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DECAY OF RED OAK: EFFECTS OF ANTAGONISTIC MICROFLORA ON WOOD DISCOLORATION AND THE EFFECT OF OXYGEN ON POLYPORUS COMPACTUS IN WOOD

The Ohio State University

Ph.D. 1982

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DECAY OF RED OAK: EFFECTS OF ANTAGONISTIC MICROFLORA ON WOOD DISCOLORATION AND THE EFFECT OF OXYGEN ON POLYPORUS COMPACTUS IN WOOD

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Thomas Johnson Hall, B.S., M.S.

* * * * *

The Ohio State University

1982

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Approved By: Adviser Department of Plant Pathology
This dissertation is dedicated to my parents, Jeanette Johnson King and Richard Laird Hall, D.V.M., whose love and support is deeply appreciated in all that I do, and to my wife, Sally Ann Lehmann Hall, whose love and devotion has aided me in bringing these studies to their conclusion.
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General: Forest and Urban Forest Pathology, Ornamental and Turfgrass Pathology, Biological Control of Plant Disease.

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INTRODUCTION

The biological deterioration of woody substrates is an important component of the carbon cycle in nature. In the forest, fallen trees, branches, and other organic debris are colonized by many types of fungi and bacteria, which use the organic residues for energy and anabolic syntheses while returning many organic skeletons and nutrients to the soil. The decay of wood and organic material is important to maintenance of soil structure, texture, and fertility. Economically, the activities of microbes on wood deterioration presents an undesirable situation in which wood products that are attacked and decayed may become hazardous and costly to replace. In living trees, decays alter the structure and physical properties of wood making the affected portions unsuitable for lumber, veneer, and other wood products. Decays can weaken a tree, making it vulnerable to wind damage, or reduce vigor, possibly affecting growth and resistance to attack by insects and disease.

In nature, communities of microorganisms are associated with wood decay in living trees (1, 3, 4, 6, 9, 25, 31, 32). The structure of these communities is dynamic, and changes in composition occur as the environment within decaying tissues is altered through physical, biological, and chemical processes (4, 25).

The manner in which a group of microorganisms affect wood discoloration and decay in living trees continues to be studied. In the past, isolation of microorganisms from columns of discolored and decayed wood has provided
the basis for the theory of successions of microorganisms and their involvement with wood decay (31, 32). Microorganisms colonize wounds, which expose the xylary and other tissues to the external environment. Freshly exposed woody tissues undergo a series of physiological changes associated with the host wound response, which may affect colonization by microorganisms (28, 30, 33). The first microorganisms to arrive at the wound and colonize the wood tissues are called "pioneer" microorganisms. However, the pioneers may not be the first group of organisms to affect discoloration and decay. Internal microbial residents at the wound surface may become active and be the first microbes to affect the exposed tissues. With time, the activities of internal residents, pioneer, and secondary colonizers, etc., affect the processes of discoloration and decay until the wound has healed (3, 31, 32). After a wound has healed, the microenvironment of the underlying tissues may return to a microaerophilic condition in which the activity of microorganisms confined to columns of discolored and/or decayed wood are reduced, or possibly stopped (11).

The fact that many types of microorganisms colonize and survive in woody substrates implies that a variety of interactions may occur between microbes (2, 4, 8, 23, 24, 37). These interactions may be beneficial or detrimental to the activities of some microbes, whereas others are not affected. The biological control of wood decays in trees may be facilitated by the presence of antagonistic microorganisms (2, 24).

Information relating the effects of specific microflora on wood decay and discoloration has been pursued, and examples of biological control have been demonstrated. Competitive exclusion of Heterobasidium annosum (Fr.) Bref. from cut pine stumps by inoculation with Peniophora gigantea
Masse has been successful in controlling the expansion of infection centers in the forest (27). Application of antagonists to pruning wounds may prevent or restrict the extent of colonization by decay fungi or other pathogens (5). Branch stub inoculation with *Trichoderma harzianum* Rifai has controlled silver leaf disease on plums caused by *Stereum purpureum* (Pers. ex Fr.) Fr. (12) and has been observed to affect colonization of wood by hymenomycetes in drill wounds on red maple (22, 34). Some fungi have been shown to hyperparasitize various decay fungi associated with decay in utility poles and other wood products (9, 12, 15, 26, 28, 37).

The interrelationships between microbes in woody substrates are affected by the microenvironment they inhabit. Fluctuations in temperature, moisture (11), nutrients (7, 16, 17, 18, 19, 20, 21, 35), and the gaseous environment (13, 14, 36) within a diseased or healthy tree will influence microbial growth and community structure. Many microbes, including decay fungi, are capable of growing and surviving in a microaerophilic environment and appear to be particularly suited to woody habitats (10, 36).

Understanding the nature of relationships between microbes in woody tissues and the control of wood decay requires knowledge of the following: 1) species composition and changes in composition over time, 2) species and community interactions over time, 3) environmental conditions associated with maintenance and changes in community structure, 4) effects of the host, and 5) physical environment on growth and interactions between microbes.

The following studies were designed to select potential decay antagonists using a variety of tests incorporating natural substrates. Field studies were used to evaluate the effects of selected antagonists and
decay fungi on wound closure and wood discoloration. In addition, the
effects of oxygen tensions on wood decay were studied, using a new method
that could be used to evaluate the effects of antagonists on wood
deterioration and to study interactions between microbes on woody substrates
in controlled gaseous environments.
INTRODUCTION

LITERATURE CITED


PART I

IN VITRO STUDY OF MICROORGANISMS ANTAGONISTIC TO WOOD DECAY FUNGI OF RED OAK

INTRODUCTION

Exposed woody tissues of trees are colonized by a variety of microorganisms. Xylophiles (8), microorganisms that colonize and survive on woody substrates, enter through wounds and become associated with wood discoloration and decay (4, 14, 15, 19, 20, 21, 24). The first organisms to invade wounds are the "Pioneers". They are followed by secondary colonizers, which may displace the pioneers to form a successional pattern. Microbial successions and their relationship to wood discoloration and decay in trees, litter, and wood products have been reviewed (9, 14, 15, 21, 24). The subject of microbial communities in tree wounds has been studied in a variety of tree species (1, 2, 3, 4, 6, 19, 20, 22, 23). Apparently the type of wound, its age, location, and time of occurrence affect species composition of invading microorganisms (4, 6, 14). After the invasion of wounds by pioneers, other microbes enter and leave the community as competition for space and nutrients intensifies (14, 16), and a successional pattern is established. At some time hymenomycete decay fungi enter, and this may lead to extensive decay.

Manipulation of xylophilic communities may influence the successional pattern of microorganisms in a manner that prevents, restricts, or promotes
wood decay. The application of specific microbial antagonists to prevent colonization of wounds by decay fungi has been tried (5, 7). Rishbeth (17, 18) used *Peniophora gigantea* (Fr.) Masse to competitively preempt *Heterobasidion annosum* (Fr.) Bref. from freshly cut pine stumps, preventing establishment of infection centers. Spore suspensions of *Trichoderma harzianum* Rifai applied to fresh pruning wounds on plum reduced infection by *Stereum purpurium* (Pers. ex Fr.) Fr. (7). Similarly, wound inoculation with *T. harzianum* on red maple prevented hymenomycete fungi from colonizing treated wounds (13, 25, 26). Several species of *Scytalidium* are hyper-parasitic on a variety of decay fungi and were effective in controlling decay in wood block tests and utility poles (8, 9, 10, 27).

The objective of this study was to isolate xylophilic microorganisms from red oak (*Quercus rubra* L.) wounds and to test them for *in vitro* antagonism to *Polyporus compactus* Overh. and other red oak decay fungi.

**MATERIALS AND METHODS**

*Isolation of microflora.* Microflora were isolated from red oak branch stub wounds made at different times of the year. Microbes were tested *in vitro* for antagonism to *Polyporus compactus* Overh. (*Pc*) on agar media and natural woody substrates.

Branches of red oak trees (4-8 cm diam) were removed at forks, leaving 15-20 cm stubs (Figure 1). Four, 16, and 14 stubs were made in August, October, and March, 1979-80, respectively, throughout the crowns at different heights (2-9 m) and aspects. After exposure for 7 wk (Figure 1), stubs were removed and placed in polyethylene bags for storage at -20 C until assayed for antagonistic microflora.
Figure 1. Fresh red oak (*Quercus rubra* L.) branch stub (Top) made in April and (Bottom) prior to harvesting after seven weeks exposure.
Wedge printing procedure. Ten disks, 6-8 mm thick, were cut serially from the exposed end of each frozen stub. The bark was removed, and each disk was quartered into wedges, which were refrozen. One frozen wedge from each disk was surface sterilized by immersing in boiling water for 6 sec (11, 12) and placed on a glass rod in a sterile petri dish containing a piece of wet (ca. 4.5 ml water) cotton gauze (1.8 cm x 3.5 cm x 0.6 cm, folded and stapled). Dishes (called "hydrator dishes") were sealed with Parafilm-M (American Can Co., Greenwich, Ct. 06830) and incubated at 24 C in a lighted room for 40-48 hr.

After incubation, the top and bottom wedge surfaces were printed (pressed for 2 min) onto the surface of oak-diffusate agar (ODA). Wedges were returned to hydrator dishes for further observation. ODA was prepared by soaking three debarked frozen red oak disks (4-5 cm diam x 0.8-10 mm) in 1 L of glass-distilled water at 7 C for 24-36 hr. Disks were removed, and powdered agar (17 g, Difco Laboratories, Detroit, Michigan 48232) was added. After autoclaving at 121 C at 2 atm for 20 min, the medium was dispensed into petri dishes (20 ml/dish). After printing each plate was seeded with an agar plug (8 mm diam) of Pc placed 1 cm from the perimeter of a wedge print in proximity to the heartwood portion. Seeded wedge prints were sealed and incubated at 24 C in continuous light for 6 days to allow wedge print microflora to grow and interact with Pc. Bacteria, yeasts, and fungi that inhibited the growth of Pc were isolated and placed in culture tubes of ODA and stored at 7 and 24 C. Pc plugs were taken from 2-3 wk old cultures on malt extract agar (malt extract, 30 g/L; glucose, 5 g/L; and agar, 20 g/L) incubated at 24 C.
Wood disk method. Decay antagonists were applied to wood disks and, after a brief incubation period, were inoculated with Pc. These tests were used to determine if an antagonist could prevent the decay fungus from colonizing the disk surface or penetrating through it.

Frozen (-20 C) debarked red oak wood disks (ca. 4-6 cm diam x 6-8 mm thick) composed of healthy sapwood and a well-defined heartwood were surface sterilized as described above and placed in a hydrator dish. A suspension (ca. 1 ml) of an antagonist was applied to one half of the top of each of three disks by means of a sterile cotton swab. Suspensions were made by crushing six agar plugs (8 mm diam) from a 2-wk-old malt extract culture (24 C and continuous light) of each antagonist in 5 ml of sterile glass-distilled water. Dishes were sealed and incubated at 24 C for 40-48 hr to allow antagonists to colonize the disks (Figure 2). The control and antagonist-treated sides of disks were each inoculated with one agar plug of Pc placed at the sapwood-heartwood boundary. Dishes were resealed, incubated at 24 C, and data were taken after 7, 10, and 14 days.

Sawdust method. In these tests, the antagonist in a sand-sawdust carrier (SSC) was layered along the center of a petri dish containing malt extract agar. Agar plugs of various decay fungi were seeded on each side of the SSC barrier, and the effect of the antagonist on the growth of the decay fungi was noted after incubation at 24 C and continuous light for 1 wk.

Fresh red oak sawdust (1.4 mm sieve) containing sapwood, heartwood, and bark was dry sterilized at 72 C for 1 wk (tests showed that all microorganisms were killed by this treatment when sawdust was placed on malt extract agar). Twenty cc of sawdust was mixed with 10 cc of sterile quartz sand (Millwood, silica sand, grade 7), placed in a petri dish, and dried
Figure 2. The wood disk method for assessing antagonist properties of microbes against *Polyporus compactus* Overh. Red oak wood disk (A), with sapwood (SW) and heartwood (HW), placed in petri dish with hydrator pad (H). Suspensions of antagonists (At) were applied to the left half of wood disks (B) and incubated at 24 C for 40-48 hr. Agar plugs of a decay fungus (DF) were placed at the sapwood-heartwood boundary (C) to test for antagonism. Plates were sealed and observed for growth of the decay fungus on the disk surface and through the disk.
again at 72°C (4 days). A 10 ml water suspension of an antagonist was added to a dish of SSC. Suspensions were made by crushing six malt extract agar plugs, 8 mm diam, of a 3-wk-old culture in 10 ml of water. Dishes were sealed and incubated at 24°C in a lighted room for 1 wk. Dish contents then were allowed to air-dry.

Portions of the SSC were placed on fresh malt extract agar to form a physical barrier (9 cm long x 2 cm wide) on the central axis of the culture dish. A plug from a 2-wk-old culture of a decay fungus was placed on each side of the SSC barrier, but not in contact with the SSC. The following decay fungi were used: Polyporus compactus Overh., Xylobus frutulatus (Pers. ex Fr.) Boidin (Stereum frutulatum), Laetiporus sulphureus (Bull. ex Fr.) Bond. & Sing. (Polyporus sulphureus), and Stereum gausapatum (Fr.) Fr. Dishes were sealed and incubated at 24°C in a lighted room.

Temperature studies. Antagonists were tested at 9, 16, 20, 24, and 28°C to determine the effectiveness of antagonists against Pc in wood disks, as described above. Six disks were inoculated with a suspension of each antagonist and a water control. Three disks from each treatment were incubated at 24°C for three days prior to inoculation with Pc. The remaining three disks from each treatment were incubated for 3 days at each test temperature and inoculated with Pc. Hydrator dishes were sealed and incubated in the dark at constant temperature for each regime. Observations were made at 9, 18, and 32 days.

Residual inhibition method. The wood disk evaluation procedure described earlier was used to test for heat-stable chemicals produced by antagonists that might be associated with inhibition of decay fungi. Each of six red oak disks was inoculated with a suspension of an antagonist, a water-treated
control, or a non-treated control. Eleven antagonists were tested (five isolates from autumn wounds and six from spring wounds). Inoculated and control disks were incubated for 2-wk at 24 C in a lighted room. After the incubation period, three disks from each treatment were placed in an autoclave and sterilized at 121 C at 2 atm for 20 min. A second group of three disks per treatment was placed in a drying oven and sterilized at 72 C for 4 days. After sterilization, each set of disks was inoculated with agar plugs of Pc and incubated at 24 C. Observations were made at 7 and 14 days. Tests were repeated.

RESULTS

Microflora from branch stubs. Xyophilic bacteria, fungi, and yeasts were isolated from sapwood and heartwood in red oak branch stubs to a depth of 8-10 cm. Also, two apparently healthy control branches were cut and at once examined for resident microflora antagonistic to Pc and other wood decay fungi. Only bacteria and yeasts were isolated from these controls and some bacterial antibiosis toward Pc was noted, but eventually Pc replaced the antagonists. Thus, resident xylophiles could be found in healthy sapwood and heartwood, but antagonistic interactions were infrequent and short-lived. Although not reported in detail, it was noted that microflora from branch stubs were more diverse than found in these healthy controls.

More than four hundred isolates were found to be antagonistic to Pc using oak-diffusate agar wedge print tests; the decay fungus did not colonize agar occupied by antagonists after 6 days at 24 C. The following genera of fungi were frequently isolated: Alternaria, Cladosporium, Epicoccum, Fusarium, Gliocladium, Macrophoma or Phoma, Penicillium, and
Trichoderma. Many non-sporulating antagonistic fungi and yeasts also
were isolated but were not readily identifiable. Most yeasts had black
or white mycelia. Bacteria, yeasts, and fungi were isolated from sapwood
and heartwood. Sapwood and the sapwood-heartwood boundary provided more
isolates than heartwood. Bacteria were most frequently isolated from
sapwood. Yeasts and fungi were frequently isolated from sapwood, from
the sapwood-heartwood boundary, and sporadically from heartwood.

Microbial interactions in vitro. Three in vitro tests were used to screen
and select for antagonists to wood decay fungi (11) associated with red
oak. Primary isolation of antagonists was made from wedge prints on
oak-diffuse agar (ODA), and final selections were based on additional
disk and sawdust tests.

The types of microorganisms and the forms of antagonism observed are
described in Table 1, which shows the sequential distribution of successful
antagonists for each assay. Of four hundred twenty-three initial isolates,
only seventeen were capable of stopping or preventing Pc and the other
decay fungi from colonizing woody substrates.

Zones of inhibition (antibiosis) and competitive exclusion were the only
forms of antagonism found in wedge print tests. Some bacteria produced zones
of inhibition on ODA that completely excluded Pc. This usually was temporary;
the decay fungus mycelium colonized the inhibition zone and eventually
replaced bacterial antagonists. However, twenty-three bacterial isolates
produced zones that were not replaced by Pc mycelium.

Competitive exclusion of Pc was found with fungal and yeast antagonists.
Pc did not grow into an area occupied by these organisms. Some xylophiles
temporarily prevented Pc from colonizing ODA wedge prints. Frequently, Pc
Table 1. Forms of antagonism to *Polyporus compactus* Overh. induced by bacteria, fungi, and yeasts in sequential screening tests.

<table>
<thead>
<tr>
<th>Form of antagonism</th>
<th>Description</th>
<th>Type</th>
<th>Primary isolation from wedge prints</th>
<th>Wood disk tests</th>
<th>Sawdust tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiosis</td>
<td>Zones of mycelial growth inhibition</td>
<td>Bacteria</td>
<td>23</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hyperparasitism</td>
<td>Antagonist parasitic on the decay fungus</td>
<td>None observed</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Exclusion</td>
<td>Competitive occupation of substrate</td>
<td>Fungi</td>
<td>274</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yeasts</td>
<td>126</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Replacement</td>
<td>Temporary exclusion followed by the displacement of an antagonist by the decay fungus</td>
<td>Fungi</td>
<td>-</td>
<td>74</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yeasts</td>
<td>-</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>Deadlock</td>
<td>Decay fungus growth restricted to a limited area of substrate by an antagonist</td>
<td>Fungi</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yeasts</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>423</strong></td>
<td><strong>93</strong></td>
<td><strong>17</strong></td>
</tr>
</tbody>
</table>

a) Sequence of tests: wedge prints (one wedge from each of ten successive disks for each stub), wood disks (three disks per isolate and repeated once), sawdust (three plates per isolate and repeated once).
mycelium would encroach into an area occupied by a xylophile and replace the antagonist. Some exclusion antagonists exhibited a zone of interaction with \( \text{Pc} \) in which the mycelia of both organisms contacted each other and growth appeared to stop. In some cases, where the mycelia of the two organisms met, a pigmented zone was formed. The zone was composed of mycelia of both fungi, which appeared to be inflated, thick-walled, and deeply pigmented. \( \text{Pc} \) did not grow beyond this zone and did not replace the antagonist.

Examples of forms of antagonism indicated in Table 1 are further described by photographs (Figures 3-7 and 9-12). Isolates 10-4 and 22-22 exhibited a weak antagonism with \( \text{Pc} \) on malt extract agar (Figure 3). The two mycelia touched, but neither appeared to grow further. \( \text{Pc} \) did not colonize agar occupied by antagonists in the sawdust carrier (SSC). Antagonist isolates 17-10 and 21-1 exhibited a moderate reaction to \( \text{Pc} \) (Figure 4). The mycelia of \( \text{Pc} \) and the antagonists were in contact, and a zone of pigmentation between them had formed. Apparently, the zone formed as a result of a physiological interaction between \( \text{Pc} \) and the antagonists and resembled zone lines that form in wood between two species of decay fungi (15, 16). Again, the organisms had contacted each other and \( \text{Pc} \) growth had ceased. Antagonist isolates 7-4 and 18-1 exhibited an intense zone of interaction with \( \text{Pc} \) (Figure 5). The zone was darker and better defined than in Figures 3 and 4. The organisms were in contact and neither microbe appeared to grow beyond the point of contact.

The interactions between \( \text{Pc} \) and \( \text{X. frustulatus} \), and \( \text{Pc} \) and the \( \text{Pc} \)-inoculated control carrier are shown in Figure 6. There was no effect between the SSC inoculated with \( \text{Pc} \) and \( \text{Pc} \) mycelium from agar plugs. The SSC was
Figure 3. Exclusion antagonists (Top, Isolate 10-4; Bottom, Isolate 22-22) in a sand-sawdust carrier placed on malt extract agar showing a weak zone of interaction with *Polyporus compactus*. 
Figure 4. Exclusion antagonists (Top, Isolate 17-10; Bottom, Isolate 21-1) in a sand-sawdust carrier placed on malt extract agar showing a moderate zone of interaction with *Polyporus compactus*. 
Figure 5. Exclusion antagonists (Top, Isolate 7-4; Bottom, Isolate 18-1) in a sand-sawdust carrier placed on malt extract agar showing an intense zone of interaction with *Polyporus compactus*. 
Figure 6. Wood decay fungi (Top, *Polyporus compactus*; Bottom, *Xylobus frustulatus*) in a sand-sawdust carrier placed on malt extract agar showing the types of interaction with *P. compactus*. *X. frustulatus* appears to be excluding *P. compactus* from the carrier and no replacement is evident.
readily colonized by Pc and no interaction occurred. X. frustulatus was antagonistic to Pc, but the interaction appeared to be weak, similar to that observed in Figure 3. Trichoderma (one isolate) appeared to prevent growth of Pc entirely (Figure 7). Trichoderma rapidly colonized the medium and the SSC and competitively excluded Pc from the substrate occupied by the antagonist. Microscopic examination of areas of mycelial contact revealed that hyperparasitism did not occur between Pc and Trichoderma, or with any of the antagonists used in these studies. The sterile SSC was readily colonized by Pc with no inhibition of growth.

Antagonist inhibition of decay fungi in wood disks. Bacteria, fungi, and yeasts from wedge prints on ODA were tested against Pc on red oak disks. Ninety-three isolates were antagonistic to Pc in disks. Thus, Pc did not appear to colonize woody tissues occupied by an antagonist, or, if Pc did colonize the upper surface of a wood disk, its mycelium did not appear to penetrate through the disk. Only one bacterium of twenty-three selected on the basis of wedge prints remained antagonistic to Pc on disks. These isolates colonized heartwood and sapwood, and many spread beyond the initial area of inoculation prior to the establishment of Pc on the control (untreated) side of disks. Effective antagonists prevented Pc from colonizing disks for up to 21 days.

Antagonists differed in their abilities to colonize sapwood or heartwood (Figure 8). Antagonists that failed to colonize heartwood or sapwood did not prevent Pc from colonizing and penetrating disks. Some fungal and yeast antagonists colonized both wood types, but mycelium density in heartwood was reduced. Most of the xylophilic microflora tested did not prevent Pc from colonizing and penetrating disks (Table 1). Some xylophiles
Figure 7. *Trichoderma* spp. in sand-sawdust carrier (Top) and sterile carrier (Bottom) placed on malt extract agar with *Polyporus compactus*. *Trichoderma* spp. prevented *P. compactus* from growing whereas the sterile carrier did not hinder growth and colonization of *P. compactus*. 
Figure 8. Red oak wood disks inoculated with antagonists and *Polyporus compactus*. Antagonists exhibited differences in their abilities to colonize sapwood and heartwood. Top row (left to right): *Fusarium*, *Phoma*, *P. compactus*. Bottom row: *Alternaria*, *Trichoderma*, unknown.
appeared to act as antagonists temporarily; eventually, encroachment and replacement by \textit{Pc} occurred. The mycelium of \textit{Alternaria} appeared to collapse and change color as \textit{Pc} replaced the antagonist (Figure 9). The mycelium of \textit{Pc} had penetrated through the xylem vessels and was colonizing the area occupied by the antagonist, a species of \textit{Alternaria} (Figure 10).

**Enriched medium and temperature studies.** In sawdust tests, encroachment followed by replacement (Figures 11 and 12) of antagonists by decay fungi occurred on malt extract agar tests, which were repeated twice. However, \textit{Pc}, \textit{L. sulphureus}, \textit{X. fruticulatus}, and \textit{S. sausapatum} did not colonize the agar or SSC that was occupied by any of sixteen selected antagonists that were effective in wedge prints and disks. Differences in the intensity of interactions between antagonists and decay fungi were found (Figures 13 and 14). Apparently, an organism that was effective against \textit{Pc} was antagonistic to the other decay fungi tested \textit{in vitro}.

Nine antagonists that were effective in disk and sawdust tests, were able to colonize and grow on wood disks and prevent or restrict the colonization of \textit{Pc} for up to 32 days when incubated at 28, 24, 20, 16, and 9 C. Two antagonists failed to prevent \textit{Pc} from penetrating disks after 32 days at 24 and 28 C. Sapwood and heartwood of water-inoculated control disks were colonized and penetrated by \textit{Pc} at all temperatures within 4 wk, and 6 wk at 9 C. There appeared to be little or no variation in the ability of selected fungal and yeast antagonists to colonize sapwood and heartwood. However, growth of antagonists and \textit{Pc} at 9 C was visibly reduced, compared with the other temperature regimes. These isolates were retained for field study because of their ability to grow on sapwood and heartwood over a wide temperature range, and their location within branch stubs (Table 2).
Figure 9. Interaction between *Polyporus compactus* and an isolate of *Alternaria* on the upper surface of a red oak wood disk in which the mycelium of *Alternaria* is colonized by the decay fungus (Top) followed by collapse of the mycelium as encroachment and eventual replacement by *P. compactus* occurs (Bottom).
Figure 10. Bottom surface of a red oak wood disk in which *Polyporus compactus* has grown through the wood disk in ray parenchyma (Top, circle) and xylem vessels (Bottom, circle). Collapse of *Alternaria* mycelium is due to the encroachment of the decay fungus into the area occupied by *Alternaria*. 
Figure 11. Encroachment by *Polyporus compactus* and *Stereum gausapatum* on an isolate of *Alternaria* suspended in a sand-sawdust carrier placed on malt extract agar and challenged with agar plugs of (Top, left) *P. compactus*, (Top, right) *Xylobus frustulatus*, (Bottom, left) *Laetiporus sulphureus*, and (Bottom, right) *S. gausapatum*. 
Figure 12. Replacement of an *Epiccocum* isolate suspended in a sand-sawdust carrier placed on malt extract agar by *Polyporus compactus* (Left). *P. compactus* is colonizing the sawdust and causing it to become decolorized (bleached to a lighter color).
Figure 13. Exclusion antagonist (Isolate 10-4) suspended in a sand-sawdust carrier placed on malt extract agar and challenged with agar plugs of (Top, left) *Polyporus compactus*, (Top, right) *Xylobus frustulatus*, (Bottom, left) *Laetiporus sulphureus*, (Bottom, right) *Stereum gausapatum*. Decay fungi mycelial growth has stopped upon contact with the antagonist.
Figure 14. Exclusion antagonist (Isolate 7-4) suspended in a sand-sawdust carrier placed on malt extract agar and challenged with agar plugs of (Top, left) *Polyporus compactus*, (Top, right) *Xylobus frustulatus*, (Bottom, left) *Laetiporus sulphureus*, and (Bottom, right) *Stereum gausapatum*. Decay fungi mycelial growth has stopped upon contact with the antagonist.
Table 2. Location of wood decay antagonists in branch stubs which were suitable for testing in red oak branch wounds.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Time collected</th>
<th>Type</th>
<th>Depth (cm)</th>
<th>Wood type</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-4</td>
<td>Autumn</td>
<td>Yeast</td>
<td>4</td>
<td>Heartwood</td>
</tr>
<tr>
<td>7-4</td>
<td>Autumn</td>
<td>Fungus</td>
<td>4</td>
<td>Sapwood</td>
</tr>
<tr>
<td>1-L</td>
<td>Autumn</td>
<td>Trichoderma</td>
<td>6</td>
<td>Sapwood</td>
</tr>
<tr>
<td>5-1</td>
<td>Autumn</td>
<td>Fusarium</td>
<td>1</td>
<td>Sapwood</td>
</tr>
<tr>
<td>1-T</td>
<td>Autumn</td>
<td>Fusarium</td>
<td>10</td>
<td>Sapwood</td>
</tr>
<tr>
<td>17-5</td>
<td>Spring</td>
<td>Yeast</td>
<td>5</td>
<td>Heartwood</td>
</tr>
<tr>
<td>17-10</td>
<td>Spring</td>
<td>Yeast</td>
<td>10</td>
<td>Heartwood</td>
</tr>
<tr>
<td>18-1</td>
<td>Spring</td>
<td>Fungus</td>
<td>1</td>
<td>Heartwood</td>
</tr>
<tr>
<td>21-1</td>
<td>Spring</td>
<td>Fungus</td>
<td>1</td>
<td>Sapwood</td>
</tr>
<tr>
<td>22-2₁</td>
<td>Spring</td>
<td>Yeast</td>
<td>2</td>
<td>Sapwood</td>
</tr>
<tr>
<td>22-2₂</td>
<td>Spring</td>
<td>Yeast</td>
<td>2</td>
<td>Sapwood</td>
</tr>
</tbody>
</table>
Antagonists failed to produced heat-stable chemicals inhibitory to decay fungi. Regardless of the method of sterilization or treatment, \( P_c \) colonised and penetrated disks within seven days after inoculation, indicating no heat-stable chemicals inhibitory to decay fungi were produced.

**DISCUSSION**

Large numbers of filamentous fungi, yeasts, and bacteria were observed in agar wedge prints of disks from autumn and spring branch stubs in red oak. These microorganisms were isolated from sapwood and heartwood at varied depths within branch stubs. Among these organisms, more than four hundred isolates were antagonistic to \( P. \) compactus. Of these, eleven isolates were selected for field studies by means of a series of laboratory tests, which included diffusate agar, wood disks, sawdust on enriched agar, and wood disks at various incubation temperatures.

Some fungi and yeast isolates colonized sapwood and heartwood and prevented \( P_c \) from colonizing and penetrating through red oak wood disks over a broad temperature range. The principal form of antagonism appeared to be competitive exclusion of decay fungi. Bacteria produced diffusible inhibitors in agar, but, except for one isolate, were not effective on wood disks. At temperatures below 16 C, bacterial growth was greatly retarded; therefore, the utility of bacteria as decay antagonists appears to be more limited than that of fungal and yeast antagonists. Decay fungi, and yeast and fungal antagonists, grew and colonized disks from 9 to 28 C.

Fungal and yeast antagonists did not produce diffusible inhibitors to decay fungi in disk or sawdust tests. These inhibitors were observed with bacteria in wedge print tests. But this effect was temporary and decay
fungi eventually colonised and replaced many bacteria. Competitive exclusion was the predominant form of antagonism observed in agar and disk tests. Hyperparasitism was not observed in agar tests and antibiosis was not effective in disk tests.

One of the aims of this study was to develop a procedure for isolating xylophilic microorganisms from woody substrates that would be rapid and accurately reflect the location of microflora within a wood sample. Large numbers of microorganisms could be isolated from wood using the wedge print method on diffusate agar, and their location within a sample could be noted. The traditional method of placing wood chips on enriched agar media may not accurately reflect the microbial diversity within a wood sample and might mask the presence of slow-growing and fastidious microorganisms.

The nature and complexity of microbial communities inhabiting exposed woody tissues of trees continues to be an important area for study by pathologists and microbial ecologists. Application of microbial antagonists to a fresh wound may afford a degree of protection from attack by wood decay fungi and other pathogens (5). The procedures used in this study provided a simple and sequential means of sampling woody tissues for xylophiles antagonistic to Pc and other wood decay fungi. The wedge print test and wood disk evaluation procedure (11, 12) provide a means for acquiring an accurate representation of the xylophilic microorganisms that colonize woody tissues associated with wounds on trees under differing environmental conditions. These methods may be useful for characterizing residents and differentiating between pioneers, residents, and secondary colonizers. They may be more efficient for isolation of xylophiles than
the traditional method of isolation from wood chips on enriched agar media, and serve as a more rigorous tool for studying xylophilic communities in trees.
PART I

LITERATURE CITED


The processes of wood discoloration and decay in trees are the result of many complex interactions among the host (33, 37, 39, 40), resident microorganisms (1, 2, 5, 7), invading xylophilic microorganisms (1, 2, 3, 4, 6, 17, 24, 25, 26), and the environment (12, 13, 15, 18). These interactions may occur for a rather short period of time with respect to the lifespan of a tree, or they may progress until the tree dies. Exposure of xylary tissues through wounding permits entry of a variety of pioneer microorganisms (4, 8, 12, 29, 30, 31) which become associated with wood discoloration and may influence decay (5, 7, 9, 26). Successions of microorganisms begin with the entry of pioneer xylophiles through wounds and appear to be involved with alteration of woody tissues leading to decay. In time, many microorganisms become associated with columns of discolored wood and can be found at different locations within these columns (1, 2, 3, 4, 7, 11, 15). Communities of microorganisms are dynamic, and in time changes in species' composition occurs (4, 9, 12, 19, 25, 26, 34, 35).

Xylophiles differ in their abilities to utilize woody tissues as an energy source (21). Bacteria, soft-rot fungi (Fungi Imperfecti), and wood decay fungi (usually hymenomycetes) are able to chemically and
physically alter woody substrates (5, 6, 7). The degree to which the wood fiber is altered depends on the capability of the xylophiles to degrade the polymer and its impregnating chemicals (21, 39). As competition for nutrients intensifies, changes in the microbial community structure occur (19, 26), allowing xylophiles of different wood deterioration capabilities to replace initial inhabitants. Eventually, xylophagus fungi (wood-decaying hymenomycetes) bring about the deterioration of wood polymers to the point at which the chemical and physical structure has been severely altered (16, 17). These changes in wood structure lead to the formation of defects which may affect the health and vigor of the tree.

Preventing entry or restricting the extent of wood colonization by decay fungi may reduce the amount of damage caused by these fungi. Establishing antagonistic microflora to wood decay fungi in wounds has been tried (10, 22, 23, 42). Biological control of wood decay by competitive exclusion was accomplished with *Paniophora gigantea* (Fr.) Masse to control stump colonization by *Heterobasidium annosum* (Fr.) Bref. (20, 27, 43). Hyperparasitism of wood decay fungi with species of *Scytalidium* has been utilized to control wood decay in wood products (20, 27, 43). *Trichoderma harzianum* Rifai has been used to exclude hymenomycetes from pruning wounds on plum (10) and drill wounds in red maple (22, 23, 24), to prevent stump colonization by *H. annosum*, and to maintain wood product quality (14).

The studies reported here were designed to examine the effects of *in vitro* decay antagonists selected on the basis of a series of experiments (Part I) associated with discoloration in field tests. Wood sections from field studies were used to determine the effects of antagonists on the *in vitro* colonization of sapwood, heartwood, and discolored wood (as a result of drill...
wounds used for these studies) in red oak-pin oak hybrids. Antagonists had been isolated from red oak branch stubs and were effective, in vitro, against *Polyporus compactus* Overh., *Xylobus frustulatus* (Pers. ex Fr.) Boidin, *Laetiporus sulphureus* (Bull. ex Fr.) Bond & Sing., and *Stereum gausapatum* (Fr.) Fr.

**MATERIALS AND METHODS**

**Field test procedure.** Field tests in Wooster, Ohio, were made on street trees that appeared to be red oak or red oak-pin oak hybrids. They were approximately 1 to 1.5 m DBH and 20-25 m tall. Four trees were used for an autumn study using five selected antagonists isolated from autumn branch stubs. *P. compactus* Overh. (Pc) also was included in these tests. In the spring, six trees were inoculated with six antagonists from spring branch stubs plus the five autumn antagonists and two decay fungi (Pc and *Xylobus frustulatus* (Pers. ex Fr.) Boidin).

**Wound procedure.** For inoculations (Figure 15), an electric drill was used to make paired wounds (12.4 mm diam x 4-6 cm deep) at each of three locations (1 m apart) along the horizontal axis of branches (ca. 6-20 cm diam). Wounds at each position were in the same vertical plane and aspect (Figure 15). The upper wound of a pair was a non-inoculated control, whereas the lower wound was either treated with an antagonist, a decay fungus, a sterile sawdust carrier, or left as a non-inoculated control. Treatment comparisons were made between upper and lower wounds of a given "wound pair".

**Inoculum preparation.** Individual antagonists or decay fungi were grown on a sterile mixture of quartz sand (Millwood, #7) and sieved red oak sawdust
Figure 15. A) Position of horizontal, paired drill wounds (12.4 mm diam) on red oak branches (6-20 cm diam). B) Cross-section through a wound pair showing the relationship of control and treated wounds to sapwood and heartwood. Treatment comparisons were made within a given wound pair.
(1.4 mm). The sand-sawdust carrier (SSC) was prepared as follows: 200 cc of dry sterile (72 C for 4 days) fresh sawdust (composed of sapwood, heartwood, and bark) and 100 cc of sand were placed in 2.1-L canning jars and mixed thoroughly. A suspension of each antagonist or decay fungus was prepared by suspending six malt extract agar (malt extract, 30 g/L; glucose, 5 g/L; and agar, 20 g/L) plugs (8 mm diam) from a 2-wk-old culture in petri dishes (24 C and continuous light) in 10 ml of sterile distilled water. Suspensions were mixed by vortexing for 3 min, brought to 100 ml with distilled water, stirred, and added to individual jars containing SSC. Jars were sealed and gently rotated on a "Rollacell" apparatus (New Brunswick Scientific, New Brunswick, N. J.) for 1 wk at 24 C and continuous light. After incubation, each jar lid was replaced with two layers of sterile paper toweling and again rotated until the SSC had dried, usually within 10-14 days. Jars were aseptically sealed and stored at 5 C.

For autumn studies, three branches per tree were used to examine eight treatments (eight wound pairs/tree). For spring studies, five branches per tree were used to examine fifteen treatments. A piece of black polyethylene plastic was wrapped loosely around each pair to reduce washing by water and to aid wound closure (41). After one growth period and before trees broke dormancy the following season, branches were removed and partitioned as described below.

Sample preparation. After carefully recording branch orientation, branch portions (Figure 16), cut approximately 30 cm on either side of a wound pair, were brought to the laboratory and stored at 5 C until dissections could be made. For each wound pair, the extent of wound closure and lengths of discolored wood columns associated with each wound were recorded.
Figure 16. Branch portion showing position of transverse saw cuts through and adjacent to wounds to provide wood disks (1 cm thick and 6-20 cm diam) for in vitro colonization studies. Disks transecting wounds and neighboring disks are called "wound disks" (WD) and "adjacent disks" (AD), respectively. Discolored wood within branch usually extended from wounds as shown above. C (upper) = control wound; T (lower) = treated wound.
Discoloration columns were measured by splitting each branch section longitudinally after removing wood disks (for colonization studies). Wood disks were removed as follows: Saw cuts were made to provide two wood disks (1 cm thick) transecting wounds (Figure 16) and two adjacent disks. Disks contained discolored wood associated with control and test (inoculated) wounds (Figure 17). Disks were cut into two quarters (Figure 17, B), one containing discolored wood from the test wound, the other containing discolored wood from the control wound. Each section contained healthy sapwood and heartwood in addition to discolored sapwood and heartwood. Sections were placed in petri dishes and stored in a plastic bag at -20 C until they were inoculated with feeder strips colonized by \textit{P. compactus}.

Discoloration column lengths for each wound pair were measured. Data for each wound pair were given as ratios. Similar ratios were obtained for wound closure measurements to indicate the extent of healing.

\textbf{Wood colonization tests.} Frozen wood quarters (Figure 17, B) were surface sterilized by immersing in boiling water for 6 sec and placed in a sterile petri plate containing a gauze pad saturated with 4.5 ml of sterile water. Hydrator dishes were sealed and incubated at 24 C for 40-48 hr to give microbial residents time to grow within discolored wood, sapwood, and heartwood before inoculation with \textit{Pc}. Following incubation, the upper and lower surfaces of each disk section were printed on oak diffusate agar (with 100 mg chlorotetracycline/L) to locate xylophilic microorganisms (See Part I). At the same time, prints were inoculated with an agar plug of \textit{Pc}, and sections were returned to hydrator dishes and inoculated with feeder strips colonized by \textit{Pc} placed as indicated in Figure 17.
Figure 17. Wood disk (A) from a branch section of red oak showing sapwood, heartwood, and discolored wood associated with control and treated wounds. Disk section used in colonization tests (B) showing placement of wood feeder strips colonized by *Polyporus compactus* Overh. Each feeder strip touches heartwood, sapwood, and discolored wood.
RESULTS

Effects on wound closure. The extent of wound closure for spring inoculations using antagonists isolated from spring and autumn branch stubs is presented in Tables 3 and 4. The effect of treatments on closure was compared to closure in adjacent controls for each wound pair. For comparison, closure in the inoculated hole (Bottom) was divided by closure in the control hole (Top) to give a wound closure ratio for each wound pair. Tables 3 and 4 list the ratio means for each treatment used in spring and autumn inoculations, respectively. There were no significant differences due to wound position along the branch axis or between branches and trees. In spring inoculations, wound closure was reduced significantly (P= 0.05) with antagonist isolates 1-T, 17-10, and 22-22 (Table 3). Isolate 1-T, a species of *Fusarium*, caused cambial death in excess of the initial drill wound diameter in several wounds and formed cankers. Autumn inoculations, using antagonists from autumn branch stubs and *Pc*, did not affect wound closure significantly (Table 4). Comparison of closure data for treatments used in autumn and spring inoculations (Table 5) indicated that closure was reduced significantly (P= 0.05) by spring inoculation with isolate 1-T and was not influenced by the other treatments.

Effects on discoloration. In spring tests, columns of discolored wood were found to extend farther from wounds inoculated with antagonists, the two decay fungi (*Pc* and *X. fraxinulatus*), and SSC than from non-inoculated controls (Table 6). Columns of discolored wood were composed of young and old sapwood and heartwood. As expected, columns diminished in width along the longitudinal axis, and older tissues were less resistant to discoloration.
Table 3. Comparison of wound closure\(^a\) in treated wounds to control wounds for wound pairs\(^b\) in red oak inoculated with selected antagonists and decay fungi, spring inoculations\(^c\).

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Time collected</th>
<th>Type</th>
<th>Category</th>
<th>Ratio(^d) means</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-4</td>
<td>Autumn Yeast</td>
<td>Antagonist</td>
<td>0.93 x</td>
<td></td>
</tr>
<tr>
<td>7-4</td>
<td>Autumn Fungus</td>
<td>Antagonist</td>
<td>1.00 x</td>
<td></td>
</tr>
<tr>
<td>1-L</td>
<td>Autumn Trichoderma</td>
<td>Antagonist</td>
<td>0.92 x</td>
<td></td>
</tr>
<tr>
<td>1-T</td>
<td>Autumn Fusarium</td>
<td>Antagonist</td>
<td>0.43 y</td>
<td></td>
</tr>
<tr>
<td>5-1</td>
<td>Autumn Fusarium</td>
<td>Antagonist</td>
<td>0.80 x</td>
<td></td>
</tr>
<tr>
<td>17-5</td>
<td>Spring Yeast</td>
<td>Antagonist</td>
<td>1.10 x</td>
<td></td>
</tr>
<tr>
<td>17-10</td>
<td>Spring Yeast</td>
<td>Antagonist</td>
<td>0.63 y</td>
<td></td>
</tr>
<tr>
<td>18-1</td>
<td>Spring Fungus</td>
<td>Antagonist</td>
<td>1.05 x</td>
<td></td>
</tr>
<tr>
<td>21-1</td>
<td>Spring Fungus</td>
<td>Antagonist</td>
<td>1.09 x</td>
<td></td>
</tr>
<tr>
<td>22-2(1)</td>
<td>Spring Yeast</td>
<td>Antagonist</td>
<td>0.82 x</td>
<td></td>
</tr>
<tr>
<td>22-2(2)</td>
<td>Spring Yeast</td>
<td>Antagonist</td>
<td>0.62 y</td>
<td></td>
</tr>
<tr>
<td>814</td>
<td>Polyporus compactus</td>
<td>Decay Fungus</td>
<td>0.92 x</td>
<td></td>
</tr>
<tr>
<td>815</td>
<td>Xylobus frustulatus</td>
<td>Decay Fungus</td>
<td>0.93 x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sawdust Carrier</td>
<td>(SSC)</td>
<td>1.00 x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>-</td>
<td>1.00 x</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Extent of callus formation associated with drill wounds (12.4 mm diam).
\(b\) See Figures 15 and 16.
\(c\) Inoculations made in May, 1980, and harvested in December, 1980.
\(d\) Ratios compare closure in treated holes to control holes for each wound pair. Means represent ratios for six wound pairs for each treatment. Means followed by the same letter are not significantly different (\(P=0.05\)).
Table 4. Comparison of wound closure\(^a\) in treated wounds to control wounds for wound pairs\(^b\) in red oak inoculated with selected antagonists and *Polyporus compactus* Overh, autumn inoculations\(^c\).

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Time collected</th>
<th>Type (Category)</th>
<th>Ratio(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-4</td>
<td>Autumn</td>
<td>Yeast Antagonist</td>
<td>1.10 x</td>
</tr>
<tr>
<td>7-4</td>
<td>Autumn</td>
<td>Fungus Antagonist</td>
<td>1.33 x</td>
</tr>
<tr>
<td>1-L</td>
<td>Autumn</td>
<td>Trichoderma Antagonist</td>
<td>1.15 x</td>
</tr>
<tr>
<td>1-T</td>
<td>Autumn</td>
<td>Fusarium Antagonist</td>
<td>1.12 x</td>
</tr>
<tr>
<td>5-1</td>
<td>Autumn</td>
<td>Fusarium Antagonist</td>
<td>1.03 x</td>
</tr>
<tr>
<td>814</td>
<td>-</td>
<td><em>Polyporus compactus</em> Decay Fungus</td>
<td>0.94 x</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Sawdust Carrier -</td>
<td>1.04 x</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Non-inoculated -</td>
<td>1.25 x</td>
</tr>
</tbody>
</table>

\(a\) Extent of callus formation associated with drill wounds (12.4 mm diam).

\(b\) See Figures 15 and 16.

\(c\) Inoculations made in October, 1979, and harvested in December, 1980.

\(d\) Ratios compare closure in treated holes to control holes for each wound pair. Means represent ratios for four wound pairs for each treatment. Means followed by the same letter are not significantly different (\(P=0.05\)).
Table 5. Comparison of wound closure<sup>a</sup> ratios<sup>b</sup> between spring and autumn inoculations<sup>c</sup> for wound pairs using selected decay antagonists from autumn branch stubs and Polyporus compactus Overh.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Type</th>
<th>Category</th>
<th>Spring inoculation</th>
<th>Autumn inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-4</td>
<td>Yeast</td>
<td>Antagonist</td>
<td>0.93 x</td>
<td>1.10 x</td>
</tr>
<tr>
<td>7-4</td>
<td>Fungus</td>
<td>Antagonist</td>
<td>1.00 x</td>
<td>1.33 x</td>
</tr>
<tr>
<td>1-L</td>
<td>Trichoderma</td>
<td>Antagonist</td>
<td>0.92 x</td>
<td>1.15 x</td>
</tr>
<tr>
<td>1-T</td>
<td>Fusarium</td>
<td>Antagonist</td>
<td>0.43 y</td>
<td>1.12 x</td>
</tr>
<tr>
<td>5-1</td>
<td>Fusarium</td>
<td>Antagonist</td>
<td>0.81 x</td>
<td>1.03 x</td>
</tr>
<tr>
<td>814</td>
<td>Polyporus compactus</td>
<td>Decay Fungus</td>
<td>0.92 x</td>
<td>0.97 x</td>
</tr>
<tr>
<td></td>
<td>Sawdust Carrier</td>
<td>-</td>
<td>1.00 x</td>
<td>1.04 x</td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>-</td>
<td>1.00 x</td>
<td>1.25 x</td>
</tr>
</tbody>
</table>

<sup>a</sup> Extent of callus formation associated with drill wounds (12.4 mm diam).

<sup>b</sup> Ratios compare closure in treated holes to control holes for each wound pair.

<sup>c</sup> Spring and autumn inoculations made in May, 1980, and October, 1979, respectively. All wound pairs harvested in December, 1980.

<sup>d</sup> Means followed by the same letter are not significantly different (P= 0.05). Comparisons apply to columns and rows.
Table 6. Comparison of wood discoloration\textsuperscript{a} in treated wounds to control wounds for wound pairs\textsuperscript{b} in red oak inoculated with selected antagonists and decay fungi, spring inoculations\textsuperscript{c}.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Time collected</th>
<th>Type</th>
<th>Category</th>
<th>Ratio Means\textsuperscript{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-4</td>
<td>Autumn</td>
<td>Yeast</td>
<td>Antagonist</td>
<td>2.18 x</td>
</tr>
<tr>
<td>7-4</td>
<td>Autumn</td>
<td>Fungus</td>
<td>Antagonist</td>
<td>2.52 x</td>
</tr>
<tr>
<td>1-L</td>
<td>Autumn</td>
<td>\textit{Trichoderma}</td>
<td>Antagonist</td>
<td>1.67 x</td>
</tr>
<tr>
<td>1-T</td>
<td>Autumn</td>
<td>\textit{Fusarium}</td>
<td>Antagonist</td>
<td>2.24 x</td>
</tr>
<tr>
<td>5-1</td>
<td>Autumn</td>
<td>\textit{Fusarium}</td>
<td>Antagonist</td>
<td>2.41 x</td>
</tr>
<tr>
<td>17-5</td>
<td>Spring</td>
<td>Yeast</td>
<td>Antagonist</td>
<td>1.57 x</td>
</tr>
<tr>
<td>17-10</td>
<td>Spring</td>
<td>Yeast</td>
<td>Antagonist</td>
<td>1.68 x</td>
</tr>
<tr>
<td>18-1</td>
<td>Spring</td>
<td>Fungus</td>
<td>Antagonist</td>
<td>1.92 x</td>
</tr>
<tr>
<td>21-1</td>
<td>Spring</td>
<td>Fungus</td>
<td>Antagonist</td>
<td>1.79 x</td>
</tr>
<tr>
<td>22-21</td>
<td>Spring</td>
<td>Yeast</td>
<td>Antagonist</td>
<td>2.32 x</td>
</tr>
<tr>
<td>22-22</td>
<td>Spring</td>
<td>Yeast</td>
<td>Antagonist</td>
<td>2.27 x</td>
</tr>
<tr>
<td>814</td>
<td>-</td>
<td>\textit{Polyporus compactus}</td>
<td>Decay Fungus</td>
<td>2.27 x</td>
</tr>
<tr>
<td>815</td>
<td>-</td>
<td>\textit{Xylobus frustulatus}</td>
<td>Decay Fungus</td>
<td>2.37 x</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Sawdust Carrier</td>
<td>-</td>
<td>2.86 x</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Non-inoculated</td>
<td>-</td>
<td>1.05 y</td>
</tr>
</tbody>
</table>

\textsuperscript{a) Total length of discolored wood above and below wounds.}
\textsuperscript{b) See Figures 15 and 16.}
\textsuperscript{c) Inoculations made in May, 1980, and harvested in December, 1980.}
\textsuperscript{d) Ratio compares length of discolored wood in treated hole to control hole for each wound pair.}
\textsuperscript{e) Means represent ratios for six wound pairs per treatment. Means followed by the same letter are not significantly different (P = 0.05).}
Columns reached their greatest length along the heartwood-sapwood transition zone. This pattern has been described by Shigo (35) for the compartmentalization of wood and wood decay in red oak.

The data in Table 6 represent the same treatments and conditions described earlier for evaluation of wound closure. For spring inoculations, there were no significant differences in discoloration column length ratios between treatments except with respect to the non-inoculated control \((P=0.05)\). However, there were significant differences in column lengths between trees. This effect was probably due to genetic diversity between trees and the site(s) on which they were growing. Discoloration column lengths were not affected by wound position along the branch axis or by wound orientation.

Autumn inoculations, using antagonists from autumn branch stubs and \(\text{Pc}\), did not affect column length ratios (Table 7) except with \text{Trichoderma}. Additionally, discoloration ratios were not affected by branch position or replication between trees. Comparison of discoloration column length ratios for treatments used in autumn and spring inoculations (Table 8) indicated that, except for \text{Trichoderma}, spring inoculation with autumn branch stub isolates, \(\text{Pc}\), and sand-sawdust carrier (SSC) caused a significant increase in ratios as compared to autumn inoculation. The discoloration ratios for \text{Trichoderma} were similar for autumn and spring inoculations. Autumn and spring ratios were similar with the non-inoculated control. Evidently, the time of inoculation with microbial agents influenced wood discoloration ratios.

In vitro colonization. Wound disk sections and adjacent disk sections (Figures 16 and 17) containing discolored wood, sapwood, and heartwood were incubated in a moisture-saturated atmosphere to provide xylophilic
Table 7. Comparison of wood discoloration\(^a\) in treated wounds to control wounds for wound pairs\(^b\) in red oak inoculated with selected antagonists and *Polyporus compactus* Overh., autumn inoculations\(^c\).

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Time collected</th>
<th>Type</th>
<th>Category</th>
<th>Ratio (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-4</td>
<td>Autumn</td>
<td>Yeast</td>
<td>Antagonist</td>
<td>0.79 (x)</td>
</tr>
<tr>
<td>7-4</td>
<td>Autumn</td>
<td>Fungus</td>
<td>Antagonist</td>
<td>1.03 (x)</td>
</tr>
<tr>
<td>1-L</td>
<td>Autumn</td>
<td><em>Trichoderma</em></td>
<td>Antagonist</td>
<td>1.64 (y)</td>
</tr>
<tr>
<td>1-T</td>
<td>Autumn</td>
<td><em>Fusarium</em></td>
<td>Antagonist</td>
<td>1.35 (x)</td>
</tr>
<tr>
<td>5-1</td>
<td>Autumn</td>
<td><em>Fusarium</em></td>
<td>Antagonist</td>
<td>0.92 (x)</td>
</tr>
<tr>
<td>814</td>
<td>-</td>
<td><em>Polyporus compactus</em></td>
<td>Decay Fungus</td>
<td>1.11 (x)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>Sawdust Carrier</td>
<td>-</td>
<td>1.06 (x)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>Non-inoculated</td>
<td>-</td>
<td>1.00 (x)</td>
</tr>
</tbody>
</table>

\(^{a}\) Total length of discolored wood above and below wounds.  
\(^{b}\) See Figures 15 and 16.  
\(^{c}\) Inoculations made in October, 1979, and harvested in December, 1980.  
\(^{d}\) Ratio compares length of discolored wood in treated hole to control hole for each wound pair.  
\(^{e}\) Means represent ratios for four wound pairs per treatment. Means followed by the same letter are not significantly different (P= 0.05).
Table 8. Comparison of wood discoloration column lengths\(^a\) between spring and autumn inoculations\(^b\) using selected decay antagonists from autumn branch stubs and *Polyporus compactus* Overh.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Type</th>
<th>Category</th>
<th>Discoloration column length: ratio(^c) of treated to control wounds for wound pairs(^d)</th>
<th>Mean(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spring inoculation</td>
<td>Autumn inoculation</td>
</tr>
<tr>
<td>10-4</td>
<td>Yeast</td>
<td>Antagonist</td>
<td>2.18</td>
<td>0.79</td>
</tr>
<tr>
<td>7-4</td>
<td>Fungus</td>
<td>Antagonist</td>
<td>2.52</td>
<td>1.03</td>
</tr>
<tr>
<td>1-L</td>
<td><em>Trichoderma</em></td>
<td>Antagonist</td>
<td>1.67</td>
<td>1.64</td>
</tr>
<tr>
<td>1-T</td>
<td><em>Fusarium</em></td>
<td>Antagonist</td>
<td>2.24</td>
<td>1.35</td>
</tr>
<tr>
<td>5-1</td>
<td><em>Fusarium</em></td>
<td>Antagonist</td>
<td>2.41</td>
<td>0.92</td>
</tr>
<tr>
<td>814</td>
<td><em>Polyporus compactus</em></td>
<td>Decay Fungus</td>
<td>2.27</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>Sawdust Carrier</td>
<td>-</td>
<td>2.86</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>-</td>
<td>1.05</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\(^{a}\) Total length of discolored wood above and below wounds.  
\(^{b}\) Spring and autumn inoculations made in May, 1980, and October, 1979, respectively. All wound pairs harvested in December, 1980.  
\(^{c}\) Ratio compares length of discolored wood in treated hole to control hole for each wound pair.  
\(^{d}\) See Figures 15 and 16.  
\(^{e}\) LSD = 0.51.
microorganisms residing within the woody tissues an opportunity to colonize
surface sterile sections and to determine the effect these microbes would
have on colonization and decay of disk sections by *P. compactus*.

Colonization and decay of sapwood, heartwood, and discolored wood in
sections were not influenced by antagonists, decay fungi, or sand-sawdust
carrier treatments. These treatments did not affect colonization and decay
by *Pc* in sapwood, heartwood, and discolored wood associated with control
and inoculated wounds of wound pairs. However, there were differences in
the colonization and decay of sapwood, heartwood, and discolored wood that
were probably due to the physiological condition of the three wood types.
Colonization and decay of wood sections from wound disks and adjacent disks
(Figure 16) of wound pairs were not affected by proximal or distal position.
Colonization frequencies were greater in adjacent disks than in wound disks
(Table 9). Also, colonization and decay frequencies were larger for
autumn-inoculated samples than for spring-inoculated samples (Tables 9 and
10). Colonization frequencies in discolored wood within wound pairs are
given in Table 11. Treatments did not seem to influence the ability of *Pc*
to colonize discolored wood as compared to wound pair controls.

Colonization frequencies of the three wood types (Table 9) and
macroscopic observations of mycelial growth indicated that *Pc* colonized
sapwood more rapidly than heartwood and discolored wood. In general,
mycelial growth of *Pc* in heartwood was diffuse, and formation of a dense
stroma was infrequent. In sapwood, colonization was rapid and a dense
stroma formed on most disk sections. Colonization of discolored wood was
not as dense as in sapwood (Table 9); *Pc* appeared to encroach into areas of
Table 9. Frequency of colonization in three wood types by *Polyporus compactus* Overh. on wood sections from autumn and spring inoculations using antagonists, *P. compactus*, and sterile sawdust.

<table>
<thead>
<tr>
<th>Time of inoculation</th>
<th>Wood section position</th>
<th>Sapwood 2 wk</th>
<th>Sapwood 4 wk</th>
<th>Heartwood 2 wk</th>
<th>Heartwood 4 wk</th>
<th>Discolored Wood 2 wk</th>
<th>Discolored Wood 4 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>Wound section</td>
<td>94</td>
<td>98</td>
<td>61</td>
<td>80</td>
<td>39</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Adjacent section</td>
<td>97</td>
<td>100</td>
<td>87</td>
<td>94</td>
<td>75</td>
<td>88</td>
</tr>
<tr>
<td>Spring</td>
<td>Wound section</td>
<td>89</td>
<td>97</td>
<td>47</td>
<td>62</td>
<td>34</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Adjacent section</td>
<td>90</td>
<td>94</td>
<td>59</td>
<td>69</td>
<td>50</td>
<td>56</td>
</tr>
</tbody>
</table>

a) Proximal and distal disk sections (Figures 16 and 17) from each wound were used for colonization studies. Frozen sections were surface sterilized by dipping in boiling water. Sections were inoculated with feeder strips colonized by *P. compactus* after two days incubation in a moisture-saturated atmosphere at 24°C.

b) Spring and autumn inoculations made in May, 1980, and October, 1979, respectively. All wound pairs were harvested in December, 1980.

c) See Table 6 for listing of antagonist isolates used.

d) Disk sections contained discolored wood, sapwood, and heartwood associated with control or treated wounds at each wound position. See Figures 16 and 17.
discolored wood from sapwood surrounding the discolored tissues. Once
colonized, \textit{Pc} eventually formed a dense stroma in discolored wood; however,
patches of non-colonized discolored wood were formed, indicating a
localized resistance to colonization by \textit{Pc} occurred. This may in part be
due to the presence of xylophiles that did not form any visible growth.

Oak diffusate agar prints of disk sections revealed areas in
discolored wood containing an active microbial community. Bacteria, yeasts,
and fungi were found in most prints. Some of these organisms were seen at
the juncture of discolored wood and healthy wood prior to inoculation and
colonization by \textit{Pc}. Some microorganisms in agar prints appeared to be
antagonistic to \textit{Pc} for a limited time, but \textit{Pc} eventually replaced most
organisms. \textit{Trichoderma} species were the most common antagonists observed
and prevented \textit{Pc} from colonizing disk sections and prints. However, some
isolates of \textit{Trichoderma} were not antagonistic and were replaced by \textit{Pc}.
Antagonists were evident in prints even though discolored wood had been
colonized by \textit{Pc}, and antagonistic reactions were found in prints from
control and inoculated wounds of wound pairs.

Frequency of wood decay caused by \textit{Pc} (Table 10) in disk sections
from autumn and spring inoculations was estimated for sapwood, heartwood,
and discolored wood by inserting a scalpel point into tissues; the point
penetrated most easily into obviously decayed wood. Sapwood was readily
decayed by \textit{Pc}. The order of decreasing decay resistance in disk sections
was heartwood \textgreater discolored wood \textgreater sapwood. Wood that had been severely
decayed was bleached to a lighter color, in most cases, than healthy wood
or non-decayed discolored wood. The extent of decay was greater in sapwood
of disk sections from autumn inoculations than in sections from spring
Table 10. Frequency of decay caused by Polyporus compactus Over., in vitro, in wood disk sections<sup>a</sup> taken from red oak branch wound pairs<sup>b</sup> inoculated with decay antagonists<sup>c</sup>, decay fungi, and sterile sawdust.

<table>
<thead>
<tr>
<th>Time of inoculation</th>
<th>Wood section position</th>
<th>Sapwood control&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Sapwood inoculated&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Heartwood control</th>
<th>Heartwood inoculated</th>
<th>Discolored wood control</th>
<th>Discolored wood inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>Wound section</td>
<td>97</td>
<td>94</td>
<td>6</td>
<td>5</td>
<td>45</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Adjacent section</td>
<td>97</td>
<td>98</td>
<td>5</td>
<td>2</td>
<td>61</td>
<td>66</td>
</tr>
<tr>
<td>Spring</td>
<td>Wound section</td>
<td>64</td>
<td>70</td>
<td>2</td>
<td>2</td>
<td>53</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Adjacent section</td>
<td>76</td>
<td>77</td>
<td>3</td>
<td>0</td>
<td>58</td>
<td>50</td>
</tr>
</tbody>
</table>

<sup>a</sup> Disk sections (see Figures 16 and 17) were frozen, then dipped in boiling water to surface sterilize. Sections were incubated in a moisture-saturated atmosphere for 48 hr and then inoculated with wood feeder strips colonized by P. compactus, and incubated at 24 C for 4 wk.

<sup>b</sup> See Figures 16 and 17.

<sup>c</sup> See Table 6 for listing of isolates used for field inoculations.

<sup>d</sup> Percent of all wound pair control wounds exhibiting decay.

<sup>e</sup> Percent of all wound pair inoculated wounds exhibiting decay.
inoculations. These differences probably reflect seasonal changes in the physiological and microbial condition of wood. Comparison of colonization frequencies for wound pairs (Table 11) after 4 wk incubation with the occurrence of decay (Table 12) shows that decay was not evident in all disk sections colonized by Pc (Table 10).

**DISCUSSION**

Inoculation of drill wounds with antagonists, and environmental conditions at the time of wounding may alter the succession of xylophilic microorganisms associated with wood discoloration and decay. In these studies, spring inoculation of drill wounds with decay fungi, antagonists, and sterile sawdust carrier increased the amount of discolored wood as compared to non-inoculated wounds. Apparently the sawdust carrier was colonized by one or more microbes and these affected discoloration. Additionally, several antagonists reduced the amount of wound closure and appeared to be pathogenic. To what extent discolored wood becomes decayed is not known. However, if discolored wood is readily colonized and decayed by hymenomycetes, or other microbes, then application of antagonists or pathogens would promote rather than retard decay. This is opposite the hoped-for result.

Alteration of the natural succession of microorganisms may not appear to decrease discoloration, whereas colonization by wood decay fungi may be impeded sufficiently to enhance the host wound response and wood compartmentalization. In these studies with red oak, addition of a specific antagonist to prevent colonization by Pc assumed a single antagonist might influence colonization by decay fungi. *In vivo* interactions of antagonists
Table 11. *Polyporus compactus* Overh. colonization of discolored wood in disk sections\(^a\) associated with paired branch wounds\(^b\) from autumn and spring inoculations\(^c\) in red oak trees.

<table>
<thead>
<tr>
<th>Time of inoculation position</th>
<th>Control (^d) 2 wk</th>
<th>Inoculated (^e) 2 wk</th>
<th>Control + Inoculated (^f) 2 wk</th>
<th>Neither Wound colonized (^g) 2 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Autumn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound section</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjacent section</td>
<td>14</td>
<td>8</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>18</td>
<td>18</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Wound section</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjacent section</td>
<td>27</td>
<td>25</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

\(a\) Disk sections were taken from proximal and distal sides of each wound for each wound pair (Figure 16).

\(b\) See Figures 16 and 17.

\(c\) Spring and autumn inoculations made in May, 1980, and October, 1979, respectively. All wound pairs harvested in December, 1980.

\(d\) Percent of wound pairs in which the decay fungus colonized discolored wood in the control wound only.

\(e\) Percent of wound pairs in which the decay fungus colonized discolored wood in the inoculated wound only.

\(f\) Percent of wound pairs in which the decay fungus colonized discolored wood in the control and inoculated wound.

\(g\) Percent of wound pairs in which the decay fungus did not colonize discolored wood in the control and inoculated wound.
Table 12. Frequency of wood decay by *Polyporus compactus* Overh. in discolored wood of disk sections\(^a\) associated with branch wounds from autumn and spring inoculations in red oak trees.

<table>
<thead>
<tr>
<th>Time of inoculation</th>
<th>Section position</th>
<th>Control(^b)</th>
<th>Inoculated(^c)</th>
<th>Control + Inoculated(^d)</th>
<th>No decay(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>Wound section</td>
<td>17</td>
<td>20</td>
<td>28</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Adjacent section</td>
<td>19</td>
<td>24</td>
<td>42</td>
<td>15</td>
</tr>
<tr>
<td>Spring</td>
<td>Wound section</td>
<td>23</td>
<td>19</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Adjacent section</td>
<td>20</td>
<td>12</td>
<td>38</td>
<td>30</td>
</tr>
</tbody>
</table>

\(^a\) Disk sections were taken from proximal and distal sides of each wound for each wound pair (see Figures 16 and 17).

\(^b\) Percent of wound pairs in which wood decay was found in control wounds only.

\(^c\) Percent of wound pairs in which wood decay was found in inoculated wounds only.

\(^d\) Percent of wound pairs in which wood decay was found in both wounds.

\(^e\) Percent of wound pairs in which wood decay was not found in both wounds.
with other xylophiles residing in the woody tissues prior to wounding and
those taking up residence after wounding may negate the antagonists’
utility as observed in vitro (3). Further study on the relationships
between discolored wood and decay are necessary to understand the role
antagonists may play in preventing or enhancing wood decay.

The extent of discolored wood was affected by the time of inoculation
with some antagonists (Table 8). Several antagonists isolated from autumn
branch stubs produced significantly longer columns of discolored wood
when used for spring inoculations in comparison to autumn inoculations
(Table 8). Antagonists from spring branch stubs were not used in autumn
inoculation tests.

Wound closure appeared to be retarded by some antagonists (Table 3),
which would make them undesirable for use as wound treatments even though
they were antagonistic to decay fungi. An isolate of Fusarium (1-T) from
an autumn branch stub caused a significant decrease in wound closure when
used for spring inoculations (Table 5), suggesting that some antagonists
may be pathogenic to the host and still influence wood discoloration.

Colonization and decay of sapwood, heartwood, and discolored wood
by Pc, in vitro, were not influenced by previous wound inoculations with
antagonists, decay fungi, or sterile sawdust carrier. However, colonization
by Pc was influenced by the wood type and the time of field inoculation.
Non-sterile sapwood was more rapidly colonized, in vitro, than was heartwood
or discolored wood. Colonization frequencies of disk sections were larger
in heartwood and discolored wood of autumn-inoculated wounds than in
spring-inoculated wounds. Colonization frequencies of disk sections
transecting wounds were lower than in adjacent sections. These differences
may be due in part to the nature of the host wound response at different times of the growth period, the chemical and physical nature of the wood tissues, and the nature of microbial communities in residence before and after wounding.

Inoculation with wood decay fungi, antagonists, or sterile sawdust carrier increased wood discoloration that may be due to increased moisture levels and altered aeration (13). The elevated moisture levels that were unavoidable as a result of inoculation may have affected wood discoloration by enhancing the host wound response and colonization by the introduced or other xylophilic microorganisms. Other wound inoculation methods (22, 42) may be more suitable than those used for these studies. In this study, the presence of microorganisms throughout discoloration columns was not examined. Microflora were observed on wedge prints of disk sections from inoculated and control wounds and would be expected to occur throughout the columns.

In vitro inoculation of disk sections from field tests with *F. c*

showed that antagonists did not prevent or restrict wood decay as anticipated. Wood decay due to natural inoculum was visible in some wounds at harvest and did not appear to be affected by in vitro antagonists. In vivo challenge inoculation of antagonist-treated wounds at various times of the host growth period with wood decay fungi would be helpful in determining the effectiveness of antagonists for biological control. However, an effective and reliable method of inoculation with decay fungi in vivo remains to be found.

Manipulation of microbial communities to achieve biological control of wood decay requires knowledge of the microbial community structure
associated with wounds throughout the host growth period (15, 19, 26, 34, 35). The effects of antagonists on wood discoloration and decay may be dependent on the effects of the environment on colonization at the time of wounding (36), competition from other xylophilic microorganisms (7, 8, 10, 16, 21, 24), and the nature of the host wound response until wound closure seals the exposed wood tissues from the external environment (33, 39, 40). Discolored wood precedes wood decay, and the extent of wood discoloration may be a measure of the minimal extent of wood decay that will follow (34). Reduction of wood discoloration by inoculation with antagonists or chemicals might provide a direct measure of potential wood decay and its control. However, wood discoloration may not be an absolute estimator of potential wood decay, and microbial inoculations which enhance discoloration may in some instances be associated with resistance to decay.
PART II

LITERATURE CITED


PART III

EFFECTS OF OXYGEN TENSION ON THE
DECAY OF RED OAK BY POLYPORUS COMPACTUS

INTRODUCTION

The study of oxygen requirements of wood decay fungi may lead to a better understanding and possible control of decays in living trees. Oxygen tensions are known to be high within trees (2, 7, 11). In vitro studies using synthetic agar media and liquid culture have shown that mycelial growth rates and dry weights decline with increasing oxygen tensions. However, more information is needed in relation to the effects of oxygen tensions on decay using natural substrates and conditions found in trees. The standard method for evaluating wood decay is the soil-wood block method (1), which is suited to testing for natural decay resistance. A new method for evaluating the effects of oxygen tensions on decay using non-autoclaved wood blocks is described. This method provides a continuous flow of specific air mixtures to decaying wood blocks, and does not require soil, which serves as a source of mineral and organic nutrition that can influence the growth of decay fungi and other xylophiles.

The effects of gaseous environments on in vitro growth and metabolism of fungi has been reviewed by Tabak and Cooke (10). In general, as oxygen levels drop below ambient levels (21%), mycelial growth (linear growth rates or dry weight increase) in many fungi declines. Additionally, interactions have been observed between oxygen and carbon dioxide levels for many fungi.
in vitro (6, 10, 12). Depending upon the concentration, carbon dioxide has been reported to exhibit stimulatory and/or inhibitory effects on fungal growth at varying oxygen tensions (10).

The response of decay fungi to increased oxygen tensions is of interest because the oxygen level within living trees is much reduced from ambient levels. Scheffer and Livingston (9) observed declining growth rates (linear growth), measured by carbon dioxide evolution and mycelial growth, of Polystictus versicolor Linn. (Fr.) under increasing oxygen tensions. Jensen (6) and Gunderson (5) studied the effects of oxygen and carbon dioxide concentrations on the growth of decay fungi in vitro. Jensen (6) observed that growth rates declined as oxygen tensions increased in Laetiporus sulphureus (Bull. ex Fr.) Bond. & Sing., Stereum gausapatum (Fr.) Fr., Xylobus frustulatus (Pers. ex Fr.) Boidin, and P. versicolor Linn. (Fr.) and that carbon dioxide inhibited growth. Gunderson (5) found that linear growth by Heterobasidium annosum (Fr.) Bref. was the same at ambient and trace levels of oxygen but that a slight elevation in carbon dioxide above ambient levels stimulated linear mycelial growth. These studies were conducted in vitro on enriched media and did not examine the effects of oxygen tension on woody substrates.

The objectives of the present study were to determine the effects of oxygen tensions on dry weight losses in red oak wood blocks by Polyporus compactus Overh. (Pc) and to develop a method that would permit the testing of wood decay under controlled conditions for ten weeks without using soil as prescribed by ASTM (1).
Selection and preparation. For decay studies, small wood blocks were cut from disks made from red oak branches, and the effects of oxygen tensions on dry weight losses caused by *Pc* in "fresh" and "dried" blocks were examined.

Freshly harvested sections from healthy red oak branches (ca. 8-9 cm diam) were placed in polyethylene bags and frozen (-20 C). Disks (8-9 cm diam x 2 cm thick) were cut serially from frozen material. Disks were numbered consecutively and placed in petri dishes, which were stored in polyethylene bags at -20 C. Prior to testing, disks were partially thawed to facilitate pencil-scribing to delineate two series of 6-8 wood blocks of similar radial position, size (2 cm x 2 cm x 0.5 cm thick), and tissue composition (sapwood and heartwood). Blocks from similar radial positions (Figure 18) of each series (left side= "fresh"; right side= "dried") represented a sample pair. Blocks were cut from disks with a band saw and separated into pairs. One block of a sample pair was used for the "fresh" treatment and the other for the "dried" treatment. Blocks were kept at -20 C until needed. Each block was thawed, debarked, and weighed to obtain a fresh weight (+/- 1 mg). "Fresh" blocks were recorded. Initial dry weight for each "fresh" block was estimated, using the dry weight of corresponding "dried" blocks. Dried blocks were rehydrated with sterile water to initial fresh weight and stored at -20 C until needed for decay tests.

Decay test procedure. Fifty block pairs (one fresh and one dry) were used in each experiment. Ten pairs were non-inoculated controls, and forty pairs were inoculated with a wooden feeder strip colonized by *Pc*.
Figure 18. Red oak branch disk (8-9 cm diam x 2 cm thick) marked into two series (left and right) of blocks (2 cm x 2 cm x 0.5 cm thick). Blocks from the left were used as "fresh" wood for decay studies, and those on the right were used for parallel "dried" tests.
Feeder strips were prepared by burying pine pot labels (15 cm x 1.5 cm x 0.2 cm thick) in soil for 2 wk to remove inhibitory chemicals. These were sectioned (ca. 1.5 cm x 1.0 cm x 0.2 cm thick) and sterilized at 121 C and 2 atm for one hr on three separate days. Fifteen strips were placed on malt extract agar (malt extract, 30 g/L; glucose, 5 g/L; and agar, 20 g/L) which was seeded with an agar plug of Pc and incubated at 24 C in a lighted room for 4 wk.

Prior to inoculation, frozen block pairs were surface sterilized by immersing in boiling water for 6 sec. Individual blocks were placed on sterile U-shaped glass rods in sterile plastic petri dishes (60 mm diam x 15 mm) at 72 C for 1 wk. Each dish (Figure 19) had an 8-mm diam vent hole cut into the top and bottom halves; holes were covered with glass fiber filter disks (Gelman Instrument Co., Ann Arbor, Michigan 48106; Type -A/E 81727, 25 mm diam) held in place with epoxy glue. Moisture levels in wood blocks were maintained by placing a moistened (4.5 ml water) gauze pad (5 cm x 5 cm, folded and stapled) in each dish. Dishes were stacked vertically on a wire rack (10 dishes/rack); this allowed gas mixtures to circulate around each dish with minimal obstruction. Two racks, one with ten fresh blocks and the other with ten corresponding dry blocks, were placed into each of five 4-L incubation jars (Figure 20) to which 125 ml of sterile water was added to maintain a high relative humidity. One jar contained ten non-inoculated wood block pairs for controls; the remaining four jars each contained ten inoculated wood block pairs.

Jars were kept under constant light at 24 C for 10 wk with an atmosphere at a given oxygen tension passing through jars continuously. After incubation, blocks were removed and dried at 72 C to constant weight.
Figure 19. Dishes used for decay studies with individual wood blocks cut from red oak branches (Figure 18). Blocks (Fresh- upper dish; Dried- lower dish) are supported on glass rods and dishes include a moist pad of cloth. Dishes, here opened, are fitted, top and bottom, with a glass fiber filter disk to allow passage of gases. Wood blocks before (Top Photo) and after (Bottom Photo) inoculation with wooden feeder strips colonized by *Polyporus compactus* Overh.
Figure 20. Jar used to provide a constant oxygen tension for decay studies. Each jar contained two racks (R) and held twenty vented dishes (Figure 19). Each dish contained a wood block inoculated with Polyporus compactus Overh., or a non-inoculated control block. Gas flow was regulated by adjusting a needle valve at the exit portal. QR= "quick-release" fitting; W= 125 ml of sterile water to maintain high humidity.
Excess fungal mycelium and pine feeder strips were gently scraped away from the exterior surfaces of each block and final dry weights were recorded.

**Controlled atmosphere system.** A controlled atmosphere incubation system (Figure 21) was designed for these tests. Prepurified nitrogen gas (N₂, Union Carbide Corp., Linde Div.) and purified air (21% O₂, Union Carbide Corp., Linde Div.) were mixed to adjust O₂ concentrations. A flow meter (Matheson, Series 7600, 150 mm, Model 601) was attached to each T-cylinder (8,456 L volume) of compressed N₂ and air with flexible copper tubing (3.175 mm diam). Air was diluted with N₂ to obtain O₂ levels (tensions) lower than ambient air (21% O₂). The gas flow at a given O₂ tension was held constant for the entire incubation period. Gas mixtures were hydrated by passage through a submerged micro-fine pore air-stone before passing through a plastic manifold (PVC-Transparent Blue, 7.6 cm diam x 120.3 cm long; Excelon, r-4000, Cadillac Plastics, Cleveland, Ohio) into six 4-L polyethylene jars. Jars were attached to the manifold with flexible copper tubing, using brass bulk-head fittings and stainless steel quick-release fittings (Swagelok; Crawford Fitting Co., Solon, Ohio 44139). Five jars were used in an experiment. Each was equipped with a brass needle valve (Nupro Co., Willoughby, Ohio 44094) to control flow rates through each jar. Gases were introduced into the system at 1.7 atm, and flow rates were maintained at ca. 12.3 L/jar/day.

**Gas analysis.** Oxygen tensions were monitored using a cabinet flue gas analyzer (Burrell Gas Analysis Apparatus, Build-Up Model No. 950-348, Burrell Corp., Pittsburgh, Pa.), which can be used to detect O₂ and carbon dioxide (CO₂) at concentrations as low as 0.2%. The device permits the
Figure 21. Diagram of controlled atmosphere system. Incubation jars (Figure 20) containing dishes with inoculated wood blocks and controls (Figure 19) were provided with a constant flow of a given moist nitrogen-air mixture to give the desired oxygen tension.
successive absorption of CO₂ in NaOH and O₂ by pyrogallol to determine the concentration of each.

The apparatus was composed of a burette sampling tube with a sample capacity of 100 ml, and three burrell contact pipettes attached in series. To obtain a 100-cc gas sample, the burette tube was filled with sterile distilled water, and gas samples were obtained by emptying water from the burette tube into a reservoir bottle held below the sampling tube. Carbon dioxide was extracted from the sample by forcing the gas contained in the burette tube into a burrell contact pipette containing a 20% NaOH solution (NaOH, 33 g; sterile distilled water, 165 ml). The gas sample was cycled four times between the contact pipette containing NaOH and the sample burette. The CO₂ concentration was determined by observing a rise of the water miniscus in the sample tube; this corresponded to the quantity of CO₂ removed by NaOH. Oxygen was extracted in the same manner with a 2.76% solution of pyrogallol, C₆H₃(OH)₃, (pyrogallol, 4.55 g; NaOH, 22.77 g; sterile distilled water, 165 ml) after removal of CO₂ from the gas sample.

RESULTS

Red oak blocks incubated for 10 wk at 24 C in oxygen tensions of 21%, 4.2%, or 1% were colonized by Polyporus compactus (Fc) with significantly different (P= 0.05) dry weight losses (20.4%, 9%, and 3.2%, respectively). This is shown in Figure 22. Dry weight losses in non-inoculated control blocks were significantly different from inoculated blocks at oxygen tensions of 21% and 4.2%, but not at 1%. There were no significant differences between non-inoculated control blocks for oxygen tensions of 21% and 4.2%, but differences were significant at tensions of 1%. Dry
Figure 22. Comparison of dry weight loss in red oak wood blocks inoculated with Polyporus compactus Overh. and non-inoculated control blocks at different oxygen tensions after 10 wk at 24 C.
weight losses in inoculated and non-inoculated "fresh" and "dried" blocks at oxygen tensions of 21%, 4.2%, and 1% are shown in Figure 23. Dry weight losses were significantly different between "fresh" and "dried" inoculated blocks at ambient O₂, but not at O₂ tensions of 4.2% and 1%. Dry weight losses in non-inoculated blocks were significantly different at O₂ tensions of 21% and 4.2% but not at 1%. At 1% O₂ "fresh" and "dried" control blocks were not significantly different from inoculated "fresh" blocks. At ambient O₂, significant differences were observed between "fresh" and "dried" controls, but there were no significant differences at 4.2% or 1% O₂. The "fresh" block controls were probably affected by resident microorganisms which grew at ambient oxygen levels and caused an 8.2% dry weight loss. The activities of these resident xyllophiles were reduced at the lower oxygen tensions.

Dry weight losses were not affected significantly by the radial position of blocks in disks or between disks. Losses were not affected by block position in incubation jars. Initial moisture contents of blocks ranged from 36-41% and did not appear to affect colonization by Pc or influence weight loss.

DISCUSSION

In vitro weight loss in red oak wood blocks caused by P. compactus declined as oxygen tensions increased. These results parallel the observations by others (6, 10) of growth of various decay fungi on enriched agar media and liquid culture in controlled atmospheres. The lower oxygen concentrations used in these studies were levels reported to occur in living trees (2, 7, 11). In healthy or sound red oak, Jenson (7) observed that oxygen concentrations were less than 2% and less than 4% in decayed
or discolored wood in trees. Dry weight losses in inoculated blocks at $O_2$
tensions of 1\% and 4.2\% were 3.2\% and 9\% (Figure 22), respectively. Thus,
the results obtained in these studies could be predicted, but they had
not been demonstrated with P. compactus in wood.

The method described is useful for testing wood decay using fresh
and dry samples, and for evaluation of wood decay in controlled gaseous
environments. Soil was not used to support the growth of decay fungi;
this is a departure from the ASTM (1) procedure used to evaluate wood
decay. Wood decays associated with branch stubs and wounds above the
soil-line do not benefit directly from the mineral and organic nutrients
in soil. Had sterile soil been used in these studies, contamination in
fresh blocks would have resulted in varying weight loss due to the large
numbers of microorganisms residing in healthy red oak tissues (Part I).
These microbial residents appeared to be active at ambient oxygen. Some
residents were observed in fresh controls, but contamination was reduced
by drying at 72 C for 4 days. The standard soil-wood block method would
have altered the moisture content, nutrient availability, and aeration of
wood samples. Additionally, the continuous flow of gas mixtures probably
allowed for removal of volatile staling products.

These results demonstrate that the activities of wood decay fungi
are affected by the oxygen concentration. Significant dry weight losses
were obtained in a gaseous environment of 4.2\% $O_2$, whereas wood decay at
1\% $O_2$ was much reduced. The activity of decays in healthy trees with $O_2$
levels of 2\% or less are probably very low, whereas activities in trees
with discolored wood and decay will be much higher in part due to the
increased availability of oxygen. However, wood decays in living trees
are affected by the physical and biological environment and proceed at a
Figure 23. Comparison of dry weight losses of "fresh" or "dried" red oak wood blocks inoculated with Polyporus compactus Overh. and non-inoculated control blocks at different oxygen tensions after 10 wk at 24°C.
slow rate for extended periods of time.

The methods used for these studies may have utility for studying the interactions between oxygen and carbon dioxide on wood decay and for studying rates of decay (3, 9) by measuring CO$_2$ evolution. Jenson (6) found that CO$_2$ inhibited the growth of various decay fungi. Inhibitory effects might appear to restrict or prevent growth of decay fungi at low O$_2$ levels (1% or less), whereas at higher levels (greater than 1%) growth can proceed slowly. Additionally, seasonal fluctuations of oxygen and carbon dioxide levels (2, 7, 11), temperature, moisture levels (4, 7), and resident microflora might be studied in relation to wood decay in trees.
PART III

LITERATURE CITED


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BIBLIOGRAPHY


