and sawnwood, mannose was overall the most depleted mono-sugar (99% depletion), followed by glucose (70% depletion). Meanwhile, no significant levels of starch-bound glucose were found both in control and in infected samples. For lipids, we more focused the content of triglycerides (TG) due to its importance in fungal nutrition. In inoculated logs, *C. coerulescens* and *Leptographium spp.* reduced TG content to very low or undetectable amounts and increased the fatty acids (FA) and resin acids (RA) content by 4 to 9 mg g^{-1} OD wood. Infection with *O. piceae* and *O. minus* resulted in the least TG reduction. This might indicate that deep stainers produce larger amounts or more robust extracellular lipases than the other fungi.

Significant pigmentation was analytically measured in the infected samples versus non-infected control wood. *C. coerulescens* infected wood was considerably more pigmented than the *Leptographium* spp. stain area. *Leptographium* isolates typically showed a non-pigmented, dead host cell zone approximately two to five cm ahead of the stained area within the logs. The kill zone of *Leptographium* was not substantially darker than the non-infected control wood. We also tested the degree of pigmentation of 4 staining species (*Leptographium, O. piliferum, C. coerulescens, O. piceae*) on defined media amended with different carbon sources. Mannose consistently yielded the densest growth and the dark color in all the tested fungi. *Leptographium* and *O. piliferum* had identical color scoring on all the carbon sources. *O. piceae* had a very similar coloring except for glucose and linoleic acid. Interestingly, *C. coerulescens* has an almost revered ranking order compared to other species. Linoleic acid yielded dense growth and dark brown color in *C. coerulescens* had negligible growth on glycerol. However, this carbon source yielded good growth and dark pigmentation in the other fungi. These results suggest that carbon source is one of factors affecting pigmentation in sapstaining fungi.

To explore the property of pigmentation in sapstain fungi, melanin genes were examined. We

amplified scytalone dehydratases (SD), THN reductase (THNR), and pentaketide synthase (PKS) gene fragments from *O. floccosum* 387N strain by PCR with degenerate primers. Genomic clones containing a full length of the three genes were isolated from an *O. floccosum* lamda genomic library using the PCR-amplified DNA fragments as probes and the three genes' sequences were determined. We confirmed that the cloned *O. floccosum* genes are melanin genes encoding SD, PKS, and THNR based on their high sequence similarity with other fungal SD or THNR genes and their ability to restore melanin production in SD- or THN- deficient mutants of *Colletotrichum* or *Magnaporthe* fungi, respectively. This work demonstrates sapstain fungi use dihydroxynaphtalene (DHN) melanin pathway for their pigment production (Eagen *et al.*, 2001; Wang, Kim, and Breuil, 2001).

To apply the use of melanin information the control of problematic sapstaining fungi, we tested a potential biocontrol agent Cartapip™, an *O. piliferum* albino strain. In a field trial in a sawmill in Alberta, Canada, the treatment of albino strain protected lodgepole pine logs from sapstain (Fig. 1).



A B

Fig. 1. An example of the effect of Cartapip treatment on log protection from sapstain. A: Cartapip-treated log, B: untreated log,

This trial involved 6-weeks-incubation periods. The success of field trial led us to develop makers that tracking the applied Cartapip in fields. For this, we tested wood samples (a total of 48 samples, 12 samples/disk) from a sawmill in Alberta where Cartapip sprayed for wood protection test. We could successfully detect the presence of Cartapip in lodgepole pine logs disks using PCR-RFLP by *Hae*III and PCR assay with Cat1-Cat2 primers. We also demonstrated that the Cat2-Cat2 primers were useful for detecting Cartapip in Germany where Cartapip was sprayed for controlling sapstain in scots pine lumber and logs (Schroeder *et al.*, 2002).

Since rRNA- or β-tubulin gene-based molecular markers for detecting Cartapip has limitation in use at some geographical regions, we assessed the feasibility of transforming with the green fluorescent protein (GFP), the sapstain fungus *Ophiostoma piceae* and a potential biocontrol agent Cartapip™, an *O. piliferum* albino strain. Transformants of the two fungal species were screened by PCR and Southern blot analyses. The GFP was expressed in spores, synnemata, and mycelia of the transformants grown in artificial media or wood. The growth, pigmentation, and wood colonization of the GFP-transformants were similar to that of the non-transformants, suggesting that the presence

of the *gfp* gene have no negative effect on the biology of the transformants. Using fluorescence and confocal microscopy, the GFP-expressing fungi were easily differentiated from the wild type strains and other fungal species in wood, even four months after inoculation (Lee, Kim and Breuil, 2002).

References

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