BIOCONTROL IN WOOD PROTECTION – CAN IT WORK?

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Summary

This paper presents an update on the Canadian biological control research that has been done on wood products in the last ten years. In particular, it concentrates on two technologies explored at Forintek Canada Corp. to address problems of stain in lumber and logs. One technology used the common mould *Gliocladium roseum* as a biocontrol agent in studies on agar media and wood wafers in the laboratory, and on lumber and logs in the field. *G. roseum* alone provided excellent results in laboratory tests. In the field, the performance of *G. roseum* was enhanced both by prior wood pasteurization and by combining it with an alkali solution. The second technology looked into the potential of using albino strains of bluestain fungi to control wild type (pigmented) staining fungi. In our initial studies on lumber we tested an albino strain of *Ophiostoma piliferum* (Cartapip®97). However results were inconsistent under field conditions. To test Cartapip® on logs an experiment was set up where Cartapip® and four challenge bluestain fungi were inoculated in different sequences onto artificially produced bark wounds. Cartapip® was able to colonize fresh lodgepole pine, penetrate up to 2 cm deep in the sapwood, and did not cause stain. The challenge fungi alone colonized large areas of the sapwood and caused significant stain. However, when the challenge fungi were applied after Cartapip®, the stain was negligible in most cases. Based on the results we concluded that there is a potential for biocontrol of stain, especially on logs using both of these technologies. However, building knowledge on optimization of their use as well as defining the conditions under which they can or cannot work is essential.

1. Introduction

A paper on biological control of wood-inhabiting fungi was last given at the CWPA 11 years ago (Seifert, 1988). The current paper will give an update on progress in biological control (biocontrol) in Canadian wood products research specifically on the protection of wood products against staining fungi.

Biological control and its place in wood products research

Biocontrol can be defined as the use of biologically based technologies and tools to manage pest populations. Biocontrol is generally thought of as the use of natural parasites, predators, pathogens, or their parts or products, to control the depredation of crops, forests, livestock and the wider environment by invertebrates, weeds and diseases. When these agents are used in concerted, synergistic combinations timed to the life cycles of the target pests and the plants and animals they attack, the approach is called

integrated pest management or IPM. Interest in studying biological control as a pest control alternative has greatly increased due to public and regulatory pressures on chemical pesticides. Generally, biocontrol is perceived to be environmentally benign, and from a regulatory point of view, biologicals (pesticides based on biocontrol agents) are considered to be reduced-risk pesticide alternatives to chemicals.

Biological control has a long track record in the agricultural sector, but is a more recent notion in forestry and especially in the forest products industry. The success of *Bacillus thuringiensis* (Bt) in combating various forest insect pests is one of few working examples of biocontrol in forestry. In the field of wood products, early attempts at biocontrol focussed on wood decay fungi using bacteria or other fungi (e.g. Etheridge, 1972; Greaves, 1970; Bruce et al., 1984). Biocontrol was also explored to control root and rot diseases (Rishbeth, 1952; 1963), and sapstain in sawn wood and logs (see Seifert, 1988). Organisms used as biocontrol agents on wood are considered to interact with pest organisms in one (or a combination) of the following ways: 1) competition for the niche, 2) antibiosis, or 3) parasitism. An ideal biocontrol organism should be efficient in primary resource capture, grow fast, and utilize available food quickly and thus inhibit colonization by other organisms. It also must be good at secondary resource capture (able to grow in substrate already colonized by other organisms) or able to prevent secondary resource capture by production of antibiotics or by mycoparasitism. The biocontrol agent must not utilize structural carbohydrates or not cause any other damage.

Antibiosis has been the approach mainly taken against wood-decaying fungi. However, as the control mechanism involves the production of secondary metabolites by the control agent, questions are raised about the nature of the mycotoxins and antibiotics involved (Seifert, 1988). Similarly, biocontrol via parasitism requires the pest organism to become established first and this is not desirable. Despite many years of working on the control of decay fungi, no effective biocontrol technologies appear to have worked consistently. The task of controlling decay fungi with another organism is made more difficult by the long-term nature of the requirement. Long term protection will require that either the biocontrol organism must stay alive in the wood for the duration of the protection needed or it must leave residual antibiotics (Seifert, 1988) and has to be equally successful against the many different organisms that it may encounter.

Attempts at the biocontrol of wood staining fungi on lumber have employed bacteria and other fungi (among others: Benko, 1988; Benko and Henningsson, 1986; Benko and Highley, 1990; Bernier *et al.*, 1986). Chakravarty and Hiratsuka, 1994; Dawson-Andoh and Morrell, 1990; Highley *et al.*, 1997; Kreber and Morrell, 1993; McAfee and Gignac, 1997; Morrell and Dawson –Andoh, 1998; Schoeman *et al.*, 1993; Seifert *et al.*, 1987, Seifert *et al.*, 1988.). The main approach to date has involved using biocontrol agents that produce antibiotics. Results have varied in their degree of success in the laboratory, but even systems that showed success in the laboratory have often failed under field conditions. This could be attributed to a lack of information about the complex

interaction between the biological control organism, the fungus being controlled, the substrate on which both are growing, and the temperature/humidity conditions.

As with control of decay fungi, mycoparasitism of staining fungi can be successful (Byrne, 1998; Croan, 1996), but development of the staining fungi before this can occur is not desirable. Thus, of the available biocontrol mechanisms, competition for the niche is the one most likely to succeed. Staining fungi are primary colonizers that utilize simple carbohydrates, lipids, proteins and resin acids (Abraham *et al.*, 1997). They are generally tolerant to the residual sapwood defense mechanisms and high moisture contents that prevail in freshly felled logs. Once they deplete readily available food sources (they do not possess the enzymes necessary to utilize cell wall constituents) they die out or are replaced by other fungi. As the biological control agent becomes established in the same or similar niche to the one used by the pest organism(s) it should not cause damage itself. An example in European forestry where this has worked well is the control of the decay fungus *Heterobasidion annosum* using another decay fungus *Phlebiopsis gigantea* (Rishbeth, 1963; Greig, 1976). *P. gigantea* has the desirable characteristics of a good biocontrol agent: fast growing on the primary resource to occupy the niche, with the ability of secondary resource capture. Despite being a decay fungus, it does not cause damage to live trees like the pathogen it controls. *Heterobasidion annosum* spreads from the decayed stump through root grafts into healthy standing trees, while *Phlebiopsis* remains in the decayed stump. Thus in the case of staining fungi, it seems logical that the best biological control organism to occupy the same niche could well be another staining fungus.

Biocontrol of stain in lumber and logs - approaches in the last ten years

Recent work in Forintek has concentrated on investigating two technologies using two biocontrol agents. One is an antagonistic mould and the other is a commercially available albino strain of a bluestain fungus. In the mid-1980's, Forintek's eastern laboratory began work on the biological protection of wood from sapstain. This work began by screening a wide variety of wood inhabiting organisms for their ability to prevent stain. (Seifert *et. al.* 1987, Seifert et al., 1991). They then carried out pre-colonization experiments on agar and small blocks of sapwood of different wood species. Selected candidates were tested for the production of metabolites. *Gliocladium roseum* was chosen for further development. It was also found that *G. roseum* is a species tolerant to alkaline conditions (capable of growth at pH 10.8) while many other fungi were sensitive to elevated pH (Yang, 1998). This property was later used, combining *G. roseum* with alkalis in attempts to enhance the performance (Yang and Rossignol, 1999).

Taking a different approach, Forintek's western laboratory tried using a commercially available albino strain of *Ophiostoma piliferum* called Cartapip® (Clariant Corp.,). This organism was developed as a pitch reducing agent for use in pulp chips destined for pulping (Blanchette *et al.*, 1992). It was also shown to be able to reduce bluestain development in red pine logs (Behrendt *et al.*, 1995, Blanchette *et al.*, 1997). Colorless

(albino) bluestain isolates can be obtained either through the selective breeding of lighter isolates or by searching for a natural mutant which occurs in 1 out of 30-40 000 individuals (Blanchette, personal comm.). Albino strains are expected to have the same aggressive growth characteristics as the wild type staining fungi and can utilize nutrient resources in the sapwood rapidly, thus preventing wild type staining fungi from establishing. The lack of pigment in their hyphae prevents the sapwood from staining. Albino (non-pigmented) staining fungi have similar ecological and biological attributes to wild-type staining fungi. As such, they are able to colonize freshly felled wood and deplete the food resources without causing aesthetic damage. Therefore, pigmented wild type fungi would not be expected to grow and cause stain in the areas first colonized by albino fungi. Our goal was to test this organism for its ability to prevent growth of wild staining fungi on Canadian wood species and then optimize its use under Canadian field conditions.

Controlling stain in lumber

There are three major challenges facing the biocontrol of fungi on Canadian softwood lumber:

1) The large number of fungi which cause stain and mould make control by a single biocontrol organism (or narrow spectrum fungicide for that matter) difficult;
2) The wood substrate is highly variable in nutrient, moisture, and extractive content, all of which affect the establishment of biological control agents;
3) The substrate is pre-infected with competing microorganisms during the long period between cutting down the trees and processing into lumber (significant infection also occurs during saw milling).

In order to overcome these challenges and to enhance the bio-activity of the biocontrol agent, Forintek's eastern laboratory used two approaches. The first approach was a physical method using wood pasteurization, and the second one was a chemical method using a combination treatment with a mild alkali. In the first approach, lumber was heat treated at 85°C for 2 hours, until the maximum internal wood temperature measured was approximately 80°C and the minimum internal wood temperature was 56°C. Then, the lumber was dipped in a spore suspension of *G. roseum*. The test on western hemlock lumber showed that after 6 months of storage, *G. roseum* treatment resulted in a high percentage of acceptability on heat pasteurized lumber, but on unpasteurized lumber successful protection against stain and mould was not achieved (McAfee and Gignac, 1997). The second approach was simultaneous application of *G. roseum* and an alkali solution. On white pine sapwood, simultaneous application of *G. roseum* and 5% sodium carbonate increased wood acceptability from 50% by the treatment with *G. roseum* alone, to 100% over an 8-week storage period. None of the untreated wood pieces in the controls were acceptable in this test. Further *in vitro* tests of the combined system were conducted on several major Canadian wood species: western hemlock, white spruce, black spruce, jack pine, lodgepole pine, white pine, red pine, Douglas-fir, amabilis fir and

balsam fir. Results showed that all wood species were fully protected by this integrated method (Yang and Rossignol, 1999). The field test on lumber showed that the treatment of jack pine with *G. roseum* spores alone was moderately effective against stain, but this treatment was ineffective on white pine. Application of *G. roseum* in an alkaline solution enhanced the activity of the fungus against stain. Use of the integrated treatment, resulted in 100% acceptable pieces of jack pine and 84% acceptable pieces of white pine. On both wood species, the treatment with *G. roseum*/alkali was superior to the reference chemical (Yang and Rossignol, 1999).

In Forintek's western laboratory we tested the ability of Cartapip® to control stain in hem-fir lumber, and obtained good results when the lumber was heat-treated and when the fungus was mixed with glucose (to encourage its establishment). However, when the trial was repeated in the same way the following year, the results were not duplicated (Byrne, 1998).

The difficulties in implementing biological control in commercial lumber have resulted in more research to understand the basic biology of staining fungi and the development of stain. To achieve this we set up alliances with the University of British Columbia Forest Products Biotechnology Group who are well placed to do research in basic biology.

Biocontrol of stain in logs

The recognition that pre-infection with competing microbes was a factor in reducing the efficacy of both biological and chemical treatments has resulted in the need to examine how these fungi get into the wood at the log stage. The increased cost of logs and the need to maximize value from them is also driving the interest in protecting logs from degrade, beginning at the point the trees are cut down. Fungal deterioration, particularly bluestain, is often associated with spiked rollers or with bark damage where the bark has been removed in log handling (Lee and Gibbs, 1996, Uzunovic et al., 1999). Modern harvesting procedures and subsequent log storage can therefore result in a significant loss in value of the product derived from the logs. Over the last two years bluestain in lodgepole pine logs had a big impact on the profits of affected sawmills. In summer of 1998, stain developed to epidemic proportions in logs being processed at some Alberta sawmills. For three sawmills visited by Forintek staff, the bluestain problem caused losses for that year of $14.3 million over and above the "normal" losses which the mills encounter. These losses were encountered because the sawn product ccannot meet Japanese requirements for stain-free wood, nor can it be used for re-manufacturing into high value products such as furniture. The fact that the year was a particularly warm one was probably the major contributor to the problem.

To date bluestain has often been considered by sawmill staff to be an inevitable occurrence in the logs processed in sawmills. However, recognition is developing to consider what might be done to reduce stain development after a tree has been felled. Spraying or sprinkling logs can provide excellent protection of softwood logs from

degradation; however, the lack of availability of water at the mill or the need to recycle the water has limited use of such techniques. Unlike other countries, there is no tradition of using chemical pesticides for the treatment of logs to prevent bluestain in Canada and it is unlikely that chemical treatments will be adapted because of negative public perceptions of the impact of pesticides on the environment.

Measures taken to control stain on logs, whether in the forest or in the sawmill yard, need to be environmentally benign. Biological control methods seem to offer the greatest practical promise as a protection method for logs in the future, and Forintek research efforts have moved from the biocontrol of lumber to the biocontrol of logs. One would expect biocontrol fungi to be able to establish more effectively in freshly felled trees (i.e. clean logs, free of microbial colonization) rather than in pre-infected lumber. Forintek is currently pursuing the use of both the fungal (e.g. albino staining fungi) and chemical/fungal integrated approaches previously mentioned. (Yang, 1998). As with chemical pesticides, such biologicals must be registered under the Pest Control Products Act administered by the federal government's Pest Management Regulatory Agency. Canada has one of the most rigorous pesticide registration processes of anywhere in the world and such registrations are expensive and lengthy so biocontrol methods will not be available over the short term.

Forintek's eastern laboratory has tested *G. roseum* technology on black spruce and jack pine logs that were cut in 1-m lengths. The logs were dipped in a spore suspension of *G. roseum* and/or in a spore suspension in an alkaline solution and compared with an untreated set of logs and a set of logs treated with a reference antisapstain chemical. Test logs were piled for four months in a yard and assessed for stain development. In general both treatments with *G. roseum* (alone or with alkaline solution) were much less stained then untreated logs during 4 months storage in summer. Treatment with the integrated *G. roseum* system provided the same level of protection as the reference antisapstain chemical.

Forintek's western laboratory used 80-year old lodgepole pine trees cut into short lengths (30-50 cm). The short logs were end-sealed to prevent fungal colonization and drying through the ends. Cartapip®97 and wild-type challenge staining fungi were inoculated onto the logs by spraying spore suspensions onto puncture and open wounds artificially produced on the logs, and similar to those found on logs that have been harvested mechanically. Challenge fungi were sprayed onto the wounds before, after or with Cartapip®. The challenge fungi used in these treatments were commonly found staining fungi. After incubation for 38 days at 20°C and 85 % RH the short logs were sliced through the mid-wound area and assessed for stain.

After studying the growth of Cartapip® in billets, it was clear that Cartapip® was able to colonize fresh lodgepole pine sapwood. It did not cause stain, but gave the wood a slightly bleached appearance. The Cartapip® did not grow as well in the short logs as did the challenge fungi, *Ceratocystis coerulescens* and *Leptographium* sp., which were

able to colonize large areas of wood and living bark and cause significant stain during the same incubation period. Cartapip® penetrated into the wood to a depth of 20 mm. For the open wounds, the average stained areas produced by *C. coerulescens*, *Leptographium* sp. and *O. minus* were 33.0, 5.7 and 8.3 cm^2 respectively; while for the puncture wound, the areas were 43.0, 10.7 and 9.4 cm^2. The success of Cartapip® as a biocontrol agent varied when it was applied simultaneously with the challenge fungi. There was significantly less stain produced by *O. minus* in both types of wound, and by *Leptographium* sp. in the open wound only. However, *C. coerulescens* managed to penetrate the wood and cause stain in both wound types. In the open wounds, the stained area was reduced to a half compared to control treatment, and Cartapip® mycelia was present across the area stained with *C. coerulescens*. However, when Cartapip® was inoculated either two or ten days prior to the challenge fungi, stain caused by *C. coerulescens, Leptographium* sp. and *O. minus* was negligible in most cases. No preventative effect on the development of stain was observed when Cartapip® was inoculated five days after the challenge fungi.

Discussion and Conclusions

Protecting wood from microbial attack is a difficult task because many organisms can cause deterioration problems. To be effective both biological and chemical pesticides need to control a wide range of these pests. Biological control of wood-deteriorating organisms is a relatively recent research goal, the focus of which has changed over the last decade or so from protecting wood in long-term service against wood-decaying fungi, to the prevention of growth of sapstain fungi. Fortunately only short-term protection is required against staining fungi and such protection is therefore probably more feasible with bio-pesticides than over the longer term required against wood-decaying fungi. Based on tests, which are intermediate between the highly controlled conditions of laboratory tests and the less controlled conditions that prevail in industrial field tests, the two technologies discussed in this paper show promise for control of stain particularly on logs. The results have clearly shown that both *G. roseum* and Cartapip®97 are able to colonize test wood and to compete with challenge fungi over the duration of the experiments. Both fungi also did not extensively colonize the phloem nor penetrate deeply into the sapwood. This reduces concerns of having a biocontrol fungus deeply established in sapwood and affecting the wood properties. When given a good start by spraying it before the bluestain fungi, both fungi established well enough to keep the wood stain-free. *G. roseum* showed excellent performance against moulds and sapstaining fungi on various wood species in the laboratory evaluation. Its bio-activity against stain can be enhanced by either pasteurization of wood or by the combination of this fungus with an alkali. Future fieldwork will include testing Cartapip® and *G. roseum* /alkali under field conditions using standard-size logs.

As Seifert said at the 1988 CWPA meeting, we should see biocontrol as just another tool against wood degradation and not as a threat to the wood preservation industry, which is

increasingly under pressure to use milder chemicals preservatives. Perhaps the solution will lay in integrated biological/chemical systems.

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