Associations Among Bacteria, Yeasts, and Basidiomycetes During Wood Decay

Robert A. Blanchette and C. Gardner Shaw

Research Assistant and Professor, respectively, Department of Plant Pathology, Washington State University, Pullman, WA 99164.

Scientific Journal Series Paper 4855, Project 0273, College of Agriculture Research Center, Washington State University, Pullman, WA 99164.

Portion of thesis to be submitted by the senior author in partial fulfillment for the Ph.D. degree, Washington State University.

The authors thank A. L. Cohen, Director, The Electron Microscope Center, for his cooperation and helpful suggestions and for use of facilities at the center; S. Gurusiddaiah, Bioanalytical Laboratory, WSU; P. Aho, Forestry Sciences Laboratory, Corvallis, OR 97331 for cultures of *Enterobacter* spp.; C. P. Kurtzman, ARS Research Fermentation Laboratory, Peoria, IL 61604, for cultures of yeasts; and F. F. Lombard, Forest Products Laboratory, Madison, WI 53705, for the basidiomycete cultures. We gratefully acknowledge technical assistance from Potlatch Corp., Lewiston, ID 83501.

Accepted for publication 27 September 1977.

ABSTRACT

BLANCHETTE, R. A., and C. G. SHAW. 1978. Associations among bacteria, yeasts, and basidiomycetes during wood decay. Phytopathology 68: 631-637.

Bacteria, yeasts, and basidiomycetes were closely associated during the decay of dead coniferous wood. In the laboratory, significant increases in decay (weight loss) and a marked stimulatory effect on mycelial growth was observed after 5 mo in wood decay treatments combining bacteria (*Enterobacter* spp.) and yeasts (*Saccharomyces bailii* var. *bailii* and *Pichia pinus*) with the basidiomycetes, *Coriolus versicolor, Hirschioporus abietinus,* or *Poria placenta*. Substrates used were wood chips from slash less than 1 yr, between 1 and 2 yr, and over 25 yr old. The amount of mycelia present was determined quantitatively by assaying for glucosamine. Up to 200% more fungal growth was obtained in treatments combining bacteria, yeasts, and a basidiomycete than with single basidiomycetes. Substantially more decay (2-10 fold) occurred with the brown-rot fungus, *P. placenta*, than with the white-rot fungi, *C. versicolor* or *H. abietinus*. The age of the chips and the species of wood used affected the rate of decomposition. Decay was more rapid in wood chips from slash less than 2 yr old than in that over 25 yr old. Bacteria and yeasts were responsible for increases of decay by the respective basidiomycete in wood chips of all age classes. Scanning electron microscopy revealed mutualistic associations among microorganisms. Bacteria and yeasts were located only in tracheid cell walls decayed by basidiomycetous hyphae. Similar associations of bacteria, yeasts, and mycelia were observed in logs naturally infected by four basidiomycetes, *C. versicolor*, *H. abietinus*, *Cryptoporus volvatus*, and *Fomitopsis pinicola*.

Additional key words: scanning electron microscopy, brown-rot fungi, white-rot fungi, synergism, deterioration, forest residue management, glucosamine analysis, conifers.

The roles microorganisms play in wood decay has been examined in cultural studies (4, 7, 14, 17, 18) and by light microscopy (15, 20, 21). Because of difficulties in isolating bacteria and yeasts and observing them in their natural state, the associations these organisms have with wood-decay fungi have not been adequately elucidated.

Bacteria may supply essential vitamins or growth-promoting substances to wood-decaying fungi while utilizing woody cell wall components modified by extracellular fungal enzymes (10, 17). In addition, the ability of some wood-inhabiting bacteria to fix atmospheric nitrogen (1, 16) could enhance mycelial growth and have a marked effect on the rate of wood decomposition.

Yeasts also form an important association with basidiomycetes during the wood decay process. Investigations indicate that yeasts are present in both initial (2, 13) and advanced stages of wood decay (19).

The scanning electron microscope (SEM) is a useful instrument in the study of wood anatomy and decay (3). The effects of microorganisms on wood and the patterns of wood cell wall lysis have been observed (8,11,12). These studies were conducted with wood blocks inoculated with one organism and consequently did not demonstrate the associations that exist between microorganisms during the wood-decay process. In a sample of ponded spruce, Jutte and Zabel (12) noted that bacteria were prevalent in the bordered pits and frequently clustered along cell wall lysis zones associated with hyphae, suggesting an interacting association between bacteria and fungi.

This study was conducted to: (i) determine with SEM techniques the associations among bacteria, yeasts, and basidiomycetes in wood during the decay process, and (ii) investigate the significance of these organisms, alone and in combination, on the rate of decay.

00032-949X/78/000 104$03.00/0

MATERIALS AND METHODS

Wood chips, approximately 0.5 × 3.0 × 2.0 cm, of three age classes: less than 1 yr old (from time of cutting), between 1 and 2 yr old, and over 25 yr old, were used. The chips of the first two age classes consisted of 50% western white pine (*Pinus monticola* Dougl.), 35% fir [*Pseudotsuga menziesii* (Mirb.) Franco and *Abies grandis* (Dougl.) Lindl.] and spruce (*Picea engelmannii* Parry ex Engelm.), and 15% western red cedar (*Thuja plicata* Donn). Chips over 25 yr old consisted of 65% cedar, 25% fir and spruce, and 10% western white pine. Chips of each age class were well mixed and used in a laboratory decay study. Approximately 50 g (dry weight) of chips were placed in quart jars stoppered with cotton. The moisture content was adjusted to 110% with distilled water and the chips were autoclaved for 2 hr at 121 C. For each age class of wood chips, the treatments included: *Coriolus* (*Polyporus*) *versicolor* (L. ex Fr.) Quél., *Hirschioporus* (*Polyporus*) *abietinus* (Dicks. ex Fr.) Donk, and *Poria placenta* (Fries) Cooke (cultures obtained from F. F. Lombard) inoculated alone and in combination with composite inocula consisting of two species of nitrogen-fixing bacteria (*Enterobacter* spp. obtained from P. Aho) and two yeasts [*Pichia pinus* (Holst) Phaff and *Saccharomyces bailii* Linder var. *bailii* obtained from C. P. Kurtzman]. Each treatment was replicated four times. The basidiomycetes were inoculated into the wood chips by "feeder strips", each consisting of one sterile wood chip, approximately 0.05 - 0.08 g (dry weight), that had been placed on an actively growing culture (Difco malt agar) until it was completely covered by mycelium. The yeasts and bacteria were grown on a malt/PDA agar (5 g Difco malt extract, 5 g Difco potato-dextrose agar, 2 g Difco yeast extract, and 15 g Difco bacto agar) in 225-ml prescription bottles placed horizontally. Cells from cultures grown at 28 C for 3 days were washed from the agar surface with sterile saline solution. Twenty ml of this suspension, containing 1×10^6 cells/ml, were added aseptically to the wood chips at the time of basidiomycete inoculation. Controls received a "feeder strip" free of mycelia as well as 20 ml of saline solution washed from noninoculated agar. One treatment included the composite inocula of bacteria and yeasts, but without the basidiomycete. Jars containing the wood chips were placed in sealed polyethylene bags and then were incubated at 24 - 26 C. A cotton plug saturated with a 0.1% solution of sodium hypochlorite solution (sufficient to prevent mold growth on the cotton) was placed in the bag to maintain the wood chips at a moisture level of 100% for the duration of the study.

After 5 mo, two samples of each treatment were dried at 105 C for 48 hr and the weight loss was determined gravimetrically. Samples from two additional jars of each treatment were used for the SEM study. Wood chips were taken from the treatments after 1 and 5 mo. Samples were vapor-fixed over 2% osmium tetroxide (O_sO_4) for 1 hr, plunged into an aqueous solution of 2% OsO_4 for an additional hour, dehydrated through a graded ethanol and Freon TF series, critical-point dried (6), and coated with gold in a Technics Hummer 2 sputtering apparatus. The specimens were examined and photographed at a 45-degree angle with an Etec Autoscan U-1 microscope at 20 Kv.

To compare the wood decayed in the laboratory with naturally decayed wood in dead trees, coniferous wood was sampled 30 cm from sporophores of *Coriolus versicolor, Hirschioporus abietinus, Cryptoporus (Polyporus) volvatus* (Peck) Shear, and *Fomitopsis* (*Fomes*) *pinicola* (Schw. ex Fr.) Karst. Nine fallen logs from three locations in northern Idaho were examined with the SEM for each fungal species. Within 24 hr after sampling, specimens of decayed wood were brought into the laboratory and sections were removed and prepared for the SEM as explained above. Additional sections of each sample were cultured on Difco trypic soy agar to detect the presence of bacteria and on a selective malt agar medium (5) with one modification (4 ml of 85% lactic acid) to determine the presence of basidiomycetes and yeasts.

Glucosamine from fungal chitin was determined in order to quantify the amounts of mycelia present in decayed wood. The samples were ground to pass a 425 μm (40-mesh) screen, hydrolyzed with 6 N hydrochloric acid for 2 hr, and the level of glucosamine was determined with an amino acid analyzer by modification (S. Gurusiddaiah, *unpublished*) of Wu and Stahmann's technique (22).

RESULTS

After 5 mo, the basidiomycetes plus composite inocula caused significantly more weight loss of inoculated wood than basidiomycetes alone (Table 1). Marked stimulatory effects on mycelial growth were evident in treatments combining bacteria and yeasts with basidiomycetous fungi (Fig. 1-A to C). The amounts of glucosamine, which reflect the amount of chitin present, (Table 1) were higher in treatments combining composite inocula with basidiomycetes than in those with basidiomycetes alone. Wood chips less than 1 yr old had the most striking differences, with over 200% increase in glucosamine observed for *Coriolus versicolor*. Although the weight losses for the composite inocula were only 1-4% greater than basidiomycetes alone (Table 1), the large increases in mycelia represent additional decomposition of substrate. Controls showed an insignificant weight loss and low levels of glucosamine. Treatments with bacteria and yeasts alone did not result in significant weight loss or detectable glucosamine.

After 5 mo, wood chips from slash less than 1 yr old or 1 and 2 yr old were substantially decayed (approx. 40%) by the brown-rot fungus *P. placenta*. The decay caused by the white-rot fungus, *C. versicolor* was approximately half as much (20%) as that caused by *P. placenta*. *Hirschioporus abietinus* caused approximately 20% weight loss in wood chips less than 1 yr old but only 10% in 1- and 2-yr-old chips. In wood chips over 25 yr old, an even more striking difference was observed between brown-and white-rot fungi; *P. placenta* caused a 10-fold increase in decay compared to that caused by treatments with the white-rot fungus. In treatments combining *P. placenta* or *H. abietinus* with composite inocula, the percent weight loss again increased substantially in wood chips over 25 yr old compared to those inoculated with basidiomycetes alone. However, the total weight loss in chips from slash over 25 yr was less for all treatments than for comparable treatments with less than 1-yr-old or 1- to 2-yr-old slash (Table 1).

The SEM study revealed a close physical association among bacteria, yeasts, and basidiomycetes in decaying wood. One mo after inoculation, basidiomycetous hyphae had proliferated in the tracheids without apparent cell wall decay. The hyphae traversed the pits from tracheid to tracheid apparently ending in the ray parenchyma cells (Fig. 2-A). Bacteria and yeasts were restricted to ray parenchyma cells (Fig. 2-B). By 5 mo, fungal hyphae were decaying the tracheid walls. In the basidiomycete and composite inocula treatments, bacteria and yeasts were found only in the immediate vicinity of hyphae decomposing the cell walls (Fig. 2-C, D). The association among these organsims was clearly observed with SEM techniques (Fig. 2-E, F). Tracheids without hyphae or with hyphae but no cell wall lysis zones did not have bacteria or yeasts associated with them. No bacteria or yeasts were observed in the composite inocula treatments after 5 mo.

In naturally infected field samples of wood colonized by *C. versicolor*, *H. abietinus*, *Cryptoporus volvatus*, or *F. pinicola* a similar association was observed. Bacteria and yeasts were present only in tracheids occupied by a

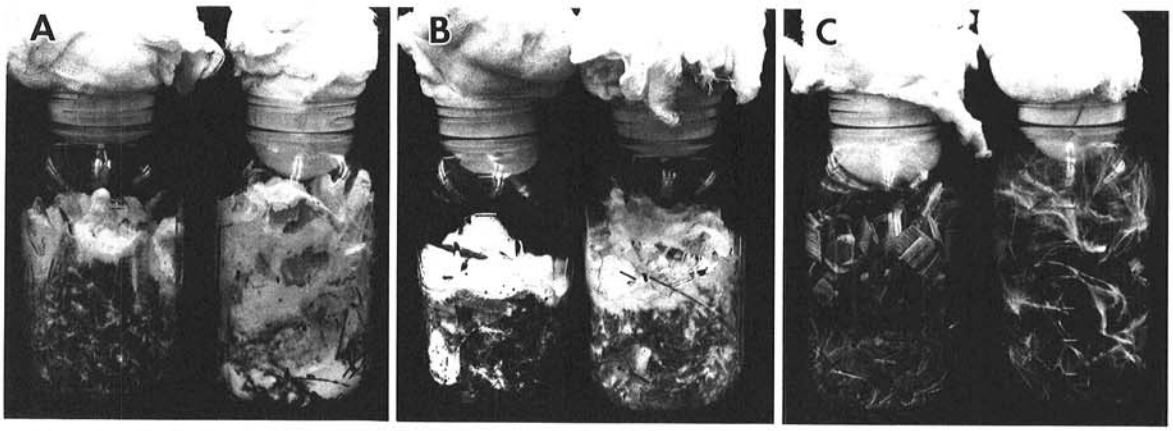

Fig. 1-(A to C). Decay, after 5 mo, of wood chips inoculated with bacteria and yeasts combined with a basidiomycete and with the basidiomycete alone. Large increases of mycelia can be seen in the composite inocula treatments (jars on right) with **A)** *Poria placenta* in chips greater than 25 yrs old; **B)** *Coriolus versicolor*, and **C)** *Hirschioporus abietinus* in chips from slash less than 1 yr old.

TABLE 1. Weight loss and level of glucosamine of sterile wood chips 150 days after inoculation with individual basidiomycetes and in combination with composite inocula[a]

	Wood chips of age:				
	Less than 1 yr		1 and 2 yr		Over 25 years[c]
Treatment	Weight loss[b] (%)	Glucosamine (μmoles/ 0.5 g)	Weight loss[b] (%)	Glucosamine (μmoles/ 0.5 g)	Weight loss[b] (%)
Poria placenta	40.12	1.85	39.88	1.57	24.77
P. placenta + composite[a]	43.63	2.04	43.32	1.59	35.06
Coriolus versicolor	18.91	1.51	20.87	1.56	3.22
C. versicolor + composite[a]	22.66	3.34	21.48	1.98	3.44
Hirschioporus abietinus	19.39	2.53	10.97	1.36	1.23
H. abietinus + composite[a]	20.31	3.55	13.92	1.50	4.56
Composite	1.17	1.16	0.96	0.60	0.88
Control	0.40	1.25	0.56	0.62	0.38

[a]Composite inocula consisted of *Saccharomyces bailii* var. *bailii, Pichia pinus,* and *Enterobacter* spp.
[b]Significantly more weight loss occurred using composite inoculum (*t*-test at $P = 0.05$) than with each basidiomycete alone.
[c]Glucosamine was not determined in wood chips over 25 yr old.

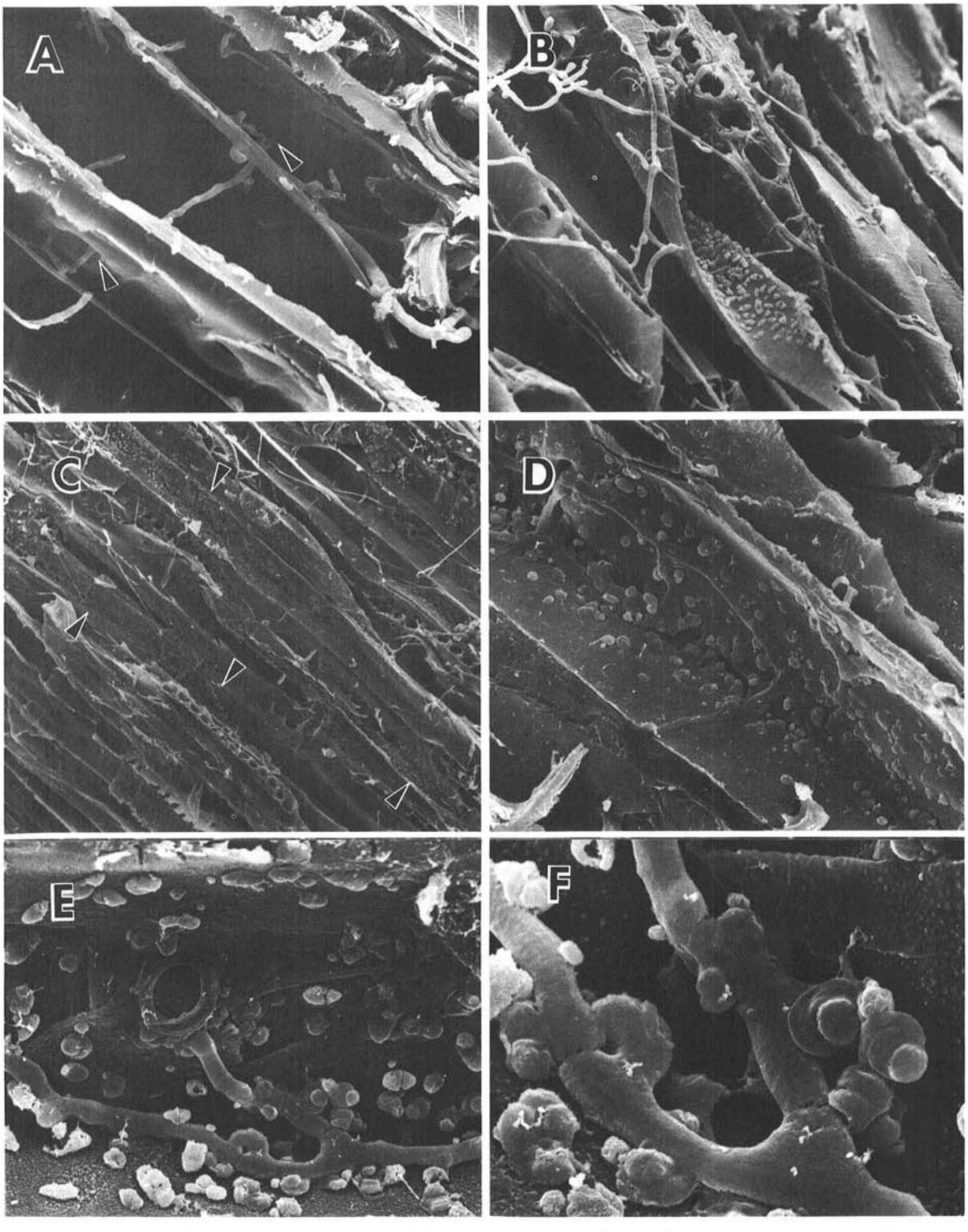

Fig. 2-(A to F). Scanning electron micrographs of coniferous wood inoculated with bacteria, yeasts, and basidiomycetes after (A,B) 1 and (C-F) 5 mo. **A)** Fungal hyphae traversing the pits of successive tracheids (arrows) ending in ray parenchyma cells (× 1,000). **B)** Bacteria and yeasts colonizing the ray parenchyma cells (× 500). **C)** Bacteria and yeasts present only in tracheids where basidiomycetous hyphae are decomposing the cell walls (arrows) (× 100). **D)** Lysis of tracheid wall around hyphae with bacteria and yeasts clustered in the immediate vicinity (× 1,400). **E,F)** The close association among bacteria, yeasts, and basidiomycetes is clearly evident (× 1,500 and × 5,000, respectively).

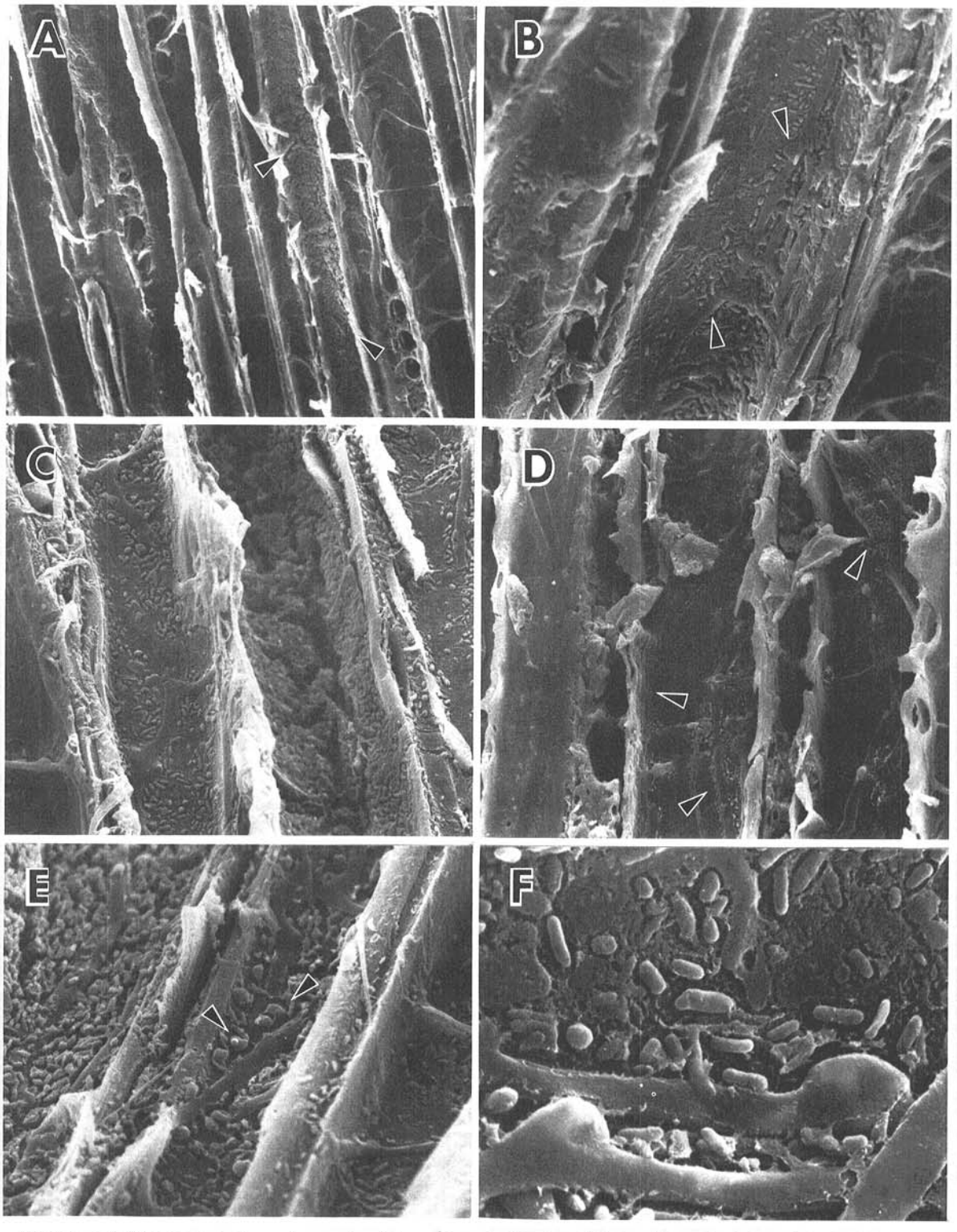

Fig. 3-(A to F). Scanning electron micrographs of decayed wood taken from fallen coniferous logs 30 cm from basidiocarps. **A)** Bacteria (arrows) in tracheids decayed by *Fomitopsis pinicola* (×400). **B)** Fungal hyphae (arrows) of *Coriolus versicolor* with bacteria closely associated (× 1,200). **C)** Tracheids severely decayed by *C. versicolor* with numerous bacteria present (× 1,100). **D)** Bacteria in the immediate vicinity of *Cryptoporus volvatus* hyphae (arrows) (×800). **E)** *Hirschioporus abietinus* decaying the cell wall with yeasts (arrows) and bacteria present (× 1,600). **F)** Lysis around the hyphae of *H. abietinus*; bacteria appear to be utilizing the modified cell wall components (× 5,000).

wood-decaying fungus (Fig. 3-A to C). Few bacteria were found in tracheids less severely decayed by fungal enzymes (Fig. 3-D). Yeasts were found in field samples of wood decayed by all four representative fungi but in much lower numbers than bacteria (Fig. 3-E). The utilization by bacteria of tracheid cell wall components modified by extracellular fungal enzymes was apparent (Fig. 3-F).

Bacteria were isolated from all logs naturally infected by each fungal type. Yeasts were isolated from only two out of nine logs of each fungal type.

DISCUSSION

Decomposition and mycelial growth are enhanced when bacteria and yeasts are combined with wood-decaying fungi. Up to 10% additional weight loss may be attributed to the basidiomycete and composite inocula treatments as compared with basidiomycetes alone. Actual decay may be higher than the figures in Table 1 indicate. The glucosamine studies indicate that as much as 5-10% of the total dry weight at the end of 5 mo may be mycelium, and the amount of mycelium in treatments involving combined inocula may be 10 to 200% greater than in treatments inoculated only with basidiomycetes (Table 1). If the amounts of glucosamine present in the respective controls for each age class of wood chips are considered, the differences in amounts of mycelium produced by a given basidiomycete alone or in conjunction with the composite inocula become even greater; e.g., 0.26 versus 2.09 μmoles/0.5 g as compared to 1.51 versus 3.34 μmoles/0.5 g for *Coriolus versicolor* without and with composite inocula, respectively, on wood chips less than 1 yr old (Table 1).

Slash less than 1 yr old has large quantities of free sugars available in parenchyma cells. As wood ages on the forest floor, these compounds are utilized by microorganisms and decreased by leaching. The rate of decomposition by *H. abietinus* is affected by the age of wood chips (Table 1). Slash over 25 yr old consisting of approximately 65% cedar still may have had decay inhibitors present in the wood (19). Nevertheless, decay by the brown-rot fungus, *P. placenta*, was higher (although only half as much as observed in wood chips of other age classes) than by the white-rot fungi. The latter could not substantially decompose the 25-yr-old wood chips. The increases in decay observed in treatments combining basidiomycetes with composite inocula indicate that bacteria and yeasts can stimulate fungal growth as well as increase the rate of decomposition in substrates not conducive to rapid fungal growth.

The micrographs presented suggest a mutualistic association among bacteria, yeasts, and basidiomycetes during the decay process. After 5 mo of decay, in laboratory samples, bacteria and yeasts were found only in tracheids occupied by the basidiomycete. Tracheid walls appear to be modified by extracellular enzymes secreted by the basidiomycetes. Utilization of this material is apparent by lysis zones around the bacteria and yeasts. Examination of naturally infected dead coniferous wood indicates that the association exists under field conditions.

The possibility of speeding up the rate of decay is currently being investigated by the authors as a means of managing forest residues. If thinning and logging residues could be decomposed more rapidly, fire hazards would be eliminated, regeneration facilitated, and nutrients rapidly recycled into the forest ecosystem (9). The potential of nitrogen-fixing bacteria associated with wood-decaying fungi to add significant nitrogen gains to the site may be of major importance.

LITERATURE CITED

1. AHO, P. E., R. J. SEIDLER, H. J. EVANS, and P. N. RAJU. 1974. Distribution, enumeration, and identification of nitrogen-fixing bacteria associated with decay in living white fir trees. Phytopathology 64:1413-1420.
2. BASHAM, J. T. 1959. Studies in forest pathology. XX. Investigations of the pathological deterioration in killed balsam fir. Can. J. Bot. 37:291-326.
3. BRAVERY, A. F. 1971. The application of scanning electron microscopy in the study of timber decay. J. Inst. Wood Sci. 30:13-19.
4. BUTCHER, J. A. 1968. The ecology of fungi infecting untreated sapwood of Pinus radiata. Can. J. Bot 46:1577-1589.
5. CASTELLO, J. D., C. G. SHAW, and M. M. Furniss. 1976. Isolation of Cryptoporus volvatus and Fomes pinicola from Dendroctonus pseudotsugae. Phytopathology 66:1431-1434.
6. COHEN, A. L. 1974. Critical-point drying. Pages 44-112 in M. Hayat, ed. Principles and techniques of scanning electron microscopy, Vol I. Van Nostrand, N. Y. 412 p.
7. ETHERIDGE, D. E., and L. A. MORIN. 1967. The microbial condition of wood of living balsam fir and black spruce in Quebec. Can. J. Bot. 45:1003-1010.
8. FINDLAY, G. W. D., and J. F. LEVY. 1969. Scanning elctron microscopy as an aid to the study of wood anatomy and decay. J. Inst. Wood Sci. 23:57-63.
9. HARVEY, A. E., M. F. JURGENSEN, and M. J. LARSEN. 1976. Intensive fiber utilization and prescribed fire: effects on the microbial ecology of forests. U.S. Dep. Agric., For. Serv., Gen. Tech. Rep. INT-28. 46 p.
10. HENNINGSSON, B. 1967. The physiology, interrelationships and effect on the wood of fungi which attack birch and aspen pulpwood. Stud. For. Suec. 53. 31 p.
11. JUTTE, S. M., and I. B. SACHS. 1976. SEM observations of brown-rot fungus Poria placenta in normal and compression wood of Picea abies. Pages 535-542 *in* Proc. 9th Scanning Electron Microscopy Symposium, Part VII Scanning Electron Microscopy, Plant Science Applications of SEM. 5-9 April 1976, Illinois Instrumentation Technology Research Institution, Chicago, IL. 708 p.
12. JUTTE, S. M., and R. A. ZABEL. 1974. Initial wood decay stages as revealed by scanning electron microscopy. Pages 445-452 *in* Proc. 7th Scanning Electron Microscopy Symposium, Part II Scanning Electron Microscopy, Plant Science Applications of SEM. 8-11 April 1974, Illinois Instrumentation Technology Research Institution, Chicago, IL. 1064 p.
13. KÄÄRIK, A. 1975. Successions of microorganisms during wood decay. Pages 39-51 *in* W. Liese, ed. Biological transformation of wood by microorganisms. Springer-Verlag. New York. 203 p.
14. MALOY, O. C., and V. S. ROBINSON. 1968. Microorganisms associated with heart rot in young grand fir. Can. J. Bot. 46:306-309.
15. SAVORY, J. G. 1954. Damage to wood caused by microorganisms. J. Appl. Bacteriol. 17:213-218.
16. SEIDLER, R. J., P. E. AHO, P. M. RAJU, and H. J. EVANS. 1972. Nitrogen fixation by bacterial isolates from decay in living white fir trees [Abies concolor (Gord.

and Glend.) Lindl.]. J. Gen. Microbiol. 73:413-416.
17. SHIGO, A. L. 1965. Organism interaction in decay and discoloration in beech, birch and maple. U.S. Dep. Agric., For. Serv., Res. Pap. NE-43. 23 p.
18. SHIGO, A. L. 1972. Successions of microorganisms and patterns of discoloration and decay after wounding in red oak and white oak. Phytopathology 62:256-259.
19. VAN DER KAMP, B. J. 1975. The distribution of microorganisms associated with decay of western red cedar. Can. J. Bot. 5:61-67.
20. WILCOX, W. W. 1968. Changes in wood microstructure through progressive stages of decay. U.S. Dep. Agric., For. Serv., Res. Pap. FPL-70. 49 p.
21. WILCOX, W. W. 1970. Anatomical changes in wood caused by microorganisms. Bot. Rev. 36:1-28.
22. WU, L., and M. A. STAHMANN. 1975. Chromatographic estimation of fungal mass in plant materials. Phytopathology 65:1032-1034.