BIOLOGICAL CONTROL AND WOOD PROTECTION

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SUMMARY

Biological control is the use of microorganisms, their metabolites, or their genes for the prevention of unwanted fungal attack on wood. A short introduction to antagonistic interactions between wood-inhabiting organisms is presented. Past and current research in biological control of biodeterioration of wood products is reviewed and strategies for improving biological control agents are outlined. Finally, the possible impact of biological control on the preservation and protection of wood products is considered.

INTRODUCTION

Biological control (abbreviated in this paper as BC) is defined as "the reduction of the amount of inoculum of disease-producing activity of a pathogen accomplished by or through one or more organisms other than man" (1). In the context of forest products, biological control is the addition of a microorganism to wood, or modification of the wood to favour a microorganism, that will reduce or prevent biodeterioration. Biological control is the manipulation of wood ecology for our own benefit.

Biological control has a history of about forty years in forestry and wood products research. For most of this time it has been considered a novelty, but the public and legislative pressure on chemical preservatives is resulting in a renaissance of BC research in all fields. The most widely used BC product is the bacterium *Bacillus thuringiensis*, better known as BT, which is used to control spruce budworm in Ontario and parts of Quebec. The bacterium produces a toxic crystal that is eaten by the budworm as it eats the leaves, eventually killing the pest. Approximately half a dozen BC systems have been marketed for agricultural use, for example DeVine, a fungal control of parasitic vines in citrus orchards. Despite these modest commercial successes, BC remains an experimental strategy for pest control.

In order to speculate what contributions biological control might make to wood protection in the year 2000, it is necessary to review past work in the field and try to extrapolate this into the future. Some BC systems have been proposed for wood products, and these will be reviewed below. At the moment, BC research in agriculture is much more sophisticated than the work

we have seen in wood products. The main lesson from this research is that the mechanisms responsible for BC are of the utmost importance. I will pay special attention to these mechanisms throughout this paper, because understanding them is the key to a successful future for BC on wood products.

EXPERIMENTAL TECHNIQUES

Many different experimental designs have been used for identifying and testing biological control agents (BCAs). Two kinds of tests are commonly used for screening; agar interactions and precolonization tests on wood. These techniques are the basis for much of the work discussed below, and they are therefore described briefly here.

The agar interaction technique has been used extensively to study the population dynamics of wood-inhabiting microorganisms (2). In this technique, two microorganisms are placed on opposite sides of a petri dish. They grow towards each other and observations are made of the zone of inhibition or line of contact to determine if one organism is inhibiting the other. This technique is simple, but is often difficult to interpret and is a poor approximation of interactions on wood. Studies have shown that interactions between organisms on agar may vary significantly under different nutrient conditions (3). Some species that are aggressive on agar do not provide BC on wood, and some species that do not appear to inhibit other fungi on agar are effective BCAs on wood.

Screening for BC candidates directly on wood is more laborious, but the results are easier to interpret and more representative of a field situation. The soil block test, designed for testing traditional wood preservatives, is also useful for screening of potential BCAs against decay. In this method, sapwood blocks are placed on sapwood feeder strips in jars containing moist soil. For testing against sapstain, we have modified ASTM standard D-4445, originally intended for screening antisapstain chemicals. In this method, small blocks of green sapwood are arranged on glass rods in petri dishes. A small amount of water is added to filter paper in the bottom of the dish to keep the relative humidity high. In "precolonization experiments" using either method, the BC candidate is grown on the wood for a certain period of time, and the sapstain or decay organisms are added afterwards. The performance of the BC organism is assessed by measuring the amount of decay or observing the amount of sapstain in the precolonized blocks after exposure to the sapstain or decay organisms for a standard incubation period.

ANTAGONISTIC INTERACTIONS BETWEEN WOOD-INHABITING MICROORGANISMS

The first observations of antagonistic interactions between microorganisms were made more than 100 years ago (4). In Germany, Harder (5) and in the United States, Zeller and Schmidt (6), studied the interactions on agar between various microorganisms isolated from wood. The latter authors observed antagonism between different species of decay fungi. Several species of the mould genus *Aspergillus* inhibited the growth of some decay fungi, in some cases before the colonies made contact, in other cases after contact. Many examples of antagonism between non-decay fungi and decay or sapstain fungi are now known, mostly based on agar interactions (7). Some microfungi, such as *Fusarium oxysporum*, actually enhance decay caused by some fungi (8).

The traditional approach to interspecific interactions was to classify them as symbiotic (harmless or beneficial to both) or antagonistic. Three types of antagonistic interactions were distinguished: competition, antibiosis and lysis, and parasitism. The introduction of modern ecological concepts to the study of fungal interactions has led to some modification of the traditional scheme (2). Three broad categories of interaction are recognized: mutualistic (both organisms benefit), neutralistic (neither benefits nor suffers) and competitive. Competitive interactions include (a) primary resource capture, in which a fast growing organism utilizes the easily assimilable nutrients in a substrate and thus inhibits colonization by other organisms, and (b) combat. Combative competitive interactions include defence and secondary resource capture. In defensive combative interactions, the organism occupying the substrate prevents colonization by other organisms by producing antibiotics or by acting as a mycoparasite. Fungi utilizing secondary resource capture are capable of growing into a substrate already occupied by other microorganisms; they may be resistant to the antibiotics or mycoparasitism of the primary colonizer, may themselves produce lethal antibiotics or act as mycoparasites of the primary colonizers, and/or may be capable of utilizing more complex nutrients, such as cellulose or lignin, that the primary colonizer is unable to use. Most of these possibilities have been demonstrated for wood-inhabiting microorganisms.

The ideal BC organism must be an efficient primary resource captor, because most BC situations in wood products dictate colonization of the wood at the time of manufacture or processing, before undesirable organisms are present. Having captured the primary resource, the BCAs should prevent colonization of the substrate by aggressive secondary colonizers. Secondary resource capture abilities are also important for potential BCAs, which must be able to invade and capture any parts of the wood already occupied by unwanted fungi. Obviously, BCAs applied to wood must not utilize structural carbohydrates, or cause a significant loss of wood strength.

BIOLOGICAL CONTROL OF ROOT-ROT DISEASES

In the 1950's, antagonistic reactions between wood-inhabiting microorganisms began to be exploited for BC purposes. Rishbeth (9) reported that *Heterobasidion annosum* (also known as *Fomes annosus*), a primary resource captor and the cause of pine root rot, was sometimes naturally replaced by *Peniophora gigantea* in the roots of cut trees. Inoculating freshly cut pine roots with *P. gigantea* prevented development of the root rot disease, demonstrating that the species was an efficient primary resource captor. Meredith (10) reported that *P. gigantea* could replace *H. annosum* in infected roots, demonstrating that *P. gigantea* could act by secondary resource capture as well. The mechanism of secondary resource capture was identified as hyphal interference (11); hyphae of *H. annosum* contacted by hyphae of *P. gigantea* develop granular cytoplasm, become highly vacuolized, then lose opacity as the cytoplasm leaks from the cell. *Peniophora gigantea* meets the criteria for a good BCA: it uses both primary and secondary resource capture, and the mechanism of action is well-understood. This BC system is now used in some pine plantations in Britain and Poland and has a stable, if modest, market (12).

BIOLOGICAL CONTROL IN TRANSMISSION POLES

In wood products, the first experimental uses of BC were against decay. The highly antagonistic nature of some species of *Trichoderma* began to receive attention during the 1960's. Species of *Trichoderma* were effective in precolonization experiments against *Poria monticola* (13), *Phellinus weirii*, a cause of Douglas fir root rot (14, 15), and *Chondrostereum purpureum*, the cause of silver leaf disease in fruit trees (16). Greaves (17) identified several bacteria that reduced or prevented decay in laboratory precolonization tests.

The most concentrated efforts to apply these observations for BC focused on decay in transmission poles. Ricard and Bollen (18) noted that wood blocks precolonized with *Scytalidium* sp. were resistant to decay by *Poria carbonica*, an important decay organism in Douglas fir utility poles in North America. Ricard subsequently obtained several patents for formulations including *Scytalidium* sp. and various *Trichoderma* spp., formed a company, Bio-Innovation AB (BINAB), and began to market BC products for the control of decay in transmission poles, dutch elm disease, and for stimulation of tree and agricultural crops (for updates of this research see 19).

Both fungal genera involved in the BINAB system for remedial treatment of transmission poles are well-known producers of antibiotics. The fungitoxic substance of *Scytalidium* was dubbed

scytalidin or scytalidic acid (20, 21). Scytalidin was inhibitory to 52 of 61 wood-inhabiting fungi tested at 50 µg/disc, including many decay and several staining fungi, and to all at 100 µg/disc (22). Vacuolization of *Lentinus lepideus* hyphae occurs at distances up to 5 mm away from growing *Scytalidium* colonies (23). Scytalidin is the major metabolite of the BINAB *Scytalidium* FY-strain, and can be isolated in comparatively large quantities using relatively simple techniques (21, 22). This compound is effective at concentrations comparable to those used for PCP, TCP and sodium arsenate in preventing wood decay by *Gloeophyllum trabeum* (24) and sapstain by *Graphium* sp. (25). Antimicrobial metabolites, including the well-known gliotoxin, have also been isolated from various *Trichoderma* species or their *Hypocrea* sexual states (26). Volatile antibiotics have also been detected (27), isolated, and identified from *Trichoderma* species (28).

The applicability of Ricard's products to wood products has been evaluated in Europe and the United States. Several field trials have been performed. In North America, Ricard (29) inoculated *Scytalidium* into 50 creosote treated Douglas fir poles, and found that after seven years, twenty percent of the poles had well-established *Scytalidium* populations that had apparently prevented growth of *P. carbonica*.

In the UK, field trials concentrated on use of the BC formulation to reduce decay caused by *Lentinus lepideus* in creosoted transmission poles. Bruce (30), in a test involving 40 poles, reported that pellets with a mixture of *Trichoderma* and *Scytalidium* spp. reduced the incidence of decay by *L. lepideus* that was introduced before or after the BCAs. Bruce and King (31) destructively sampled 166 model poles inoculated with *Trichoderma* at three field locations in the UK, and demonstrated that the *Trichoderma* became established in 94% of the poles and could be reisolated for up to 4 years following inoculation. The incidence of decay was reduced by these treatments, but establishment of the BC organisms was somewhat inhibited by other microfungi resident in the poles, particularly *Hormoconis resinae* (32).

The results of Morris et al. (33) were less promising. They inoculated thirty model poles with *L. lepideus*, allowed the decay to become established, then attempted various remedial treatments. Instead of employing destructive sampling, they periodically examined dowel baits inserted into the poles. Although *Trichoderma* effectively colonized the poles, *L. lepideus* was detected as frequently in treated as in untreated poles after fourteen months. *Scytalidium* was usually detected only after *L. lepideus* was established. It was effective at colonizing wood already invaded by *L. lepideus*, and was an ineffective primary resource colonizer itself. These authors concluded that the BINAB system was an ineffective remedial treatment, but might be

useful as a protective treatment if the ability of *Scytalidium* to colonize undecayed wood could be enhanced.

Recently, Morrell and Sexton (34) performed a laboratory evaluation of a BINAB system for the prevention of decay of douglas fir and southern yellow pine. They found that the BC was effective against brown rot fungi in precolonization tests, but had little effect on the white rot fungi common in decaying poles.

BIOLOGICAL CONTROL IN ROUNDWOOD

A second possible application of microorganisms antagonistic to decay fungi was identified in round wood in storage. Shields (35) reported that decay by *Bjerkandera adusta*, *Coriolus hirsutus* and *C. versicolor* was inhibited in wood blocks precolonized with *Trichoderma harzianum* or an unidentified strain (now known to be *Scytalidium lignicola*). The strain of *T. harzianum* was later used in a field test on birch bolts (36, 37), where a conidial suspension was sprayed onto fresh cut ends of birch bolts. After a two week precolonization period, *Bjerkandera adusta* was inoculated onto the bolts. After six months, very little *B. adusta* was reisolated from the bolts.

Stilwell (38) isolated a strain of *Cryptosporiopsis* sp. from yellow birch that inhibited the growth of 31 decay fungi in agar interactions. Decay of blocks by *Fomes fomentarius* was inhibited in precolonization experiments. In a field test, decay was reduced in peeled birch logs inoculated with a water suspension of *Cryptosporiopsis* sp., but no significant difference was noted in unpeeled logs. Culture filtrates of *Cryptosporiopsis* also inhibited growth of *F. fomentarius*. The antibiotic metabolite was purified, characterized and given the name cryptosporiopsin (39).

BIOLOGICAL CONTROL AND WOOD CHIPS

Decay of wood chips during storage was also considered as a possible target for antagonistic microorganisms. Bergman and Nilsson (40) tested several mould fungi isolated from wood chips for their ability to inhibit chip decay in laboratory experiments, and found that most decay fungi were inhibited. *Gliocladium viride*, a mycoparasite frequently isolated from chips, was tested on spruce chips in the field, and inhibited decay at temperatures less than 30°C, but failed at higher temperatures.

Conifer chips inoculated with the antibiotic-producing *Cryptosporiopsis* sp. and stored outdoors for 12-15 months yielded an improved quality of pulp although decay was not completely eliminated (41). The results of trials using the antibiotic as a

chemical preservative, and of a proposed field test, have not been published.

Bacteria were also considered as BCAs in chip piles. Some bacteria isolated from hardwood chips were inhibitory to selected decay fungi in agar interactions (42, 43). Lapetite (44) found that the antagonism was only effective on wood when the bacteria were inoculated onto the wood several weeks before the decay fungi. The results of a planned field trial have not been published.

BIOLOGICAL CONTROL OF SAPSTAIN

Early studies on BC on wood products concentrated on decay, but the possibility of controlling sapstain by using antagonistic organisms has also received attention. The early work of Stilwell and his colleagues (20, 22, 38, 41) demonstrated the antagonism of some microorganisms towards some sapstain fungi. Stranks (25) found that 0.25% and 0.50% solutions of the antibiotic hyalodendrin, applied to white pine blocks by dipping, were effective at preventing sapstain by *Graphium* sp., while cryptosporiopsin was ineffective.

Bacteria have also been considered as BCAs against sapstain. Vasil'ev (45) isolated an unidentified bacterium that inhibited the growth of several sapstain fungi on agar. Bernier et al. (46) showed that an isolate of *Bacillus subtilus* prevented sapstain when wooden blocks dipped into a cell suspension were placed on agar plates inoculated with sapstaining fungi. Our work with the same culture of *B. subtilus* showed that although it strongly inhibited sapstaining fungi on agar, it did not inhibit sapstain when the wood was not placed on agar. The bacterium colonized wood very poorly and this prevented effective BC (47). Benko (48) has recently screened many bacteria for antagonism towards sapstain fungi in agar interactions, and has selected some strains of *Pseudomonas* for further study.

Some innovative approaches towards biocontrol of sapstain have also been tried. Johnson (49) studied polyoxin, an antibiotic that inhibits the synthesis of chitin, a major component of fungal cell walls. The eight sapstain and mould species tested were sensitive to this compound but at concentrations too high to be economical on a practical scale. Benko (50) demonstrated that crude culture extracts of some antibiotic producing mycorrhizal fungi prevented growth of several sapstaining fungi on blocks of pine.

Our recent work at Forintek has concentrated on screening a wide variety of wood inhabiting microorganisms for their ability to prevent sapstain in laboratory precolonization experiments (51).

We are presently eliminating those that have undesirable properties, such as the ability to produce soft rot.

MECHANISMS: THE KEY TO IMPROVING BIOLOGICAL CONTROL

Biological control is notorious for working well in the laboratory, but failing in the field. These failures are not difficult to explain. Unlike chemicals, BCAs must grow to produce their effect, and this requires adequate nutrients, moisture and the proper temperature. BCAs fail when they are rushed into the field without adequate study, and without proper consideration to formulation and application technology.

It is important to study the biology of potential BCAs as carefully as possible, to weed out species with undesirable side affects. *Scytalidium*, for example, is known to cause weight loss in some woods (52), sometimes causes sapstain (53), and is an ineffective primary colonizer of wood (33). Its usefulness as a BCA for protection of wood, therefore, must be questioned.

Strain improvement is the key to improving BC, but has scarcely been considered in research on BC in wood products. Preliminary screening to select the agents that have promise as BCAs is just the first step. Once an organism has been selected, there are several strategies to improve its usefulness. The two main strategies are enhancing the antagonistic activity, and enhancing the ability of the strain to grow in the desired conditions.

In order to enhance antagonistic activity, it is important to understand what mechanisms the BCA is using to inhibit or kill the unwanted organisms. In the review above, I have tried to include some details of the mechanisms behind the BC if they are known. Most systems probably rely on more than one kind of mechanism. Antibiotics have been implicated in most BC systems and many examples are given above. Two other mechanisms may be involved in formulations including *Trichoderma* species. The first is primary colonization; *Trichoderma* species grow rapidly, and by utilizing easily metabolized nutrients in the wood, can grow into the substrate very quickly (54). Species of *Trichoderma* are also well-known mycoparasites. Hyphae of most isolates of several tested *Trichoderma* species grow towards hyphae of *H. annosum*, attach to and coil around them, and kill them (55). Mycoparasitism by *Trichoderma* in agricultural BC systems has been well studied, but it is uncertain whether mycoparasitism by *Trichoderma* is a factor in BC systems in wood products.

The use of benign isolates of normally undesirable species is a possibility that has received little attention in BC on wood products, although it is an important consideration in BC of some plant pathogens. Micales and Highley (56) have recently reported on some physiological characteristics of a strain of *Poria*

placenta that is unable to decay wood, but this has not been extrapolated to consider the BC possibilities.

Once the mechanisms are known, it is possible to select isolates or create mutants that colonize wood faster, produce increased amounts of antibiotic, are more aggressive mycoparasites, or are resistant to chemical preservatives. The ability of the BCA to grow in the desired conditions is a function of the speed that it is able to colonize the wood under field conditions, as well as its ability to remain effective in a broad range of temperature and moisture content. BCAs that are resistant to chemical wood preservatives can be used in combination with chemicals. In some agricultural systems, such integrated controls are as effective as chemical controls, but use only 10% the amount of chemicals.

Formulation of BCAs into marketable products is a challenge that has hardly been addressed in wood products. The biological pesticide must be packaged so that the living components remain viable under normal storage conditions for an acceptable period of time. Most biological pesticides marketed to date are composed of spores diluted with some sort of inert material that protect them from drying. Inclusion of selective nutrients that might give the BCA a head start, encapsulation of the spores to give a controlled release, and the inclusion of emulsifying agents to enhance the stability of suspensions should be considered. It has been suggested that with chemical pesticides, 50% of the research time is directed towards developing the optimum formulation. We have scarcely entered this phase in BC research for wood products.

THE FUTURE OF BIOLOGICAL CONTROL IN WOOD PRODUCTS

The title of this paper indicates my bias that BC may have some application for short term protection of wood. The implication is that I believe it will be of limited use for long term preservation. BCAs must not damage wood, yet if they are going to continue to live, produce antibiotics, and act as mycoparasites, there must be food for them. There is a limited supply of simple nutrients in wood. When these are depleted, the BC organism will become dormant and eventually die. Logic suggests, therefore, that BC can be effective only as long as the agent is alive, or as long as its antibiotics remain active in the wood. There is some evidence that some antibiotics are leached out of the wood or become detoxified after the BCA dies (57). This indicates to me that short term wood protection is a more attainable objective.

Pesticide science is changing. The environmental concerns of the public are manifested by a general distrust of chemicals, particularly those that are broad spectrum biocides. New legislation being considered by the federal government, particularly the Environmental Protection Act, will make field

testing and registration of all pesticides much more difficult. Biological control agents are not going to be a panacea for pesticide science. In fact, they will encounter the same problems that new chemicals will. Most BC systems will operate through the production of one or more antibiotics, and these toxins will be subject to the same scrutiny as the toxins in chemical preservatives. The advantage will be that the toxins will be produced where they are needed, in the wood, and will not be sprayed. They also presumably will be biodegradable.

Users of wood products have always battled against fungi, and persuasive arguments, combined with subtle reeducation, will be required before they will be willing to put beneficial fungi on their wood. Biological control systems will also face resistance from the public because many BCAs will be genetically engineered. Public attitude against the environmental release of genetically engineered organisms may be as strong as the fear of chemicals. Biological controls will also be evaluated using some criteria that do not apply to chemicals. Because they are living organisms, some BCAs may have the potential to cause human, animal and plant disease, or to cause allergic responses. Because they are capable of self propagation, they may spread in the environment, causing unforeseen side effects. For these reasons, BCAs will have to be carefully selected, and carefully evaluated.

At the very least, BC research will further our understanding of the ecology of wood and wood degradation. This understanding will help us protect and preserve wood better, whatever the technology. The wood preservation industry should not view BC as a threat, as a competing technology that will erode its market. BC will become one more weapon in our arsenal against wood degradation, used in harmony with the gentler chemical preservatives of the future.

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