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NITROGENASE ACTIVITY ASSOCIATED WITH DECAYED WOOD OF LIVING NORTHERN IDAHO CONIFERS

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ABSTRACT

Nitrogen fixation, as determined by the acetylene reduction technique, was demonstrated in decay columns of western larch and western white pine caused by *Phellinus pini,* western red cedar caused by *Postia sericeomollis,* and western hemlock and grand fir caused by *Echinodontium tinctorium.* Nitrogenase activity vaned with tree species, fungal pathogen, wood decay stage, and seasonal moisture-temperature regimes within decaying stems. Nitrogen fixation potential in the productive forest ecosystems of northern Idaho was calculated between 0.06 kg/ha/yr and 4.91 kg/ha/yr and was primarily dependent on volume of decay in live standing trees on site.

Key Words: acetylene reduction, asymbiotic nitrogen fixation, site productivity, nitrogen input

Decay of heartwood in live, standing trees by wood-rotting fungi continues to be a significant problem to forest management. Both hardwoods and conifers are affected (Boyce, **1961;** Wagener and Davidson, **1954).** Numerous reports have shown that other microorganisms are associated with heartrot fungi during wood decay, *e.g.,* yeasts, bacteria, and imperfect fungi (Blanchette and Shaw, **1978;** Shigo and Hillis, **1973;** Maloy and Robinson, **1968;** Good and Nelson, **1962).** It is also common to find bacteria, yeasts, streptomycetes, and other non-basidiomycetes in apparently sound and healthy trees (Bagley *et al.,* **1978;** Greaves, **1971;** Etheridge and Morin, **1967;** Bouchier, **1961, 1967).** Greaves **(1970)** demonstrated both inhibition and stimulation of decay by bacteria and actinomycetes. Blanchette *et al.* **(1978)** showed enhancement of decay in wood blocks inoculated with a filamentous white-rot fungus growing in association with bacteria or yeasts. Bacon and Mead **(1971),** studying the occurrence of bacteria in living aspen, pine, and alder, noted that many of the species they encountered could be regarded as soil-inhabiting organisms. Aho and Hutchins **(1977)** reported extensive microbial colonization of the pith region in healthy though suppressed grand fir *[Abies grandis* (Dougl. *ex* D. Don) Lindl.] trees in Oregon. Included among these organisms were nitrogen (N)-fixing bacteria.

The role of heterotrophic N-fixing bacteria associated with the cycling of nitrogen during wood decay is particularly interesting from an ecosystem perspective. Presence and activity of N-fixing bacteria have been demonstrated in a wide variety of both large and small hardwood

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and conifer forest residues (Jurgensen *et al.,* **1979, 1984,1987;** Larsen *et al.,* **1982;** Roskoski, **1980;** Cornaby and Waide, **1973),** fungal fruiting bodies on decaying trees and residues (Larsen *et al.,* **1978),** and with certain insects that invade and utilize dead woody materials (Bridges, **1981;** Potrikus and Breznak, **1977).** Rates of N fixation have been shown to differ with change of substrate, *i.e.,* wood species, decay stages, fungal species, and seasonal moisture-temperature regimes (Jurgensen *et al.,* **1984;** Roskoski, **1980;** Larsen *et al.,* **1978;** Aho *et al.,* **1974).** Nitrogen-fixing bacteria have been isolated directly from decaying woody residues (Spano *et al.,* **1982;** Aho *et al.,* **1974)** and have been shown to penetrate blocks of wood in contact with forest soil (Baines and Millbank, **1978).**

Nonsymbiotic N-fixing bacteria are also associated with living trees. Nitrogen fixation has been reported in stem wetwood of western black cottonwood (*Populus trichocarpa* Torr. and Gray) (van der Kamp, **1986)** and associated with decay by *Hericium abietis* (Weir *ex* Hubert) K. Harrison and *Phellinus pini* var. *cancriformans* Larsen *et al.* [reported under the name *Phellinus chrysoloma* (Fr.) Donk] in living white fir *[Abies concolor* (Gord. *et* Glend.) Lindl.] trees (Aho *et al.,* **1974).** It has also been associated with decay of living western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] caused by *Echinodontium tinctorium* (Ell. *et* Ev.) Ell. *et* Ev. (Larsen *et al.,* **1978).** Nitrogen fixation was not found in decay columns of white fir caused by *Heterobasidion annosum* (Fr.) Bref., or *Armillaria mellea* (Vahl: Fr.) Quél. (Aho *et al.,* **1974;** Seidler *et al.,* **1972).** However, none of these studies were designed to examine seasonal N fixation associated with major heartwood decay (brown *vs* white) fungi, or to assess the contributions of N-fixing bacteria to stand N economy during heartwood decay.

The objectives of this investigation were: 1) to measure N fixation associated with pathological decay of heartwood in live standing conifers; 2) to examine potential differences in N fixation that may exist among fundamentally different kinds of heartwood decay, *e.g.,* brown-rot, white pocket rot, and white stringy rot; and 3) to estimate the N accretion to forest sites associated with heartwood decay. Tree and fungus species chosen for study were representative of major timber-producing tree species and common heartrot fungi in the highly productive forests of the northern Idaho Panhandle.

MATERIALS AND METHODS

The study site is on the Priest River Experimental Forest (USDA Forest Service), Kaniksu National Forest in northern Idaho. Timber type is generally dominated by western hemlock and western red cedar (*Thuja plicata* Donn), with grand fir well represented. Also present are western larch (*Larix occidentalis* Nutt.), western white pine (*Pinus monticola* Dougl. *ex* D. Don), and Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franc ~]The habitat type is *Tsuga heterophylla-Pachistima myrsinites* (Cooper *et al.,* **1987;** Daubenmire and Daubenmire, **1968).** Slope is less than 10%, and trees selected for sampling were in or near riparian sites.

Climate is transitional between northern Pacific and continental, characterized by wet, cloudy winters and dry, sunny summers. Elevation of the experimental site is 725 m. Annual precipitation averages 817 mm. Monthly mean temperatures range from −4C in January to 18 C in July, and the annual mean temperature is 7 C (Finklin, **1983).**

Criteria for selection of trees with suitable decay columns were presence of fruiting bodies and detection of heartrot with increment borings. This selection process resulted in five fungus–treehost combinations: *Phellinus pini* causing a white pocket decay in western larch and western white pine; *Postia sericeomollis* (Rom.) Jülich causing a brown, cubical decay in western red cedar; and *Echinodontium tinctorium* causing a white, stringy decay in western hemlock and grand fir. Diameter of western red cedar averaged 40 cm, diameter of the other species averaged 29 cm. Small pieces of woody tissue from characteristic decays were flame sterilized, placed on 2% malt agar (w/v), and incubated at 18C. Fungal growth typical of the respective organisms assured presence of active hyphae,

Three trees from each of the five fungus–host combinations were sampled in July, August, September, and October, 1977. Five samples of decayed heartwood (approximately 50 cm^2) were obtained from the centers of each tree bole with a chainsaw and were separated into three decay stages: 1) incipient decay; 2) intermediate decay; and 3) advanced decay.

Each sample was immediately chipped into small pieces and placed into five 20-ml Vacutainer tubes (Becton-Dickinson Div., Becton Dickinson[1]). Tree samples were replaced with matching wood blocks to seal the stem until all four monthly samples had been taken. A total of 900 samples were analyzed over the course of this study [3 trees × 3 decay stages × 5 tree host–fungus combinations × 5 replications (one control without injected acetylene) × 4 sampling times].

Nitrogen-fixation rates were estimated using an adaptation of the acetylene reduction technique reported by Hardy et al. **(1968).** Because N-fixing bacteria associated with wood decay are reported to be either microaerophilic or anaerobic (Aho et al., **1974;** Schink *et al.,* **1981;** Silvester *et al.,* **1982),** atmospheric oxygen introduced into the sample when trees were cut open had to be removed. Therefore, sample tubes were evacuated and flushed five times with pure argon gas prior to injection. Four of the five tubes from each decay type were then injected with 1.5 ml of acetylene gas. The non-injected tube was used as a control to monitor endogenous ethylene production in each decay type/fungus combination. Evacuation and flushing lowered oxygen concentration in the tubes to the 1 to 3% range. All tubes were incubated for 24 hours at the ambient temperature of the decay column at the time of plug removal (July 14C, August 17C, September 13C, October 9 C). After incubation, gas samples were drawn from the incubation tube with 2-ml glass Vacutainers for analysis. Samples were dried at 105 C for 24 hours to determine dry weight and moisture content. These samples were then analyzed for lignin and decayed wood solubility in alkali (TAPPI, **1983;** Effland, **1977;** Nelson, **1944).**

Gas samples were analyzed for acetylene and ethylene concentrations using a Varian series 2800 gas chromatograph fitted with an 80- to 100-mesh poropak R column. The column was operated at 50 C using N as the carrier gas (Jurgensen *et al.,* **1987).** Background ethylene in the acetylene gas used for sample injection was also determined. Acetylene concentration used in the experiment also provided an internal standard for the correction of air space variations in the tubes and for gas leakage (McNabb and Geist, **1979).**

An analysis of variance was used to determine effects of fungus, host tree, sampling time, and decay type on both ethylene production and moisture content. When significant differences were found ($P = 0.05$), the Duncan multiple range test was used for grouping data.

RESULTS AND DISCUSSION

Nitrogen fixation, as expressed by acetylene reduction to ethylene, was detected and measured in all fungus–host tree species combinations and decay stages (TABLE I). Depending on specific fungus–host associations, ethylene production rates vaned with sample date (TABLE II). Mean ethylene production rates (for all decay types by host-species, fungus, and sample date) were higher for Postia sericeomollis and Phellinus pini compared to Echinodontium tinctorium (TABLES I, II). With all fungus–host combinations the fixation rates were highest in July, declining to a low point in October (TABLE II).

Moisture content of heart-rotted wood vaned with fungus–host species combinations, decay type, and decay stage (TABLE III). The relationship between ethylene production rates and moisture content of heart-rotted wood appears to be affected by fungus species (TABLES I, III). An inverse relationship between nitrogenase activity and wood moisture content was evident for *P. sericeomollis* decaying western red cedar and *E. tinctorium* decaying western hemlock. In the other host tree–fungus associations, nitrogenase activity and moisture content generally declined or increased together, supporting an earlier study that indicated nitrogenase activity was a function of moisture content in decaying wood (Larsen et al., **1978).** General decline in wood moisture content with decay progression found for host–fungus combinations in this study, except *E. tinctorium*–grand fir, suggests that processes associated with heartwood decay by these fungi in living trees may remove a physical or chemical barrier that otherwise could maintain internal moisture.

Phellinus pini causes a white pocket rot, or decayed heartwood with voids in advanced stages, constituting a major structural discontinuity. This provides major avenues for gas (including oxy-

[1] The use of trade or firm names in this paper is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

TABLE I

ETHYLENE PRODUCTION FROM HEART-ROTTED WOOD OF LIVE STANDING CONIFERS IN NORTHERN IDAHO[a]

Heartrot fungus	Tree species	Ethylene production[b] Incipient decay	Intermediate decay	Advanced decay
		(nm/g/odw/24 hr)		
P. sericeomollis	w. red cedar	1.43[a]	1.47[a]	1.90[b]
E. tinctorium	grand fir	1.38[e]	1.60[f]	1.81[g]
	w. hemlock	1.67[l]	1.18[m]	1.84[n]
P. pini	w. larch	1.25[s]	1.21[s]	1.10[s]
	w. white pine	1.38[x]	1.21[x]	1.16[x]

[a] Average values for the four sampling dates.
[b] Values denoted by different letters within rows are significantly different at the P = 0.05 level.

gen and water vapor) exchange and may account for the low moisture content and associated nitrogenase activity for this host–fungus association (TABLES II, III). *Echinodontium tinctorium* causes a decay that delaminates wood, both axially and radially, producing a white stringy rot. Major structural discontinuity occurs only in late stages of decay. Brown-rotted wood caused by *Postia sericeomollis,* from the incipient to advanced decay stages, maintains its structural integrity. Shrinkage and collapse provide only minor structural discontinuity even very late in the decay process.

Investigations on N fixation in downed woody residues have generally shown high ethylene production rates associated with advanced wood decay and high moisture content (Jurgensen *et al.,* **1984;** Spano *et al.,* **1982,** Larsen *et al.,* **1978).** In heartwood decay of living trees this ethylene production–moisture relationship is more variable (TABLES I, II of this study; Larsen *et al.,* **1978;** Aho *et al.,* **1974;** Seidler *et al.,* **1972).** Therefore, it seems likely that other wood variables, in addition to moisture content, are controlling the nature or activity of N-fixing bacteria associated with specific fungus–host combinations. These may include concentrations of O_2 and CO_2 (Highley *et al.,* **1983;** Highley and Kirk, **1979),** tree species and site effects on moisture level (Etheridge, **1958),** or wood pH (Gray, **1958).**

Relationships between decay stage, chemical nature of the decay, and fungus–host combinations in heart-rotted wood are presented in TABLE IV. Other studies on N fixation in brown-rotted wood have shown that chemical characterization (lignin and carbohydrate) of substrate can provide a means for defining decay classes (Jurgensen *et al.,* **1984;** Spano *et al.,* **1982).** In this study, visual field estimates and chemical analyses agreed for decay classes of brown-rotted wood. However, white-rot fungi remove lignin and cellulose (including hemicellulose) at equal rates. Thus, chemical properties did not provide a clear picture of decay progression. Because some white-rot fungi selectively remove more lignin than cellulose (Blanchette, **1984),** the determi-

TABLE II

SEASONAL EFFECT ON ETHYLENE PRODUCTION FROM HEART-ROTTED WOOD OF LIVE STANDING CONIFERS IN NORTHERN IDAHO[a]

Heartrot fungus	Tree species	Ethylene production[b] July	August	September	October	Average
		(nm/g/odw/24 hr)				
P. sericeomollis	w. red cedar	1.62	1.66	1.77	1.27	1.60[x]
E. tinctorium	grand fir	2.17	1.48	1.42	1.25	1.60[x]
	w. hemlock	1.65	1.62	1.49	1.52	1.57[x]
P. pini	w. larch	1.58	1.02	0.88	1.16	1.19[y]
	w. white pine	1.62	0.99	1.24	1.16	1.25[y]
		1.68[a]	1.34[b]	1.36[b]	1.28[b]	

[a] Average values for the three decay stages.
[b] Values denoted by different letters are significantly different at the P = 0.05 level.

TABLE III

WOOD MOISTURE CONTENT (%) ASSOCIATED WITH HEART-ROTTED WOOD OF LIVE STANDING CONIFERS IN NORTHERN IDAHO[1]

Heartrot fungus	Tree species	Decay class[b] Incipient	Intermediate	Advanced
		%		
P. sericeomollis	w. red cedar	173[a]	39[b]	69[c]
E. tinctorium	grand fir	112[e]	125[e]	156[f]
	w. hmelock	100[l]	76[m]	75[m]
P. pini	w. larch	78[s]	42[t]	44[t]
	w. white pine	84[x]	46[y]	46[y]

[a] Average values for the four sampling dates.
[b] Values denoted by different letters across rows are significantly different at the P = 0.05 level.

nation of alkali solubility (primarily the solubilization of cellulose and hemicellulose) for white-rotted wood should provide an added means for interpreting decay progression (**TABLE** IV). This analysis did provide limited success for discriminating incipient from intermediate and advanced decays of *P. pini* and *E. tinctorium*, and substantiated decay classes with *P. sericeomollis*.

Data presented here can be used for approximating potential N gains associated with pathological decays of living trees in northern Idaho forests. Representative heartwood volumes for this region were obtained from records on stand volumes and cull percentages of the Panhandle National Forest in northern Idaho (TABLE V). Cull is defined primarily as stem decay, in minor instances as stem deformities (1–2% of the cases), that render the tree unsuitable for commercial products. These values were based on site records from more than 4700 stands covering the six major, commercial habitat types in eastern Washington, northern Idaho, and western Montana.

Although significant differences in ethylene production were noted among decay stages, organisms, etc., these differences were modest. Overall average ethylene production rate of decaying heartwood in living trees (species, date, and decay stage inclusive) was 1.43 nm C_2H_4/g (dry weight) of infected wood per day. A theoretical conversion factor of acetylene reduction to N fixation of 3:1 was used for this calculation to facilitate comparison with other studies, even though it may overestimate N conversion (Silvester *et al.*, **1982;** Roskoski, **1981).**

Assuming N-fixing bacteria are active in decay columns at least 6 mo of the year (180 days), the majority of N gains in decaying, live timber on an average northern Idaho site would amount to <0.1 kg/ha/yr. In stands where cull volume is large, such as on many wet, cool riparian sites and habitat types, N additions could be greater than 1 kg/ha/yr, particularly if the season is extended beyond the 180-day estimates used above. Such large N gains on some sites could equal or surpass the amounts of N fixed in downed woody residues on forest soils in this region (Jurgensen

TABLE IV

LIGNIN, TOTAL CARBOHYDRATE, AND ALKALI SOLUBILITY VALUES (%) OF HEART-ROTTED WOOD FROM LIVE STANDING CONIFERS IN NORTHERN IDAHO

Decay class	*P. sericeomollis* (western red cedar)	*E. tinctorium*[a] (grand fir & w. hemlock)	*P. pini*[b] (w. larch & w. w. pine)
Lignin		Percentage[c]	
Incipient	35.1[a]	32.2[e]	29.6[l]
Intermediate	37.9[a]	35.5[e]	31.1[l]
Advanced	51.3[b]	33.6[e]	29.0[l]
Total carbohydrate			
Incipient	61.2[a]	63.7[e]	63.8[l]
Intermediate	58.5[a]	58.4[f]	62.9[l]
Advanced	42.0[b]	57.5[f]	64.1[l]
Alkali solubility			
Incipient	14.2[a]	13.9[e]	19.0[l]
Intermediate	18.4[a]	21.3[ef]	25.3[m]
Advanced	31.8[b]	29.0[f]	27.9[m]

[a] Values for grand fir and western hemlock combined due to lack of significant differences.
[b] Values for western larch and western white pine combined due to lack of significant differences.
[c] Values denoted by different letters down columns are significantly different at the P = 0.05 level.

TABLE V

LIVE, STANDING VOLUME, PERCENT CULL, AND NITROGEN FIXATION FOR COMMERCIAL FOREST HABITATS IN NORTHERN IDAHO[a]

Habitat series	Stands sampled	Live volume		Cull		N fixation	
		Mean	Range	Mean	Range	Mean	Range
		(m^3/ha)		(%)		(gN/ha/180 days)	
PSME[b]	148	175	26–563	6	<1–95	14	<1–515
ABGR	467	243	7–1108	9	1–100	21	<1–1060
THPL	942	272	8–1433	8	1–99	21	<1–1360
TSHE	2260	298	8–1294	11	<1–100	31	<1–1240
ABLA	372	208	6–5523	7	<1–90	14	<1–4765
TSME	557	219	1–4156	8	1–100	17	<1–3985

[a] Data on file at the Forest Supervisor's Office, Idaho Panhandle National Forest, USDA Forest Service, Coeur d'Alene, Idaho.

[b] Habitat series designations, Douglas-fir, grand fir, western red cedar, western hemlock, subalpine fir, and mountain hemlock, respectively (Daubenmire and Daubenmire, 1968).

et al., **1989).** Because cull volume estimates include a small amount of non-decay-related defect, these calculations may slightly overestimate N fixation. This error would be at least partially counterbalanced by N fixation in heartwood decay of trees showing no external symptoms that were not included in cull estimates.

This study shows that N-fixing bacterial populations are likely to accompany common fungal-initiated wood decay processes of living trees. The presence of N-fixing bacteria in decay columns is a considerable advantage for decay fungi colonizing these high C/N substrates. While amounts of N fixed in decaying heartwood of most forested stands in northern Idaho are low, appreciable amounts can be added to sites with large cull volumes. This source of N should be considered in calculating N budgets, particularly for the highly defect-prone habitat types in northern Idaho and adjacent areas.

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