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Respirometric Testing of Decay Resistance of Discolored Root Wood

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ABSTRACT

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Oxygen uptake by decay fungi on milled wood samples was used as a measure of decay resistance of healthy versus discolored wood in roots of *Picea rubens* and *Abies balsamea*. Healthy and discolored zones were excised, milled, sterilized, placed on water agar, and inoculated with a mycelial homogenate of the test fungus (usually *Resinicium bicolor*). Oxygen uptake rose rapidly on healthy samples and reached a peak at about day 10, when starch was depleted and degradation of structural polymers had begun. Discolored samples varied but generally supported much lower levels of oxygen uptake than did healthy samples. This supports the concept that these discolored tissues are best described as reaction zones rather than zones of fungal colonization or incipient decay. Low starch levels as well as wood extractives appear to play important roles in controlling fungal colonization. Bioassay of discolored zones in roots of spruce and fir produced in response to field inoculations with *R. bicolor* and *Scytinostroma galactinum* showed that the host response was maximally effective within 4 wk after inoculation. Large roots, but not small roots, of *A. balsamea* have a weaker, inconsistent wound response compared with roots of *P. rubens*. This may account for the greater susceptibility of *A. balsamea* to root disease.

Additional keywords: respirometry, wood decay.

When an infection court becomes available, resistance of trees to many wood-decay diseases involves alteration of living xylem, rendering it relatively decay-resistant. In heartwood, this alteration takes place during the normal conversion of sapwood to heartwood and may be considered preformed resistance (20). Decay resistance of living sapwood, however, involves a dynamic response; wounding and invasion by microorganisms results in the conversion of the adjacent wood into a nonconducting, discolored zone that appears to be important in resistance (21). Reaction zone is a term used for this discolored, extractive-rich zone between infected and healthy wood (21). Alternatively, some workers place emphasis on the role of microorganisms in directly causing or enhancing discoloration (23). Thus, discolored wood may represent a zone of fungal colonization or incipient decay rather than evidence of a host response.

A comparison of the host responses of resistant and susceptible species should help in clarifying the significance of discolored wood and in identifying features of the host response important in

resistance. Red spruce (*Picea rubens* Sarg.) is highly resistant and balsam fir (*Abies balsamea* (L.) Mill.) is highly susceptible to root and butt rot (3,14,19). Both red spruce and balsam fir have discolored zones around root wounds with no obvious decay or around decay columns. Isolations from discolored wood in these hosts have generally yielded no fungi (19), so the discolorations apparently do not indicate presence of fungi or incipient decay but may instead be reaction zones. The objectives of this work were to: 1) develop a technique for investigating the nature of discolored wood, 2) determine quantitatively the effect of the host response on fungal colonization, and 3) compare the development of the host response in resistant and susceptible hosts (red spruce and balsam fir, respectively).

MATERIALS AND METHODS

Samples. Woody roots, usually 2–6 cm diameter, were collected from mature red spruce and balsam fir in the White Mountain National Forest, New Hampshire. Bark was removed and the outer, moist, nondiscolored conducting wood was sampled and designated healthy. Discolored wood around wounds or infections by wood decay fungi was excised carefully. Samples were cut into small pieces, air-dried, ground in a Wiley Mill to pass a 10-mesh screen, and stored at −20 C until use.

Respirometry. Studies of wood decay and decay resistance generally involve measurement of relative weight loss during decay of wood samples. Unfortunately, standard tests (1,2) require several months and blocks of wood larger than those commonly encountered in reaction zones. Also, removal of extractives from wood blocks is difficult. For our study, we used milled wood samples (6) bioassayed in a respirometric procedure (4,10,25,26,28). The technique is amenable to working with small and irregular volumes of tissue, samples are homogeneous, extractives can be easily removed or added, and assays can be completed in 10 days. However, a number of parameters of the technique required evaluation. Methods of sterilizing samples before inoculation were compared to determine if the methods differed with respect to subsequent fungal O_2 uptake. Healthy and discolored spruce wood samples were autoclaved at 121 C for 15 min or exposed to propylene oxide fumes in a closed chamber for 4 hr, followed by airing in a laminar flow hood for 1 hr. Results between methods were compared with a Student's *t* test.

An earlier assay method (28) was modified by using a 6-cm-diameter petri dish containing solidified water agar as a base, the addition of approximately 0.5 g of sterile milled wood sample, followed by 1 ml of fungus inoculum. Inoculum was prepared by adding one half of a malt extract agar (1.5% malt extract and 1.5% agar) plate just covered by the test fungus to 100 ml of sterile distilled water and homogenizing it twice for 5 sec in a Sorvall Omnimixer at half speed. The inoculum was mixed into the sample with a spatula. Samples were incubated at 25 C in the dark.

Oxygen consumption by the test fungus was measured with a Gilson respirometer after various periods of incubation. The colonized sample of milled wood was transferred from the agar surface into the respirometry flask, 0.3 ml of water was added to the sample to maintain moisture, and 0.3 ml of KOH was placed in the center well with a folded piece of filter paper to increase surface area for CO_2 absorption. Flasks were then sealed on the instrument and placed in a water bath at 25 C. The Gilson respirometer is a closed system in which changes in gas volume are measured manometrically. Because CO_2 is absorbed in the KOH reservoir, O_2 uptake by the fungus can be measured by the decrease in gas volume.

To determine if discolored wood is more inhibitory to nonpathogens than to pathogens and if inhibition depends on decay type, we tested a number of fungi. *Gloeophyllum trabeum* (Fr.) Murr. (Mad 617 R), *Coniophora puteana* (Schum: Fr.) Karst. (ME 715), *Tyromyces balsameus* (Pk.) Murr. (B90) (brown rotters), *Heterobasidion annosum* (Fr.) Bref. (Mod 14F), *Phanerochaete chrysosporium* Burds. (ME 446), *Resinicium bicolor* (A. & S.: Fr.) Parm (B46), *Scytinostroma galactinum* (Fr.) Donk (B41), and *Coriolus versicolor* (L.:Fr.) Quel. (Mad 697) (white rotters) were used.

Oxygen uptake and wood degradation. Because respirometry provides an instantaneous rather than a cumulative measure of fungal activity, we followed changes over time in O_2 uptake of the test fungus and the accompanying catabolism of wood components. We inoculated a large number of replicate samples with *R. bicolor* and removed samples periodically for measurement of O_2 uptake. Replicate samples were then bulked within each harvest date and a portion used for analysis of acid-insoluble lignin (7) and of wood sugars following acid hydrolysis (17). The figures for total glucose and other sugars thus include sugars from cellulose and hemicelluloses. Starch was determined colorimetrically (24) in perchloric acid extracts (13).

Extractives. For some experiments, 5 g of milled samples was extracted by shaking in three successive 50-ml volumes of ether, 95% ethanol, or acetone for 1 hr at room temperature. Extracted samples were thoroughly air-dried before further use.

Host response in inoculated roots. For measurement of the degree and timing of inhibitory responses after controlled inoculations, samples of discolored root wood were obtained from a separate study (19). In that study, mature red spruce and balsam fir (14–24 cm diameter at 1.4 m height) were inoculated at the Bartlett Experimental Forest in the White Mountain National Forest. Inoculum cylinders (1 cm long, 5 mm diameter) were prepared from a balsam fir board, autoclaved in 10% malt extract for 1 hr, placed in jars, autoclaved again for 20 min, and incubated for 12 wk with either *S. galactinum* or *R. bicolor*. Three or four roots per tree were inoculated in early August 1985. Each root was inoculated at two points with the same fungus, at 15 cm from the root collar (diameter >8 cm) and again at a point where the diameter was 2–3 cm. The root surface was washed with 95% ethanol before a hole was drilled aseptically 1 cm deep in the upper surface. An inoculum cylinder was inserted, the wound was sealed with molten paraffin, and the soil was replaced.

Roots were harvested at 1, 2, or 9 mo after inoculation. After chips were removed for fungal isolation (19), the associated discolored wood was carefully excised and processed for respirometric analysis as described above.

RESULTS

Respirometry. Immediately after transferring colonized samples of healthy spruce wood to the respirometry flasks, oxygen consumption by *R. bicolor* was high and declined rapidly during the first 2 hr (Fig. 1). After 4 hr, readings remained constant, and oxygen consumption was routinely measured between 4 and 5 hr after transfer of colonized samples to respirometry flasks. This initial peak in O_2 uptake could be observed in all respirometry tests with *R. bicolor*. The same pattern was observed in *Phanerochaete chrysosporium*, but the initially high rate of O_2 consumption was less apparent in other test fungi such as *Scytinostroma galactinum* (data not shown).

Ten days after inoculation, O_2 uptake by *R. bicolor* on samples previously sterilized by the two methods (autoclave or propylene oxide) did not differ significantly ($P > 0.3$). Mean (and standard error) rates of O_2 uptake following autoclaving and propylene oxide, respectively, were 343 ± 3 and 343 ± 10 $\mu l\ hr^{-1}\ g^{-1}$ on healthy samples of three spruce roots and 14 ± 6 and 10 ± 2 $\mu l\ hr^{-1}\ g^{-1}$ on discolored samples. Autoclaving was more convenient than fumigation and was used in subsequent experiments.

Oxygen uptake and wood degradation. On healthy spruce and fir wood, O_2 uptake by *R. bicolor* rose rapidly within several days after inoculation and reached a peak at about day 10 (Fig. 2A and B). It then dropped and reached a steady level at 15–20 days. On discolored fir wood (Fig. 2B), O_2 uptake rose slightly and entered a lag phase before rising to a peak and then decreasing late in the experiment. A lag was less evident on discolored spruce (Fig. 2A), and O_2 uptake reached a maximum several days earlier. The same general pattern of O_2 uptake with time was observed in other experiments (e.g., *R. bicolor* in Fig. 3).

Original starch concentrations (w/w, dry weight basis) in sample materials were: healthy spruce 0.58%, discolored spruce 0.05%,

healthy fir 0.63%, discolored fir 0.01%. The rapid rise in O_2 uptake on healthy samples of spruce and fir wood corresponded with a rapid decrease in starch content (Fig. 2C and D). When starch content reached its lowest levels, O_2 uptake dropped off to a steady rate, at which point O_2 uptake was apparently supported almost exclusively by structural polymers. Degradation of structural polymers in fir wood was detectable as early as day 5, when total glucose and other sugars (in acid-hydrolyzed samples) declined (Fig. 2D). A decrease in lignin and sugars other than glucose was evident in spruce by day 14. In both woods, a greater proportion of lignin than of total glucose (primarily from cellulose) was lost by day 40, indicating selective delignification. Likewise, other sugars appeared to be catabolized at a rate faster than total glucose.

For subsequent studies, samples were incubated with the test fungus for 10 days before respirometry. At 10 days, readily available starch was virtually depleted, degradation of structural polymers was under way, and O_2 uptake on healthy samples was nearly maximal.

Fungal inhibition on discolored wood. Discolored spruce roots gave quite variable results, with O_2 uptake values ranging from less than one tenth to almost one half those on healthy wood excised from the same root (Table 1). There was no clear relationship between decay resistance as measured by respirometry and the nature of the discoloration (color, intensity, association with wound, or decay), although the three most inhibitory spruce samples (S12, S18, and S19) were the only ones that were obviously resinous. Discolored fir root wood was less variable and generally supported less fungal O_2 uptake than did discolored spruce wood (Table 1). Much of the fir discoloration had the characteristics of wetwood (e.g., water-soaked appearance, browning after exposure, and fermentative odor) and occupied the inner core of the roots (29).

Eight species of wood decay fungi varied widely in their rates of O_2 uptake on healthy and discolored spruce wood (Fig. 3). Oxygen uptake by four of the fungi (*H. annosum*, *C. puteana*, *T. balsameus*, and *P. chrysosporium*) on discolored wood remained fairly low relative to that on healthy wood throughout the

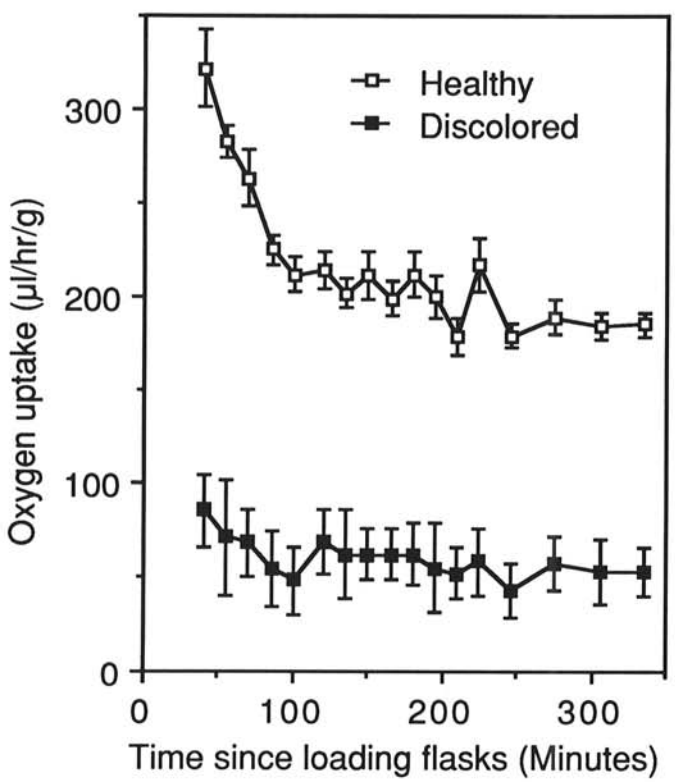

Fig. 1. Oxygen uptake by *Resinicium bicolor* on healthy and discolored red spruce root wood, measured at frequent intervals after transfer of colonized samples to respirometry flasks. Each point is the mean (± standard error) of three replicates.

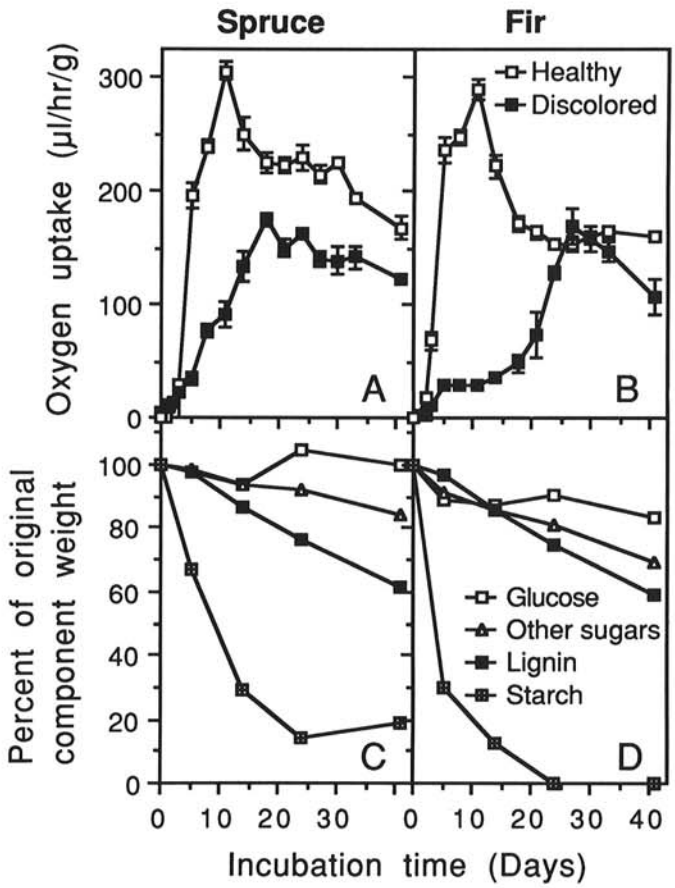

Fig. 2. A and B, Oxygen uptake by *Resinicium bicolor* on healthy and discolored milled root wood samples harvested at various intervals after inoculation. **A,** Red spruce. **B,** Balsam fir. **C and D,** Changes in components of root wood samples during the same experiment. **C,** Healthy spruce. **D,** Healthy fir. Original concentrations (w/w) in spruce were: glucose 47%, other sugars 25%, lignin 28%, and starch 0.58%. Concentrations in fir were 50, 24, 28, and 0.63%, respectively. A and B, each point is the mean of three replicates. C and D, each point is the result of a single analysis.

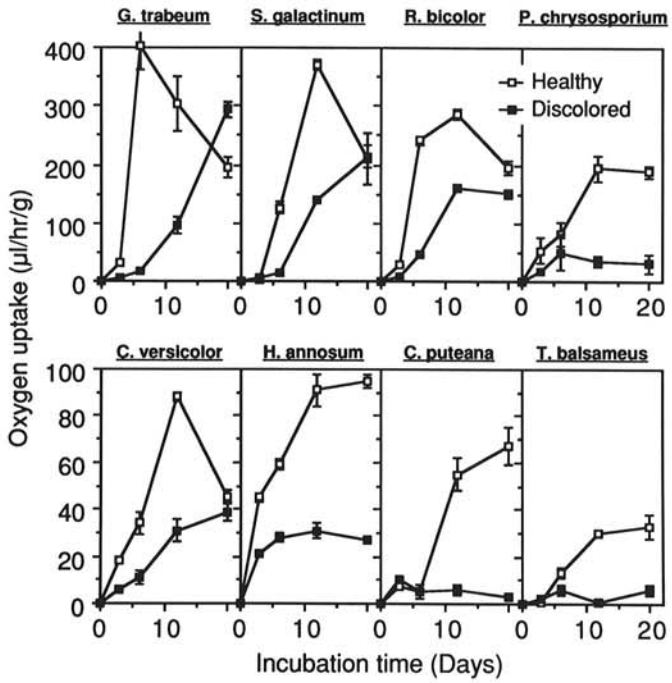

Fig. 3. Changes in oxygen uptake rates by eight test fungi after inoculation of healthy and discolored spruce root wood samples. Each point is the mean of three replicates. Note that the two tiers use different scales for oxygen uptake.

experiment. Oxygen uptake by the other four fungi (*R. bicolor*, *S. galactinum*, *G. trabeum*, and *C. versicolor*) slowly increased on discolored wood to approximately that on healthy wood by day 20. Monitoring of O_2 uptake over time was repeated for several of the fungi with similar results.

Detoxification. The delayed increase in O_2 uptake by some fungi growing on discolored wood suggested that those fungi were in some way able to overcome physical or toxic barriers. Several experiments were conducted to investigate the phenomenon. It was first considered that the delayed rise in O_2 uptake may be a result of gradual loss of inhibitors during the course of the incubation, either by leaching or by volatilization. Leaching was tested by preincubating one group of sterile samples on sterile agar for 8 days before inoculation, but subsequent O_2 uptake was actually somewhat lower on leached, discolored samples than on discolored controls. Volatilization was tested by processing fresh samples in the frozen state, oven-drying (105 C, to constant weight) a portion of each sample, and bringing all sample portions to 60% moisture content before autoclaving. Removal of volatile materials by this procedure generally did not increase subsequent O_2 uptake by *R. bicolor* on discolored samples (Table 2). The experiment was repeated with similar results.

An alternative hypothesis, that the delayed increase in O_2 uptake on discolored wood was a result of fungal metabolism that detoxifies inhibitory compounds or degrades physical barriers, was tested by two consecutive incubations. Samples of spruce root wood were preincubated with *Resinicium bicolor* for 0, 5, 10, 15, and 20 days, then dried, stored, autoclaved, and inoculated a second time. Oxygen uptake by *R. bicolor* on healthy wood during the second incubation was lower with the longer periods of preincubation (Fig. 4), presumably because some of the readily available nutrients (e.g., starch) were consumed during the first incubation. The opposite was true for discolored spruce wood; the samples that had been preincubated for longer periods supported higher O_2 uptake than those with shorter initial incubations, indicating that prior fungal metabolism under these conditions increases decay susceptibility of discolored wood.

Extractives. Our first step in identifying chemical mechanisms of resistance was extraction of discolored root wood with various solvents and testing of fungal O_2 uptake rates on extracted samples. Results of several extraction experiments are shown in Table 3. Oxygen uptake on discolored spruce wood was approximately doubled by ether extraction, but subsequent acetone or ethanol extraction had little or no effect. However, ethanol or acetone was generally more effective than ether in removing the inhibitory qualities of discolored fir samples.

Oxygen uptake on extracted samples of discolored fir was three or more times greater than on unextracted samples. Oxygen uptake on extracted samples of discolored tissues was proportional to that on the preextracted samples. For instance, the discolored fir sample in experiment 1 was very inhibitory and supported little more O_2 uptake after extraction. The discolored spruce sample in experiment 3 was mildly inhibitory, and after extraction, the O_2 uptake rate was comparable to that on healthy samples. Extraction of healthy samples had no consistent effect on O_2 uptake (Table 3).

Host response in inoculated roots. Inhibitory properties of wood discolored in response to inoculation were less variable than that of the naturally occurring discolored samples tested earlier. Within 4

TABLE 1. Oxygen uptake by *Resinicium bicolor* after 10 days' growth on healthy and discolored spruce and fir root samples

Sampled tree	O_2 uptake ($\mu l\ hr^{-1}\ g^{-1}$)	
	Healthy	Discolored
Spruce		
S 11	205 ± 29[a]	50 ± 6
S 12	184 ± 4	37 ± 2
S 13	165 ± 6	55 ± 2
S 14	215 ± 10	107 ± 5
S 15	NT[b]	67 ± 3
S 16	NT	119 ± 9
S 17	229 ± 5	50 ± 4
S 18	NT	13 ± 3
S 19	NT	38 ± 2
Fir		
F2	NT	27 ± 1
F 11	NT	1 ± 1
F 12	252 ± 16	2 ± 3
F 13	189 ± 9	7 ± 2
F 14	NT	29 ± 1
F 15	251 ± 8	0 ± 0
F 16	217 ± 8	2 ± 2

[a] Each value is a mean of three determinations from the same discolored or healthy area, followed by the standard error.
[b] NT = not tested.

TABLE 2. Effect of oven-drying before autoclaving milled wood samples on respiraton of *Resinicium bicolor* 10 days after inoculation

Sampled tree	O_2 uptake ($\mu l\ hr^{-1}\ g^{-1}$)	
	Not dried[a]	Oven-dried[a]
Fir		
F 16 Healthy	186 ± 2[b]	241 ± 4
F 12 Discolored	10 ± 3	3 ± 2
F 13 Discolored	11 ± 3	0 ± 0
Spruce		
S 14 Healthy	251 ± 8	275 ± 6
S 12 Discolored	60 ± 2	55 ± 3
S 18 Discolored	13 ± 0	19 ± 1

[a] Samples were all brought to 60% moisture content after drying but before autoclaving.
[b] Each value is the mean of three replicates followed by the standard error.

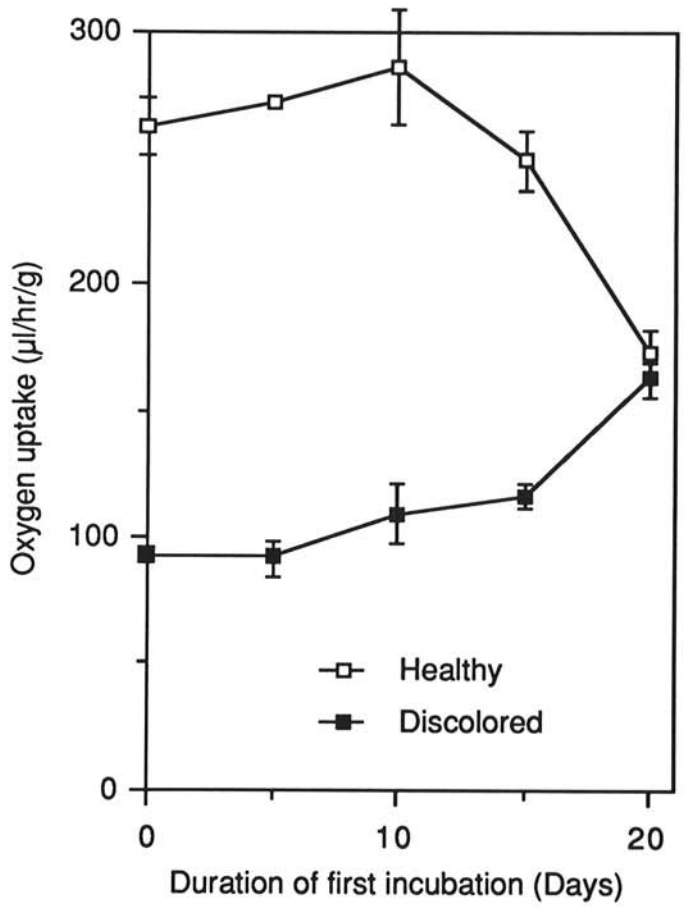

Fig. 4. Effect of varying periods of incubation of healthy and discolored spruce root wood samples with *Resinicium bicolor* on oxygen uptake measured after 10 days of a second incubation with *R. bicolor*. Each point is the mean of three replicates.

wk after inoculation of spruce roots, O_2 uptake by *R. bicolor* on discolored wood excised from around the inoculation site had dropped to about 25% of that on the uninoculated samples (Fig. 5). Standard errors were low. *Scytinostroma galactinum*, the more virulent of the two inoculated fungi (19), induced discoloration that was somewhat more inhibitory than that resulting from inoculation with *R. bicolor*. This level of inhibition was maintained in samples harvested up to 39 wk after inoculation, and did not differ between small and large roots.

The results of fir inoculations (Fig. 5) differed in several important respects. Mean levels of inhibition in discolored zones from small fir roots were comparable to those from spruce roots, but discolored samples from large fir roots supported higher O_2 uptake rates than did those from other roots. Also, much higher standard errors were obtained on discolored samples from fir roots as compared with spruce roots.

DISCUSSION

Discolored zones in these hosts generally yielded no fungi to isolation attempts (19). Because the zones are inhibitory to fungal colonization and contain antifungal extractives, they are best considered reaction zones (21) rather than areas of fungal colonization or incipient decay.

A respirometric bioassay using milled samples can be employed with small irregular samples, such as those associated with wounds and infections. It permits ready removal or incorporation of extractives, is considerably faster than traditional methods, and is amenable to a number of test organisms. Despite these advantages, there are several drawbacks. There is potential for disruption of physical barriers (e.g., resin) by milling, and oxygen levels in milled samples may be considerably higher than in intact tissues. It must also be recognized that, although O_2 uptake may reflect degradation of structural components within the experimental time period, it is not a direct measure of wood decay. It is, however, a sensitive measure of fungal activity and, in conjunction with other methods, can provide valuable information on development and nature of the host response in woody tissues.

We observed high initial rates of O_2 uptake by the test fungi followed by a rapid decline after transfer of samples to respirometry flasks. This is commonly found in respiration by higher plants and is attributed to wound respiration (16). Disruption of cells releases oxidases and stimulates metabolic activity, which then declines. Behr (4) noted the phenomenon when measuring fungal respiration in wood blocks but attributed it to respiration of external mycelium that soon died due to lack of nutrients.

The changes over time of O_2 uptake on healthy samples can be interpreted with reference to utilization of wood components. During initial colonization by *R. bicolor*, O_2 uptake rose and starch content declined rapidly. When starch was virtually depleted, O_2 uptake reached a peak and declined to somewhat lower levels. At this time, there was evidence of degradation of structural polymers, especially lignin, which continued as O_2 uptake remained more or less constant. Toole (26) and Smith (25) observed similar patterns of fungal respiration in wood blocks, though their peaks were slower to develop than our peaks with milled samples.

With both hosts, O_2 uptake by *R. bicolor* on discolored samples was lower than that on healthy samples but gradually rose later in the experiment. In fir, this rise occurred after a lag; in spruce, it occurred slowly but steadily. At least two factors, lack of starch and presence of extractives, appear to be important in leading to lower O_2 uptake on discolored samples.

On healthy samples, starch apparently supported rapid initial growth until degradation of structural components was under way. In discolored wood, levels of starch were negligible, corresponding with the absence of an early peak in O_2 uptake. Starch utilization may be a critical event early in the interaction of host and

TABLE 3. Effect of extraction of fir and spruce wood samples on respiration of *Resinicium bicolor* after a 10-day incubation period

Sample	Expt.	O_2 uptake (μl hr^{-1} g^{-1}) None[a]	Ether	Ether; ethanol	Ether; acetone
Fir					
Healthy	1	204 ± 9[b]	242 ± 9	NT[c]	226 ± 2
	2	236 ± 5	231 ± 19	200 ± 10	NT
	3	334 ± 1	277 ± 20	264 ± 26	NT
Discolored	1	8 ± 1	10 ± 5	NT	23 ± 1
	2	35 ± 3	55 ± 7	113 ± 15	NT
	3	23 ± 4	50 ± 4	86 ± 6	NT
Spruce					
Healthy	1	208 ± 8	218 ± 4	NT	213 ± 3
	2	262[d]	237 ± 12	183 ± 16	NT
	3	273 ± 5	293 ± 9	281 ± 14	NT
Discolored	1	48 ± 5	92 ± 3	NT	102 ± 1
	2	30 ± 4	50 ± 7	54 ± 11	NT
	3	107 ± 6	222 ± 18	209 ± 15	NT

[a] Milled wood samples were not extracted, extracted with ether only, extracted with ether followed by an ethanol extraction, or extracted with ether followed by an acetone extraction.

[b] Each value is the mean of three independently extracted replicates followed by the standard error.

[c] NT = not tested.

[d] One replicate only.

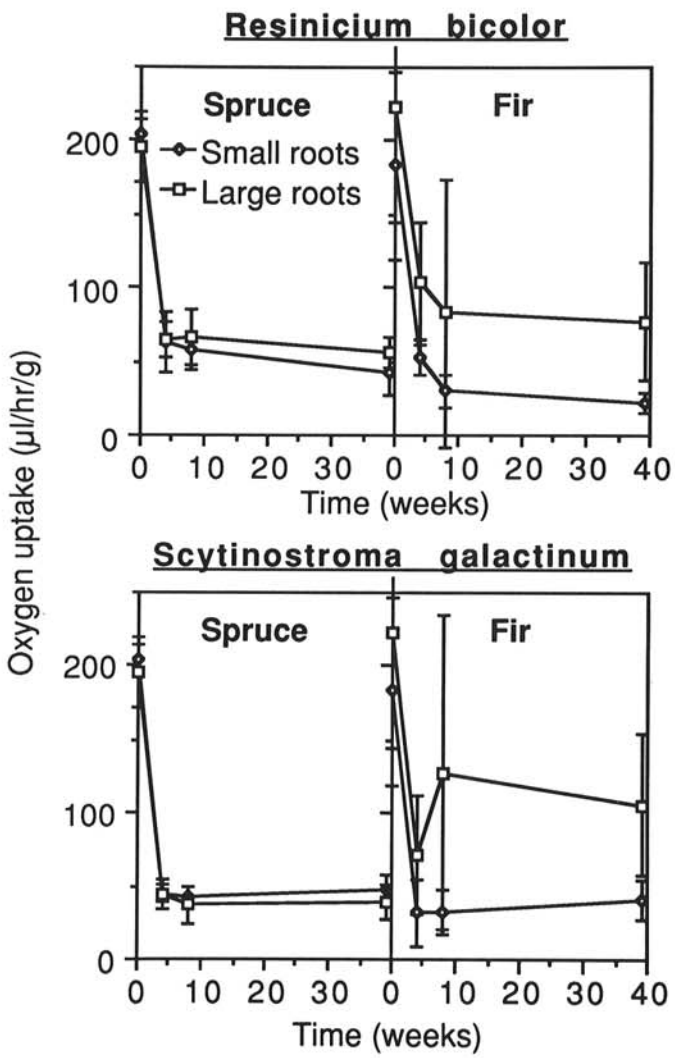

Fig. 5. Oxygen uptake rates of *Resinicium bicolor* on discolored wood from small and large roots of spruce and fir that had been previously inoculated in the field with *R. bicolor* or *Scytinostroma galactinum* and harvested at three dates. Data for week 0 is from healthy wood that had not been inoculated and was collected at the 8-wk harvest date. Each point is the mean of four or five replicate roots.

pathogen. If the host response is successful, starch is used in the production of defensive compounds and thus made unavailable to pathogens (15). If, however, a pathogen kills and colonizes tissues before any host response occurs, it may gain access to the starch, enabling further rapid colonization.

But, because O_2 uptake on discolored wood generally remained lower than that on healthy sapwood, even when growth on healthy samples was supported solely by structural polymers, inhibitory factors in discolored zones that slow structural degradation appear to also be important. This conclusion is supported by the increase in O_2 uptake resulting from extraction of discolored wood.

In fir, discolored reaction zones had the characteristics of wetwood. Wetwood in *Abies* spp. has been implicated in resistance to butt rots (5,29) and may render wood decay-resistant in intact trees largely because of accumulation of antifungal extractives, such as low-molecular-weight organic acids, as well as the alteration of the physical environment by water-soaking and anaerobic conditions (29). Although detection of decay resistance contributed by reduced aeration was precluded by sample preparation procedures, other inhibitory mechanisms were detected. Extractions of discolored fir samples with the more polar solvents (acetone or ethanol) were somewhat more effective than ether extraction in removing inhibitory extractives. This is consistent with direct inhibition by soluble inhibitors such as organic acids, which have been found in high concentrations in wetwood of fir and are known to be toxic to decay fungi (29).

In spruce, ether removed more inhibitory extractives than did subsequent extraction with polar solvents, suggesting the importance of nonpolar extractives such as oleoresin. Although there has been much interest in detecting direct antibiotic activity of extractives such as oleoresin components, experiments of that nature have given conflicting results in the case of *Picea* spp. (8,9,12,18,22). Oleoresin components may instead, or in addition, function as an inert, hydrophobic barrier, protecting the walls from enzymes (27).

All of the eight fungi tested with healthy and discolored spruce samples used less O_2 on the discolored sample over most of the experiment. For some species, growth on discolored wood eventually approximated that on healthy wood, but others were strongly inhibited by discolored wood throughout the experiment. These differences did not coincide with groupings of the fungi based on pathogenicity or type of decay.

Three hypotheses were considered to explain the increase in growth during incubation of discolored samples with test fungi: 1) inhibitory materials were leached from the sample into the agar during incubation, 2) they were lost by volatilization, or 3) they were detoxified or bridged by fungal activity. The experiments devised to test these hypotheses ruled out 1) and 2) and supported 3). Prior fungal growth on discolored spruce samples permitted higher O_2 uptake rates during the second incubation, whereas the opposite was true for healthy samples. This indicates that during the first incubation, the fungus was able to detoxify inhibitory materials in the discolored wood or circumvent a physical barrier. If inhibition results from binding of fungal extracellular enzymes to extractives, as suggested for ellagitannins in *Quercus alba* (11), detoxification could result from saturation of binding sites. The lag period observed in some cases could represent the initial period of binding. Detoxification is also suggested by studies on the effects of lignans (18), polar extractives and resin acids (8) from *Picea abies*, where inhibition was also observed to decline during incubation. However, there is reason to suspect that such in vitro detoxification may in some cases be an artifact (8).

Based on surveys of root and butt rot and on controlled inoculations, balsam fir is much more susceptible to root-rotting hymenomycetes than is red spruce (19). However, our collections of naturally occurring discolored fir and spruce roots generally showed discolored fir wood to be more decay resistant than discolored spruce wood. Detoxification of the spruce samples during incubation may explain part of this discrepancy. In addition, mechanisms that rely on alteration of the physical environment, such as low O_2 levels in wetwood, or physical barriers, such as that presented by resin, may be disrupted by milling, drying, and other sample preparation processes, introducing an artifact. Finally, the sample of naturally occurring discolored zones may be biased by not including unsuccessful discolored zones, i.e., those that were decayed. The ability of hosts to rapidly deliver an effective response at the site of injury can be compared only by controlled inoculations with the same pathogen, same type of wound and initiated at the same time.

Bioassays of inoculated roots harvested after various periods revealed important differences between fir and spruce. Within 4 wk of inoculation, affected wood in small roots of both hosts supported about 75% lower O_2 uptake than did healthy wood. In spruce, large roots responded essentially the same as small roots. In fir, however, large roots usually showed a weaker response than small roots, and the response was much more variable than in spruce. Consistent with the respirometry data, Rizzo (19) found that *R. bicolor* and *S. galactinum* were able to successfully infect and colonize the large fir roots but not the small fir roots that were sampled for our bioassay. Also consistent with the respirometry data was the greater resistance of red spruce than balsam fir to colonization by these fungi after 2 mo (19).

These data lead to several conclusions regarding the susceptibility of balsam fir to root disease relative to that of red spruce. First, the greater susceptibility of balsam fir appears to be a function of the weaker, inconsistent host response of large fir roots. Thus, susceptibility is apparently expressed in large rather than in small roots. Second, because individual firs may be found with host response in large roots that is as effective as that of spruce, substantial variation exists in this trait in fir, much more than in spruce.

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