A COOPERATIVE POLE RESEARCH PROGRAM

CONSERVING ENERGY BY SAFE AND ENVIRONMENTALLY ACCEPTABLE PRACTICES IN MAINTAINING AND PROCURING TRANSMISSION POLES FOR LONG SERVICE

SECOND ANNUAL REPORT
June 1982

by

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and

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ABSTRACT

Improved Fumigants

Chloropicrin, Vorlex and Vapam continue to control internal decay of pressure-treated Douglas-fir poles 12 years after application. Fungitoxic quantities of chloropicrin are present in the wood as high as 2.4 m (8 feet) above the groundline. Solid methylisothiocyanate (MIT), which goes directly to a vapor, is somewhat more effective in controlling decay than is Vorlex MIT is the active ingredient of Vorlex.

Gelatin capsules containing chloropicrin or MIT offer new opportunities for improved fumigation of poles. MIT is readily released in moist wood, but chloropicrin capsules require wetting after they have been placed in the wood or they must be mechanically ruptured for maximum effectiveness.

Although chloropicrin and MIT are adsorbed by wood after adequate aeration, the fumigant-treated wood may be attacked by decay fungi.

Encapsulated MIT, is being compared to Vapam in pressure-treated Douglas-fir transmission poles near Buffalo, NY.

Controlling decay of cedar sapwood

Six decay fungi have been identified from sapwood and three from heartwood, of decay in cedar poles.

Five waterborne chemicals have been selected as potential substitutes for the 10% pentachlorophenol-diesel oil solution currently used to spray cedar sapwood. Of these, 3-iodo-2-propynyl-butylcarbamate equalled or exceeded the pentachlorophenol solution in effectiveness at depths to 15 mm in the endgrain of weathered blocks.

Seven chemicals judged to be the most promising were applied to cedar poles at the Northwest Forest Genetics Center last fall. Other promising chemicals detected by a bioassay will be applied to poles this summer.

Bolt-hole protection

Twenty-eight Douglas-fir poles 18 feet long were Boulton dried in a pentachlorophenol-heavy petroleum solution, drilled with 8 alternating 3/4-inch and 1-inch diameter holes in a spiral pattern and installed at the Northwest Forest Genetics Center. Dry or liquid waterborne chemicals with low mammalian toxicity or a 10% pentachlorophenol solution were applied in the holes before the bolts were inserted. Patox washers between the pole and crossarm attachment also are being tested.

Cores will be removed below unprotected bolt holes in control poles and cultured for decay fungi as a guide for removal of cores below protected bolt holes.

Detecting decay and estimating residual strength.

Testing plugs from Douglas-fir poles for radial compression strength (RCS) was more promising than chemical tests that colored the wood or a needle-scratch test for detecting early decay. RCS, which measures the strength of the weakest springwood layer was highly correlated with weight loss caused by Poria placenta, an important brown-rotter of Douglas-fir poles. RCS losses were detected before weight losses could be measured. Sapwood was lower in RCS than heartwood, probably because of differences in extractive content of cell walls.

Infrared spectographic analysis of dried warm water extracts from wood beams decayed to low weight losses and tested in bending is being evaluated as a means of detecting early stages of decay that might cause significant strength losses.

As a means of estimating residual strength of poles, RCS values, bending strength properties, toughness and sonic properties of small beams cut from pole sections exposed at the four Northwest air-seasoning sites are being evaluated.

Decay of Douglas-fir poles prior to pressure treatment

Fourteen cores were removed from 229 unpeeled poles and 752 peeled poles stored in seasoning yards from 0 to over 24 months and cultured for fungi. About 50% of the probable decay fungi isolated from the cores have been identified. Atleast 30 of the unpeeled poles (13%) contained decay fungi. The incidence of decay fungi, especially Poria carbonica, increases significantly as the air seasoning time is prolonged. This summer cores will be removed from freshly cut poles in the forest and from additional poles during air seasoning, especially those seasoned for over 1 year.

Laboratory studies indicate that spores and fungal fragments, alone or attached to soil particles and air borne, play an important role in the infection of poles. Exposure of sterilized pole sections at the four Northwest air seasoning sites for successive 3-month periods indicates that infection increases dramatically during the November-January period at three sites. Airborne spores appear to play an important role in the infection process.

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COOPERATORS

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OBJECTIVE I

DEVELOP SAFE AND ENVIRONMENTALLY ACCEPTABLE FUMIGANT TREATMENTS TO CONTROL INTERNAL DECAY OF DOUGLAS-FIR POLES AT AND ABOVE THE GROUNDLINE

PREVIOUS ONGOING RESEARCH

The evaluation of fumigants placed in decaying pressure-treated Douglas-fir poles in 1969 and 1977 is being continued. Results of this ongoing research are presented as background information for the development of improved fumigant treatments for the future.

Douglas-fir poles treated in 1969 with Chloropicrin, Vapam or Vorlex.

Forty pressure-treated poles from 18 to 24 m long with internal decay and located in a pole line near Corvallis, Oregon, were randomly assigned to five test groups. No fumigants were applied to one control group. Poles in the other groups were treated with 1 liter of chloropicrin, Vapam or Vorlex distributed among four holes near the groundline and three holes 1 m above the ground. The 2-cm diameter holes were plugged with treated dowels. A temporary laminated paper-polyethylene film wrap applied to poles after treatment deterioriated within 1 to 2 years. A group of Vapam-treated poles and the controls were not wrapped.

To evaluate effectiveness of the treatments, three cores equally spaced around each pole starting near the widest check were removed at -0.3, 0, 0.6, and 1.2 m from the groundline and bioassayed for decay fungi. Three additional cores were removed at 0, 1.2, 1.8, and 2.4, m above the groundline to determine distribution of residual fumigant by the closed-tube bioassay.

Results

Within 1 year all fumigants greatly reduced the number of poles from which decay fungi could be cultured (Table 1) and the population of decay fungi (Figure 1). Chloropicrin and Vorlex have been more effective than Vapam in maintaining the population of decay fungi at very low levels. The persistence of chloropicrin vapors in the poles as far as 2.4 m above the groundline (Table 2) suggests that retreating cycles with this chemical may exceed 15 years. A retreating cycle of 10 years with Vapam, which is least persistent, and 15 years with the more persistent Vorlex appear reasonable for this quantity of chemical and size of poles.

The steadily decreasing number of untreated poles from which decay fungi could be cultured reflects the increasing amounts of rotten wood in these poles which no longer contains viable decay fungi. The occasional presence of decay fungi in fumigant-treated poles indicates that decay fungi were not completely eliminated or have reinfected the poles and consequently persistence will be an important characteristic of efficient fumigants.

Douglas-fir poles treated in 1977 with Allyl Alcohol, Methylisothiocyanate or Vorlex

Methylisothiocyanate (MIT) and allyl alcohol found effective for controlling decay fungi in our laboratory bioassay, for fumigants, were compared with Vorlex in poles in service. Internally decaying poles pressure-treated with pentachlorophenol in heavy oil were selected for treatment by removing three cores equally spaced around the poles at -0.3, 0, 0.6, and 1.2 m from the groundline and culturing the cores for decay fungi. Because of the prevalence of decay fungi at 1.2 m, cores also were removed at 1.8 and 2.4 m for bioassay.

TABLE 1

UNTREATED AND FUMIGANT-TREATED DOUGLAS-FIR POLES WITH DECAY FUNGI

	NUMBER OF POLES[1]				
		VAPAM		VORLEX	CHLOROPICRIN
YEAR	UNTREATED	WRAPPED	UNWRAPPED	WRAPPED	WRAPPED
1968	8	8	8	8	8
1969		POLES TREATED WITH FUMIGANT			
1970	8	4	4	0	1
1971	8	1	1	0	0
1972	8	0	1	0	0
1973	8	0	0	0	0
1974	7	4^{7}	4^{7}	0^{7}	1^{6}
1975	7	1	0	0	0
1976	5	2	3	1	0
1977	5	2	1	0	0
1978	5	3	2	0	0
1979	5	3	2	0	1
1980	5	2	3	2	0
1981	3	2	2^{6}	1	0

[1] All untreated poles contained decay fungi.
The superscript denotes fumigant-treated poles remaining in test; other poles were inadvertantly replaced.

TABLE 2

CLOSED-TUBE BIOASSAY FOR RESIDUAL FUMIGANT VAPORS IN PRESSURE-PENTACHLOROPHENOL-TREATED DOUGLAS-FIR POLES TWELVE YEARS AFTER APPLICATION[1]

METERS ABOVE GROUND	SEGMENT LOCATION FROM SURFACE (cm)	AVERAGE GROWTH OF ASSAY FUNGUS, (mm)			
		NO FUMIGANT	VAPAM	VORLEX	CHLOROPICRIN
2.4	0-2.5	19	11	8	2
	5.1-7.6	22	20	17	8
	12.5-15	22	20	18	4
1.8	0-2.5	12	9	9	3
	5.1-7.6	24	20	12	0
	12.5-15	24	19	16	0
1.2	0-2.5	19	8	8	2
	5.1-7.6	20	21	15	2
	12.5-15	--	23	11	2
0	0-2.5	12	9	7	4
	5.1-7.6	21	17	12	7
	12.5-15	24	15	15	7

[1]Suppressed growth of the assay fungus compared to poles with no fumigant indicates that fungitoxic vapors are present. Lower values in the outer zone reflect the presence of pentachlorophenol.

Figure 1. Changes in fungal population of internally decaying pressure-treated Douglas-fir poles treated with fumigants. Each value is based on 12 cores removed each year times the number of poles in test (Table 1).

The poles were randomly assigned to groups for treatment with 1 pint of chemical which was distriburted between four holes. MIT was melted for pouring but not all of the chemical could be applied because it solidified too rapidly in the holes. Annually thereafter three cores equally spaced around the pole were removed at each of five levels and were cultured for fungi. Additional cores were assayed for residual fumigant vapor by the closed-tube bioassay. Three or four poles per test group were deleted from the test because they were inadvertently treated with Vapam during 1980 by a commercial applicator.

Results

Solid MIT continues to be the most effective chemical for controlling decay (Table 3) and the most persistent (Table 4). Because we cannot explain the poor performance of allyl alcohol, a relatively inexpensive chemical, we are continuing to study this chemical in laboratory tests.

TABLE 3

POPULATION OF DECAY FUNGI IN DOUGLAS-FIR POLES PRIOR TO AND AFTER TREATMENT WITH FUMIGANTS IN 1977

CHEMICAL AND METERS ABOVE GROUNDLINE	POLES IN TEST[1]	POLES WITH DECAY FUNGI					CORES WITH DECAY FUNGI, PERCENT				
		1977	1978	1979	1980	1981	1977	1978	1979	1980	1981
METHYL ISOTHIOCYANATE (SOLID, 100%)	8, 5										
1.8 to 2.4		5	1	0	0	0	40	2	0	0	0
-0.3 to 1.2		8	1	0	0	0	61	1	0	0	0
METHYL ISOTHIOCYANATE (20%) IN DIESEL OIL	9, 5										
1.8 to 2.4		8	6	4	2	1	54	24	15	2	3
-0.3 to 1.2		9	1	0	1	0	65	1	0	1	0
VORLEX	7, 4										
1.8 to 2.4		7	3	3	2	0	55	17	12	8	0
-0.3 to 1.2		7	0	1	2	0	73	0	1	12	0
ALLYL ALCOHOL	9, 5										
1.8 to 2.4		8	8	7	8	4	51	54	52	16	19
-0.3 to 1.2		9	9	9	9	5	67	25	30	20	21
NO FUMIGANT	9, 5										
1.8 to 2.4		8	7	8	9	4	61	59	68	15	20
-0.3 to 1.2		9	9	9	9	5	56	55	55	22	21

[1]First number is for poles initially in test. Second number is for poles remaining in test in 1981. Others were inadvertently treated with Vapam by a commercial applicator.

TABLE 4

CLOSED-TUBE BIOASSAY FOR RESIDUAL FUMIGANT VAPORS IN DOUGLAS-FIR POLES 4 YEARS AFTER APPLICATION

METERS ABOVE GROUND	SEGMENT LOCATION FROM SURFACE (cm)	AVERAGE GROWTH OF ASSAY FUNGUS-IN (mm)				
		METHYL ISO-THIOCYANATE (MIT)	MIT IN DIESEL OIL	VORLEX	ALLYL ALCOHOL	NO FUMIGANT
2.4	0-2.5	7	17	16	15	16
	5.1-7.6	4	17	11	23	23
	12.7-15.2	0	13	13	20	23
1.8	0-2.5	7	15	18	17	20
	5.1-7.6	0	14	11	20	21
	12.7-15.2	0	18	12	22	25
1.2	0-2.5	0	13	16	19	24
	5.1-7.6	0	11	5	20	23
	12.7-15.2	1	12	0	22	17
0.6	0-2.5	0	6	5	17	20
	5.1-7.6	0	8	1	13	18
	12.7-15.2	3	13	5	16	22
	NO WOOD					26[2]

[1]A core was removed at each height from 4 to 6 test poles. The assay fungus was Poria placenta. Suppression of fungal growth is a measure of fumigant effectiveness and, in the 0-2.5 cm zone, of pentachlorophenol with which the poles were pressure treated.
[2]Average growth in 30 tubes without wood.

A. PREPARATION AND EVALUATION OF ENCAPSULATED FUMIGANTS IN LABORATORY WOOD-BLOCK TESTS.

In studies reported last year, we demonstrated that MIT and ammonium bifluoride (NH_4HF_2) could be sealed in gelatin capsules that retained the fumigants without siginificant vapor loss for up to 55 days. We further demonstrated that when the fumigants were released from the capsules in wood by addition of small quantities of water, the encapsulated treatments were just as effective as when the fumigants were injected directly into the wood.

MIT loss from capsules during dry storage

Studies evaluating the use of gelatin encapsulated fumigants for control of wood decay fungi have continued to show promising results. The impermeability of gelatin capsules to MIT loss under dry storage conditions now has been well established. Four large capsules of a size suitable for pole fumigation (0.75 x 3.25 inches) containing about 17g of MIT each have been monitored for fumigant loss for about 6 months. The capsules were open to the air in a fume hood at 21°C and were periodically weighted to determine any weight changes with time. During 181 days of storage the capsules lost an average of 0.38% of their MIT content (Table 5) which is a loss that should pose no difficult problems during storage.

Effect of wood moisture on MIT loss from capsules.

The minimum wood moisture content necessary for efficient release of MIT from gelatin capsules placed in wood without subsequent addition of water has been investigated. Douglas-fir heartwood blocks (2.5 x 1.5 x 1.5 inches) were drilled to accommodate gelatin capsules (0.375 x 1.0 inches) containing about 1.0g of MIT. The wood blocks were oven dried, weighed, and then adjusted in groups of five to the following moisture contents (MC) based

TABLE 5

METHYLISOTHIOCYANATE (MIT) LOSS FROM LARGE CAPSULES DURING DRY STORAGE

CAPSULE NO.	WEIGHT OF FILLED CAPSULES IN GRAMS[1] 2ND DAY[2]	181ST DAY	PERCENT MIT LOSS
1	20.2122	20.1500	0.36
2	20.3766	20.3005	0.44
3	20.1151	20.0616	0.32
4	20.5414	20.4731	0.39

[1]Empty capsule wt. about 3.1g.
2Initial capsule weight recorded 2 days after filling to allow the capsules to thoroughly dry and equilibrate after sealing.

on their original oven dry weight: 20%, 23%, 26%, 30%, 40%, and 80%. The blocks were allowed to equilibrate for 6 days at the three higher moisture levels and for 14 days at the three lower moisture levels before the capsules were placed in the wood and the holes sealed with serum caps. The blocks were stored in plastic bags (water impermeable but MIT permeable) for 7 days after which the capsules were removed, air dried to prevent further MIT loss and reweighed. Wood block moisture contents were calculated for each block both before the capsules were sealed in the wood and after they were removed by comparing the weights with their original oven dry weights. There was generally a 1-2% decrease in moisture content for each block after the 7 day period, with the high and low moisture values in each group within a 3% MC range.

Capsules incubated in wood at the lowest moisture content (i.e. 18%) lost very little of their MIT (Table 6). As the moisture content of the blocks increased the rate of MIT loss rapidly increased until a near maximal loss of 74% was obtained in wood at 29% MC. The capsules in wood at 77% MC showed only a 59% loss of MIT probably because at this high moisture level there is restricted diffusion of the MIT into the wood and away from the capsule.

The results of this test indicate that between 24 and 29% MC there is sufficient moisture for a maximal rate of MIT release from the gelatin capsules. This corresponds closely with the fiber saturation point of wood and suggest that a relatively small amount of water added along with capsule treatments should be sufficient for adequate release of encapsulated MIT in wood.

TABLE 6

INFLUENCE QF WOOD MOISTURE CONTENT ON THE LOSS OF MIT FROM GELATIN CAPSULES

PERCENT WOOD MOISTURE[1]	LOSS OF MIT FROM CAPSULES DURING A 1 WEEK INCUBATION PERIOD IN WOOD	
	AVERAGE WT. LOSS IN mg	PERCENT MIT LOST
Control[2]	2	0.2
19	20	2.0
21	90	9.2
24	304	30
29	753	74
38	762	77
77	586	59

[1]Moisture content of the blocks is the average of the initial and final moisture contents for the period the capsules were present.

[2]Capsules incubated in air in a fume hood.

Fungitoxicity of encapsulated MIT and chloropicrin

Following our initial success with MIT in controlling P. carbonica in a laboratory assay (1981 Ann. Rpt.) we tested gelatin encapsulation as a means of improving the handling of chloropicrin. Gelatin capsules retained chloropicrin as effectively as they did MIT and so we evaluated encapsulated chloropicrin against P. carbonica in our standard fumigant assay.

Test blocks (2.5 x 2.5 x 10 cm long) of Douglas-fir heartwood at 30% MC were autoclaved and the sides of the blocks were coated with paraffin wax. The ends were inoculated with P. carbonica and the blocks were incubated 1 month while the fungus grew into the wood. A 4 mm diameter hole was drilled 22 mm deep at midlength and then No. 4 capsules containing MIT or No. 5 capsules containing chloropicrin were placed in the wood, 200 µl of water was added to aid release of the fumigants, and the holes were sealed with serum caps. Fumigant effectiveness was estimated by the viability of Poria near the ends of the treated blocks.

As in our earlier tests, MIT was as effective when applied in capsules as when injected directly into the wood (Table 7). Similarly, chloropicrin was equally effective injected or when applied in capsules that were either partially dissolved by added water or physically ruptured when placed in the wood. Encapsulation in gelatin capsules offers new opportunities for improved usage of chloropicrin in treating utility poles with decay problems.

Influence of wood moisture on loss of chloropicrin from capsules

Chloropicrin was tested in an experiment similar to that used to determine the influence of wood moisture content on release of MIT from gelatin capsules incubated in the wood. In the chloropicrin experiment, however, there were no blocks prepared to have about an 80% MC.

TABLE 7

THE FUNGITOXICITY OF MIT AND CHLOROPICRIN APPLIED IN GELATIN CAPSULES TO WOOD INFESTED WITH Poria carbonica

FUMIGANT	TREATMENT	PERCENTAGE INHIBITION OF PORIA IN WOOD AT VARIOUS FUMIGANT CONCENTRATIONS (mg/BLOCK)[1]				
		203 mg	102 mg	50 mg	25 mg	5mg
MIT	capsules[2]	84	78	37	19	
MIT	Injected	87	75	44	6	
Chloropicrin	capsules[2]			100		35
Chloropicrin	capsules[3]			97		40
Chloropicrin	injected			97		47

[1]Each value is based on recovery of Poria from 32 cubes cut from four blocks

[2]After the fumigant capsules were placed in the wood blocks, 300 µl of water was added and the holes were immediately sealed with serum caps.

[3]After the fumigant capsules were placed in the wood blocks, the capsules were physically ruptured and the treatment cavities were immediately sealed with serum caps.

Chloropicrin was far less readily released from the gelatin capsules than was MIT (Table 6 vs. Table 8). Even at the highest wood moisture content, i.e. 39%, only about 16% of the chloropicrin was lost from the capsules. These results suggest that chloropicrin capsules will require added water or physical rupturing at treatment time for optimum effectiveness.

Movement and fungitoxicity of encapsulated MIT and NH_4HF_2 in poles

A test was conducted to compare the movement and toxicity of encapsulated and nonencapsulated MIT and NH_4HF_2 in Douglas-fir poles. The fumigants were applied in holes drilled at the midlength of 5-foot long pole sections. Water (20 ml) was pipetted into all capsule treatments and the nonencap-

TABLE 8

INFLUENCE OF WOOD MOISTURE CONTENT ON THE LOSS OF CHLOROPICRIN FROM GELATIN CAPSULES

PERCENT WOOD MOISTURE[1]	LOSS OF CHLOROPICRIN FROM CAPSULES DURING A 1 WEEK INCUBATION PERIOD IN WOOD	
	AVERAGE WT. CHANGE IN mg	PERCENT CHLOROPICRIN LOST
Control[2]	+ 2	0.0
19	+ 2	0.0
22	- 4.4	0.3
25	- 12	0.8
28	- 160	10
39	- 255	16

[1]Moisture content of the blocks is the average of the initial and final moisture contents for the period the capsules were present.

[2]Capsules incubated in air in a fume hood.

sulated NH_4HF_2 treatments before sealing the holes with rubber stoppers. MIT concentrations were monitored at 1 and 2 feet above and below the treatment holes at 8 day intervals by both the closed tube bioassays and gas chromatographic techniques using ethyl acetate extractions of core sections. Both techniques detected highly fungitoxic levels of MIT moving through the wood at similar rates although, in general, the concentrations were slightly lower with encapsulated MIT.

After 5 weeks, the capsules were removed and inspected for fumigant content. MIT capsules lost over one half the fumigant and were rubbery and still releasing MIT. In contrast, the NH_4HF_2 capsules had lost all their contents but NH_4HF_2 movement was not detected by the closed tube bioassay from either the nonencapsulated or the encapsulated chemical. Although the bioassay proved inconclusive for detecting NH_4HF_2, this experiment demonstrated that HN_4HF_2 can be released effectively from gelatin capsules.

Current studies

In current studies, we will more accurately monitor MIT in wood using vapor sampling techniques to estimate concentrations at fixed sampling sites in pole sections. Results from encapsulated MIT treatments will be compared with equivalent treatments using Vapam (which breaks down to MIT), and nonencapsulated MIT. The results from these tests and concentration-time curves for MIT fungitoxicity should give a comparative basis for evaluating expected effectiveness of encapsulated MIT in the field.

B. EVALUATE NEW FUMIGANTS IN THE LABORATORY

No new fumigants were evaluated in the laboratory during the past year.

C. INVESTIGATE THE INFLUENCE OF ENVIRONMENTAL FACTORS ON EFFECTIVENESS AND PERSISTENCE OF FUMIGANTS

The influence of environmental factors on the fungitoxicity of MIT is being studied through the establishment of concentration-time (CT) relationships for the toxicity of this fumigant to P. carbonica. Fungitoxicity curves are being generated for P. carbonica grown and treated in Douglas-fir heartwood at different moisture contents and temperatures.

Retention of MIT by sound wood

Studies to date indicate that wood adsorbs relatively large amounts of MIT from vapor treatments. For example, wood blocks fumigated with 48µg of MIT/cc of air adsorbed sufficient MIT to build up concentrations of about 28,000 µg MIT/g oven dry weight of wood. As a comparison, the volume of air contained in 1 g oven dry weight of springwood (i.e. 4.2 cc) could at most hold 200 µg MIT, or less than 1% of the MIT actually adsorbed. Even in very wet wood, (80% water), the solubility of MIT in the aqueous phase can account for only a limited portion of the adsorbed MIT. Of the 28,000 µg of MIT recovered only about 6,000 µg could exist in the aqueous phase based on the maximum solubility of MIT in water at 23°C of 7,600 µg/ml.

Most of the adsorbed MIT is loosely bound to the wood and is lost rapidly during aeration, especially from the springwood.

Retention of MIT by decayed wood

The retention of MIT in decayed Douglas-fir heartwood has been studied to help establish aeration times necessary for wood blocks used in C-T fumigation studies. Douglas-fir blocks (2.5 x 2.5 x 0.5 cm) were decayed by

P. carbonica for 8 weeks before the blocks were fumigated in an MIT saturated atmosphere (48 µg MIT/cc air) for 27 hrs and then removed and aired in a fume hood. At specified time periods, both spring- and summerwood bands were cut from the blocks and extracted in ethyl acetate for 4 hours to remove MIT. MIT concentrations were determined by a gas chromatographic technique.

While the majority of the MIT was lost rapidly during aeration of the small blocks (Table 9), the summerwood maintained a substantial amount of MIT even after 24 hours aeration. One gram of summerwood has a volume of about 1.3 cc and the 0.24 mg of MIT still present in the summerwood is over four times the amount that can be carried by the air at saturation. Differences in the retention of MIT by spring- and summerwood point out the differences that can occur when comparisons are made between treatments of wood with differing proportions of spring- and summerwood.

TABLE 9

RATE OF DESORPTION OF MIT FROM SMALL BLOCKS OF DECAYED DOUGLAS-FIR HEARTWOOD.

AERATION TIME	MIT (mg) PER GRAM OF OVEN DRY WOOD, SPRINGWOOD	SUMMERWOOD
15 sec.	32	26
60 sec.	24	26
5 min.	11.3	22
15 min.	3.0	18
60 min.	0.41	6.6
2 hrs.	0.09	1.5
8 hrs.	0.04	0.32
24 hrs.	0.02	0.24

Viability of Poria carbonica in wood fumigated with MIT

Techniques for determining fungal viability in fumigated wood have been modified several times during the course of our studies. Wood block

colonization by decay fungi was originally accomplished by first sterlizing Douglas-fir heartwood blocks (2.5 x 2.5 x 0.5 cm), and adjusting them to about 30% MC. The blocks were then inoculated by placing them on glass rods on malt agar plates containing a growing colony of P. carbonica. In all cases, the fungus rapidly grew across the plate and up onto the blocks, but after 8 weeks incubation the blocks varied greatly in moisture content (i.e. 60 - 140%) and amount of decay. In many cases the fungus acted as a wick between the agar medium and the wood to increase the moisture content to the point where internal colonization of the wood was severely limited.

To overcome this problem a new wood block decay procedure was devised that better controlled the wood moisture content during the decay period. The oven dry weight of each block was obtained by drying for 20 hours at 110°C, and then the blocks were sterilized by saturating them with water and autoclaving for 30 minutes. The blocks were adjusted to 0.05 g less than the 80% MC weight by aeration in a laminar flow hood, and then placed on glass rods over moist filter paper in petri plates. The wood was then inoculated with 0.05 ml of a water suspension of framented P. carbonica mycelium and the plates were sealed with parafilm and incubated at 30°C.

Two different fungitoxicity values have been used for comparing fumigant effectiveness in wood. The lethal dose (LD) for a specified level of kill (usually LD_{50} or LD_{95} concentration), or the minimum lethal dose, i.e. the amount of fumigant necessary for 100% kill of decay fungi in wood. For comparative studies, the LD values are more desirable because they can be more accurately determined than the minimum lethal dose and they are not confounded by the presence of low numbers of fumigant resistant fungal cells found in most cultures.

In our initial fumigation studies, modified LD values were used to evaluate fumigant effectiveness. The dose necessary to kill 50% of the small fungal subpopulations contained in a series of small wood slivers chipped from blocks after fumigation was determined. In a number of tests, the fumigation time necessary to reach LD_{50} values at different MIT vapor concentrations was measured. Groups of three blocks were fumigated for various time periods in a continuous flow apparatus, the blocks were aerated, and then a series of 24 slivers (subpopulations) were chipped out of each block and plated for fungal variability. Control blocks from the same wood sample were not fumigated.

Results from four different fumigation experiments with blocks decayed at 80% MC and treated at 25 µg MIT/cc air for various time periods were highly variable (Table 10). Variation occurred between replications and within the same time period of the same replication (e.g. 2 hour fumigation for experiment 3, one block had 22/24 survival while another block had only 1/24 survival). To accurately determine LD values, these experiments should give results that are nearly linear with time. Their failure to do so makes it impossible to accurately determine LD values.

These data further illustrate the problem in determining minimal lethal doses. In the first three experiments 100% kill was obtained between the 9th and 12th hours of fumigation. However, in the 4th experiment after 12 hours of fumigation, one of the 72 slivers from the three original blocks contained viable P. carbonica and thus the minimal lethal dose at this concentration was not reached until sometime over 12 hours.

New procedure for evaluating fungal viability

A new sampling procedure has been developed to overcome the problem of excessive variation in estimating decay fungus populations in wood. In the

TABLE 10

TOXICITY OF MIT TO *P. carbonica* IN WOOD AFTER EXPOSURE TO 25µg MIT PER ml OF AIR FOR VARIOUS TIME PERIODS

FUMIGATION TIME (HRS)	PERCENTAGE KILL OF *P. carbonica* IN FOUR FUMIGATION EXPERIMENTS[1]			
	1	2	3	4
0	0	0	0	0
2	2	37	51	33
4	22	88	75	21
6	47	95	92	50
9	55	87	100	54
12	100	100	100	99

[1]Percentages based on three blocks per treatment with 24 slivers cut from each block then cultured for viability of the *P. carbonica*.

new method, after inoculation and 8 weeks incubation, two thirds of the blocks are readjusted to 40% MC and incubated for an additional 2 weeks. Then one half of these blocks are lowered to 18% MC. This gives three groups of blocks at three different mositure contents that can be treated with MIT and compared to determine the influence of moisture content on fungitoxicity. The initial decay fungus population in each block is determined and only those blocks with uniform populations are selected for the experiment.

To determine the fungal population, the blocks are first scraped to remove all external mycelium. Then six 60 µm-thick sections 1.5 cm long are cut from a radial face of each block with a sliding microtome under sterile conditions. The six sections are cut after first removing 1-2 mm of wood from the edge of the block. One section was discarded between each of the six test sections. This removes any edge drying effects and spreads the sampling

over a larger area of the block. The wood sections were then fragmented by blending in 10 ml of sterile distilled water for 2.5 minutes in an omnimixer. The suspension of wood fragments was then added to potato dextrose agar medium at 45°C to make 75 ml of medium that was evenly distributed between five petri plates. The blocks were then fumigated, aired, and resampled using the same procedure to determine the decay fungus population. The post fumigation sections were taken from the same area as originally sampled. After incubating the plates for 2-3 weeks, Poria colonies were counted and used as an estimate of the fungal population in the wood. Comparisons can then be made between the initial fungal populations and the final population after fumigation for each block to determine percentage kill. Results from these tests are being used to establish the LD values necessary to formulate C-T curves for the comparison of the effectiveness of MIT for decay control under various environmental conditions.

D. EVALUATE MOST PROMISING FUMIGANTS IN POLES

New York field tests

Field treatments comparing the effectiveness of gelatin encapsulated MIT with standard Vapam treatments were initiated in New York State Electric and Gas Co. poles on lines near Hamburg, NY. The poles were 21 to 23.5 inches DBH, incised, pressure-treated with CCA, and placed in service in 1972. In the spring of 1981, six cores were removed from each pole for culturing to detect decay fungi. Of 81 poles, 24 which contained decay fungi in at least three cores were divided into four groups to receive the following treatments per pole: 1 pint of encapsulated MIT, 2 pints of encapsulated MIT, 2 pints of Vapam, and untreated controls.

All fumigants were applied equally between a series of five 1-inch diameter by 26 inches deep holes drilled at a steep angle into the pole.

The holes were drilled in a spiral pattern offset by about 70° around the pole and at about 0, 6, 12, 18 and 24 inches above the groundline. Either 15 or 30 gelatin capsules containing MIT were placed in each pole. The capsules were 1 inch in diameter by 3.5 inches long and contained 30 ml of MIT. Water was added to all MIT treatment holes before sealing with treated wooden dowels.

Poles were treated in early October between rain showers on cool and windy days. The light rain caused no problems with the gelatin capsule treatments. In general there was minimal chemical odor during MIT treatments except from a few capsules that cracked during shipment from Oregon. In contrast, the Vapam treatments produced a strong chemical odor and also involved the contamination of gloves with liquid Vapam which was not a possiblity with the encapsulated MIT treatments.

The treated poles will be resampled this summer to determine the decay fungus population as an estimate of fumigant effectiveness. In addition, a capsule will be removed from each pole to determine if all the MIT has been released into the wood.

E. MICRODISTRIBUTION AND RETENTION OF CHLOROPICRIN IN SOUND AND DECAYED WOOD.

The chlorine content, a measure of the amount of chloropicrin present, of sound Douglas-fir wood through which chloropicrin diffused was 921 to 532 ppm after 3 days aeration. Chlorine content of ground sound wood mixed with chloropicrin and aerated for 34 days stabilized at about 30 to 45 ppm, enough to inhibit growth of a decay fungus in the closed-tube bioassay. When sound wood and wood decayed to weight losses up to 30% were treated with chloropicrin, there was no strong correlation between chloropicrin retention and the extent of decay.

Chemical bonds

To determine if weak chemical bonds could be detected between wood and chloropicrin, pine cellobiose, the basic repeating unit of cellulose, was treated with chloropicrin and analyzed for a shift in infrared spectra. Spectral peaks for chloropicrin were not present and no shift was detected. Heat generated in the preparation of the sample and during analysis may have driven off the chloropicrin.

Chemical breakdown of chloropicrin

Douglas-fir wafers were exposed to a saturated atmosphere of chloropicrin and cores were removed from a southern pine laminated arch 4 years after treatment with chloropicrin. Matched segments from the cores and the wafers were assayed for chloropicrin vapors by the closed-tube bioassay and by gas chromotography-mass spectroscopy (GC/MS).

In the GC/MS analysis only one major peak was observed that is either chloropicrin or, less likely, carbon tetrachloride. Further analysis will identify the cause of the peak. Minor peaks, too small for identification, were present.

Frequently in the closed-tube bioassay, inhibited growth of the decay fungus indicated the presence of chloropicrin but the concentration of its vapors in the closed-tubes was too low to be detected by the GC/MS analysis. The closed tube-bioassay appears to be a simple, sensitive test for detecting chloropicrin

Decay resistance of chloropicrin-treated wood after aeration

Douglas-fir and ponderosa pine wafers treated either with liquid chloropicrin or its vapors were aerated for varying periods of time and implanted in agar tubes inoculated with brown rot, white rot and soft rot fungi. Wafers were

removed periodically and examined for attack by light microscopy and scanning and transmission electron microscopy.

An initial aeration period was necessary to allow the chloropicrin in the wafers to dissipate below fungitoxic levels. Preliminary results show that the brown rot fungus Poria carbonica attacked treated and untreated wafers similarly following aeration. Attack by the white rot fungus Gonoderma applanatum and the soft rot fungus Chaetomium globosom was limited in both treated and untreated wafers. Attack by the soft rot fungus Phiolophora lagerbeigii produced numerous boreholes in the cell walls but not the cavities typical of this fungus. The extent of its attack in treated and untreated wood is being determined.

Summary

- Chemical bonding of chloropicrin to cellobiose, if present at all, is so weak that the bonds apparently can be broken by heating.
- Chloropicrin apparently does not break down as it diffuses through wood.
- After sufficient aeration to allow the chloropicrin to disssipate, wood exposed to attack by brown rot, white rot and soft rot fungi under laboratory conditions appears to decay equally as well as untreated wood.

OBJECTIVE II

DEVELOP ENVIRONMENTALLY ACCEPTABLE PRESERVATIVE TREATMENTS FOR SAFELY CONTROLLING ABOVE-GROUND SAPWOOD DECAY OF CEDAR POLES

A. BASIDIOMYCETES ASSOCIATED WITH ROT IN CEDAR POLES

Pieces of wood adjacent to typical shell rot in the sapwood and to decay zones in the heartwood were cultured on nutrient media. The following Basidiomycetes were isolated and then identified with the help of Mrs. F. Lombard, U. S. Forest Products Laboratory:

SAPWOOD	HEARTWOOD
Aleurodiscus lividocoerulous	Aleurodiscus lividocoerulous
Coriolus versicolor	Coriolus versicolor
Gloeophyllum saepiarium	Poria rivulosa
Peniophora pseudo-pini	
Poria latemarginata	
Schizophyllum commune	

B. SCREENING TESTS FOR PRESERVATIVES

An Aspergillus niger (mold) bioassay* was used to screen chemicals for their ability to control decay fungi. Effectiveness of a chemical is based on its ability to diffuse from treated wood in nutrient agar to inhibit the growth of the mold and development of its dark-colored spores.

The chemicals also are being evaluated for their ability to prevent wood decay using a modified ASTM soil-block procedure. Effective preservatives that are fixed in wood might not be detected in the mold test.

* Scheffer, T. C. and L. Gollob. 1978. A bioassay for appraising preservative protection of wood above ground. Holzforschung 32(5):137-161.

Mold Spore Bioassay

Blocks of ponderosa pine sapwood (1.25 x 2.75 x 1.5 inches along the grain) were soaked for 30 minutes with the endgrain in contact with the preservative solution. The blocks were stored at room temperature for 2 days and then heated at 52°C for 13 days. One-half of the blocks were bioassayed without being weathered. The remaining blocks were placed in a weatherometer with the treated end facing a water spray at 1 minute intervals for 6 hours each day. Continuous heating by infrared lamps produced a temperature of 43°C at the wood surface .

Two and 4 weeks later a 9.5 mm diameter plug was removed through the endgrain surface and crosscut into four 4 mm thick wafers representing depths of 0, 5, 10 and 15 mm from the end. The four wafers in each set were equally spaced in a petri dish on nutrient agar seeded with Aspergillus spores and incubated for 1 week. Some chemicals produced circular clear zones and/or white zones of fungal mycelium around the wafers surrounded by agar covered with dark-colored spores (Figure 2). The distance from the wafer to the dark-colored agar is called the total zone of effect (TZE). The larger the TZE the more effective is the chemical in preventing fungal spore development. Thirteen chemicals were evaluated and four additional chemicals are being tested (Table 11).

Results. Chemical A, 10% pentachlorophenol in diesel oil, produced zones of effect (TZE) at all wood depths and was affected least by weathering (Table 12). Chemical B, 5% tribulyltinoxide in diesel oil, was comparable to A after weathering for 4 weeks. The other oil-borne preservative, Copper-8-quinolinolate (chemical C), was less effective at wood depths of 5 to 15 mm.

Five waterborne formulations, chemicals D, E, I, J and K, appear promising. Of these, chemical E, 3-iodo-2-propynyl-butylcarbamate (Troysan)

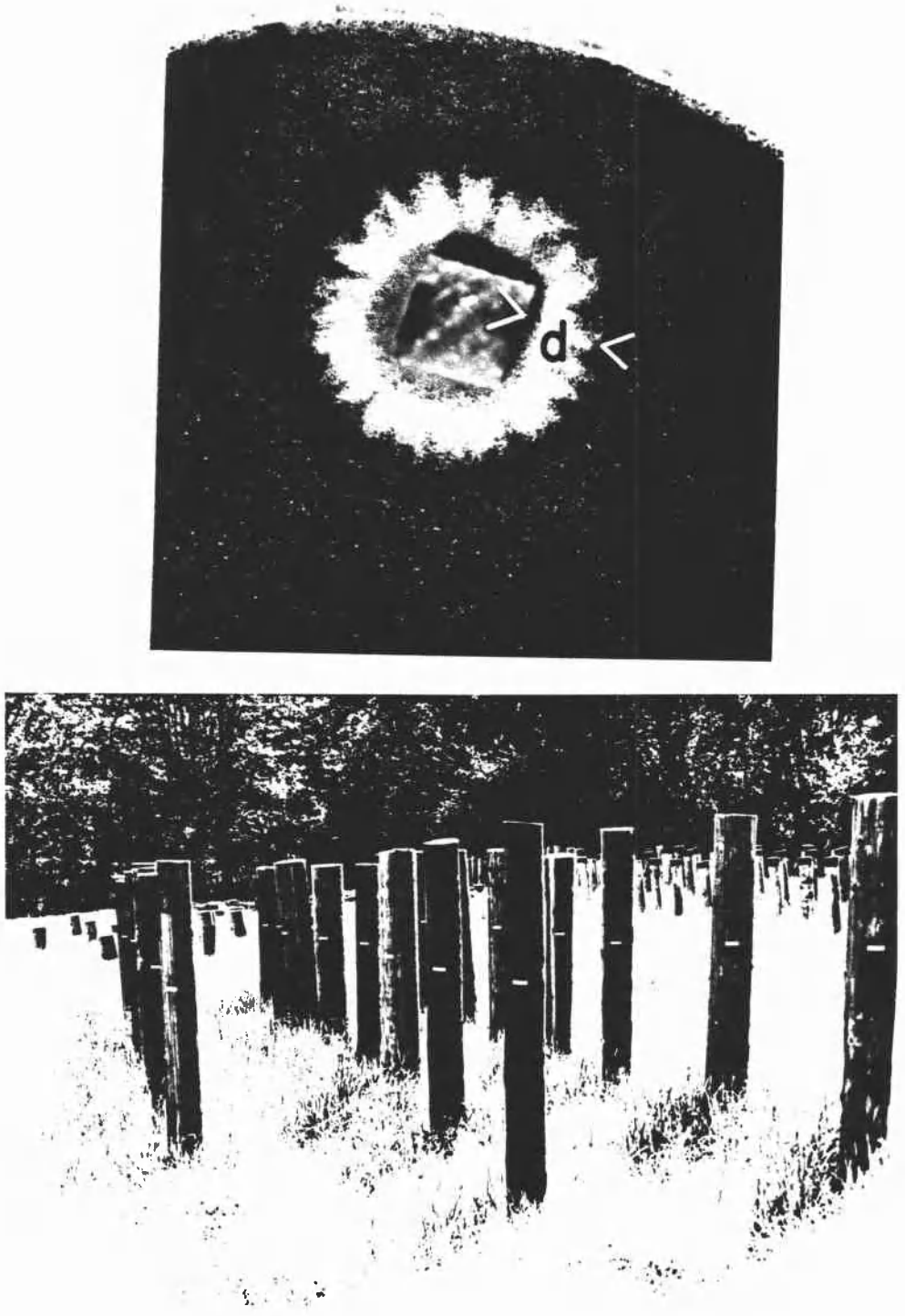

Figure 2. Total zone of effect (d) in Aspergillus mold test (top) and cedar poles in field test (bottom) to evaluate chemicals for controlling sapwood decay of western red cedar poles.

TABLE 11. CHEMICALS IN SCREENING TESTS

*A. Pentachlorophenol, 10% in diesel oil

B. Tributyltinoxide, 5% in diesel oil

*C. Copper-8-quinolinolate, Pole Spray 675, 0.121% copper in diesel oil, Chapman Chemical Co.

D. 3-iodo-2-propynyl-butylcarbamate, Woodtreat WB, 2% in water, Koppers Co., Inc.

*E. 3-iodo-2-propynyl-butylcarbamate, Troysan, 2% in water, Troy Chemical Corp.

*F. Copperamine formulation, CWP-44, 10% in water, Chapman Chemical Co.

H. Copper-8-quinolinolate, Nylate, 9% + 1% diesel oil in water, Seymour Chemicals.

*I. Water-dispersible pentachlorophenol, DuraTreat No. 2, 10% in water, Idacon, Inc.

*J. Water-dispersible pentachlorophenol, Formula 49-162, 2.5% in water + ammonia, Reichhold Chemicals, Inc.

K. Tetrachlorophenol + copper, formula 111380-3, not diluted, Reichhold Chemicals, Inc.

*M. Ammonium bifluoride, 20% in water

N. Ammoniacal copper borate, 10.9% in water

O. 3-trimethyl cocammonium chloride, Arquade C 50, 5% in water, Armak Chemical Co.

P. Solubilized copper naphthenate, Cunapsol, 2% copper in water, Chapman Chemical Co.

Q. Fluor-chrome-arsenic-phenol, 5% in water.

R. Chromic acid, 5% in water

S. Ammoniacal copper arsenate, 3% in water, J. H. Baxter & Co.

* Applied also to weathered cedar poles.

TABLE 12

CHEMICAL EFFECTIVENESS FOR PREVENTING DEVELOPMENT OF *Aspergillus niger* SPORES

ZONES OF EFFECT (mm) OF WAFERS CUT AT DISTANCES SHOWN FROM ENDS OF UNWEATHERED AND WEATHERED WOOD BLOCKS[1]

	0 mm			5 mm			10 mm			15 mm		
CHEMICAL	U	W2	W4	U	W2	W4	U	W2	W4	U	W2	W4
A	8	7	6	8	6	6	7	5	5	7	5	6
B	12	15	7	7	8	6	6	7	5	6	7	5
C	7	6	5	6	1	3	4	1	3	2	1	2
D	18	11	10	10	5	8	2	0	2	1	0	0
E	22	15	13	14	11	11	10	9	9	6	5	7
F	2	0	0	1	0	0	0	0	0	0	0	0
H	10	4	7	1	0	1	0	0	0	0	1	0
I	16	13	10	13	8	9	5	2	2	1	0	1
J	14	7	7	5	4	4	1	1	1	0	0	0
K	24	14	9	9	11	11	2	5	2	0	5	0
M	30^+	28	6	30^+	28	20	30^+	28	0	30^+	28	0
N	8	0	0	2	0	0	2	0	0	2	0	0
O	3	0	0	0	0	0	0	0	0	0	0	0

[1]Sample size was four blocks. U - unweathered, W_2 - weathered 2 weeks, W4 - weathered 4 weeks.

equalled or exceeded chemical A in effectiveness at all depths. Ammonium bifluoride, chemical N, produced the largest TZEs in blocks unweathered or weathered for 2 weeks but its effectiveness abruptly decreased after 4 weeks of weathering.

C. FIELD TESTS

Twenty-three 10-foot long, weathered western redcedar poles were kerfed full-length to a depth of 4 cm to divide the pole surface equally into three sections, one untreated, and two for flooding treatment by different chemicals. The poles were set in the ground to a depth of 3 feet and the holes were backfilled with pea gravel with one section facing north (Figure 2).

Seven chemicals judged to be the most promising were selected for the field test (Table 11). The chemicals were assigned systematically to five poles. Metal dividers were inserted into the kerfs and a pole section was flooded with the appropriate chemical during the fall of 1981. Rainfall was unusually heavy and continuous that fall and winter.

Additional weathered cedar poles will be installed for other chemical treatments that appear promising in the screening tests.

Objective III

PREVENT DECAY INITIATED IN FIELD-DRILLED BOLT HOLES IN DOUGLAS-FIR POLES

Twenty-eight poles 18 feet long were Boulton-dried with a commercial charge of poles treated with pentachlorophenol in heavy petroleum and removed prior to the pressure phase. Starting 1½ feet below the top, increment borer cores were removed at eight locations 1½ feet apart in a downward spiral pattern (45° offset). Then alternate 1-inch and 3/4-inch diameter holes were drilled at the coring sites. Poles were randomly distributed among seven groups of four poles each for application of the protection methods.

The poles were set in holes which were back filled with crushed rock on Sept. 28, 1981. The protection methods and dates of application and installation of galvanized hardware for crossarms but no arms were as follows:

(Figure 1)

.* Patox washers between pole and attachments:	Dec. 29, 1981
. Polybor as dry chemical	Dec. 29, 1981
. Ammonium bifluoride as dry chemical	Dec. 29, 1981
. Pentachlorophenol (10%) in mineral spirits	Mar. 18, 1982
. Boracol 40 plus ethylene glycol (50-50)	Mar. 18, 1982
. Unprotected holes	Dec. 29, 1981
. Unprotected holes	Dec. 29, 1981

An extra pole with holes was installed without hardware. Delays in application of the protections were due to an extremely wet fall and winter.

* Pole number 4, top bolt hole, contained a decay fungus when installed.

Figure 3. Field test of methods for protecting field-drilled bolt holes.

Next fall, cores will be removed and moisture meter readings will be made in line with and 6 inches directly below the holes in one set of the poles with unprotected bolt holes. If decay fungi are prevalent, cores will be removed from all test poles with protected bolt holes. Meanwhile a sprinkling system is being installed to wet the poles 1 hour per day from June 1 to September 30.

OBJECTIVE IV

DETECT EARLY DECAY IN WOOD AND ESTIMATE THE RESIDUAL STRENGTH OF POLES IN SERVICE

A. DETECTING EARLY DECAY IN DOUGLAS-FIR USING CRUSHING STRENGTH, CHEMICAL INDICATORS AND FRACTURE TESTS

Testing 0.375-inch diameter plugs from Douglas-fir for radial compression strength (RCS) was more promising for detecting early decay (Annual Report, August 1981) than chemical tests that colored the wood or a needle fracture test. RCS, which measures the strength of the weakest earlywood layer was highly correlated with weight loss caused by Poria placenta, an important brown rotter in Douglas-fir poles. Strength losses occurred before weight loss could be detected, reaching 55% at a 10% weight loss. At this early stage of decay, the presence of a decay fungus is still difficult to detect with a microscope.

These initial studies, using plugs cut from a 3-foot long section of a Douglas-fir pole, also indicated that sapwood was lower in RCS than adjacent heartwood. Subsequent tests of 46 radial pairs of sound, green, sapwood and heartwood plugs from 23 poles representing four pole yards from northern Washington to northern California confirmed that sapwood was lower than heartwood in RCS. The difference may be due to extractives.

The study also provided the following equation for predicting the RCS of adjacent sound heartwood from RCS of the sapwood at a 95% confidence level:

$$\text{Heartwood RCS} = 128 + 0.781 \text{ sapwood RCS}$$

Using a prediction interval at a 95% confidence level computed from this equation, it may be possible to detect all plugs having 7.5% or more weight loss and 50% of the plugs with weight losses as low as 3.5%. To test this

model, plugs will be removed from poles, tested for RCS, designated as sound or containing early decay, and then flamed and cultured on nutrient agar for the presence of decay fungi.

B. DETECTING EARLY DECAY BY MICROSCOPIC AND ANALYTICAL METHODS AND ESTIMATING RESIDUAL STRENGTH OF WOOD*

Small Douglas-fir and southern pine beams decayed to weight losses of 2 to 14% suffered large reductions in modulus of rupture (16 to 70%) and modulus of elasticity (5 to 29%). Examination of the wood by fluorescent microscopy using an acridine orange stain permitted differentiation between undecayed wood and wood decayed to small weight losses. However, the mixture of the green color of undecayed wood and orange color of decayed wood observed by image analysis as well as anatomical features posed problems in quantifying the amount of decay. Alkali solubility was a poor indicator of the extent of early decay.

A second set of decayed and nondecayed beams has been tested in static bending and the data are being analyzed for strength-weight loss relationships. Microtome sections of the beams will be stained with acridine orange to re-evaluate the ability of the fluorescent microscopy technique to detect the extent of early decay.

Preliminary work on infrared spectroscopic analysis (IR) of decayed and undecayed wood has begun using dried warm water extracts of wood mixed in KBr pellets. Also, microtomed sections of wood were held between NaCl crystals and analyzed with IR. Some differences in the spectra of decayed and undecayed wood were detected. The IR analyses will continue.

*In cooperation with Dr. R. DeGroot, U.S. Forest Products Laboratory

C. ESTIMATING RESIDUAL STRENGTH OF POLES

The increasing frequency of decay fungi in Douglas-fir poles with increasing air seasoning time found in Part V of this study, provided an opportunity for evaluating the significance of various stages of decay on pole strength using RCS, bending strength properties, toughness and sonic properties. The objectives of this study are to determine:

- The significance of early internal decay and advanced internal decay on pole strength.
- The predictive value of RCS for estimating effects of decay on bending strength properties.
- The relationships between bending and sonic properties.

Using pole sections that have been air seasoning for 1 year (decay development study, Part V), two sets of specimens 1 x 1 x 18-inches long will be cut at three radial positions from two locations on each pole section and conditioned to 18% moisture. These specimens will provide smaller specimens for RCS, static bending (modulus of rupture, modulus of elasticity, warp to maximum load), toughness and sonic tests. Cross sections near the breaks will be used for moisture content and specific gravity measurements and cultured for decay fungi.

OBJECTIVE V

CONSERVE ENERGY BY PROCURING DOUGLAS-FIR POLES THAT HAVE BEEN SEASONED BY THE MOST EFFICIENT METHODS AND THAT ARE AND WILL REMAIN FREE OF VIABLE DECAY IN SERVICE

A. DETERMINE THE INCIDENCE AND SPECIES OF DECAY FUNGI IN FRESHLY CUT POLES AND IN POLES STORED IN WIDELY SCATTERED AIR-SEASONING YARDS ONE, TWO OR MORE SUMMERS.

Studies under this objective were intiated in the summer and fall of 1981 when air-seasoning poles in 11 pole yards in the Pacific Northwest were sampled. Fourteen 6-inch long cores distributed along the length of each pole were removed, placed in plastic straws, and stored in a refrigerator at the end of each day. In the laboratory, the cores were flamed, plated on malt agar medium and subsequently the cultures were examined microscopically to detect the presence of Basidiomycetes that might initiate decay.

In general, the longer the poles were in air-seasoning the greater the percentage of poles infected by Basidiomycetes and fungi suspected of being Basidiomycetes (Table 13). Suspect fungi are isolates that have most of the morphological characteristics of Basidiomycetes but positive identification is pending. Often these isolates turn out to be monokaryons of decay fungi that lack clamp connections, a microscopic characteristic used to identify decay fungi.

TABLE 13

INCIDENCE OF DECAY FUNGI IN DOUGLAS-FIR POLES AIR SEASONED FOR VARIOUS TIME PERIODS IN ELEVEN POLE YARD IN THE PACIFIC NORTHWEST

	MONTHS IN AIR-SEASONING						
	Unpeeled	0-6	7-12	13-18	19-24	25+	Total
POLES SAMPLED	229	251	230	56	89	126	981
POLES W/BASIDIOMYCETES	30	106	147	53	70	108	514
POLES X/SUSPECT FUNGI[1]	29	57	59	24	27	45	241
CORES REMOVED	2650	3662	3221	824	1257	1726	13340
CORES W/BASIDIOMYCETES	61	254	393	196	260	288	1452
CORES W/SUSPECT FUNGI[1]	42	84	84	30	40	58	338

[1]Suspect fungi are isolates that have most of the morphological characteristics of Basidiomycetes but positive identification is pending.

A little over one half of the Basidiomycetes isolated from the air-seasoning poles have been identified (Table 14), but until more poles in the older groups are sampled it is difficult to make valid comparisons between population changes with time for the different fungi. Nevertheless, it is apparent that Poria carbonica, the primary decay fungus in Douglas-fir poles, is building up significantly as the air seasoning period is prolonged. Some of the isolates identified are known decay fungi. These isolates as well as the unidentified Basidiomycetes and suspect fungi are being tested for their ability to decay wood.

This coming summer we will sample additional pole yards to increase our sample size of poles over 1 year in air seasoning. Because there was significant infection in the fresh unpeeled poles, we will extend our sampling to freshly cut poles in the forest.

TABLE 14

NUMBER OF IDENTIFIED BASIDIOMYCETE ISOLATES FROM CORES REMOVED FROM DOUGLAS-FIR POLES AIR SEASONED FOR VARIOUS TIME PERIODS IN ELEVEN POLE YARDS IN THE PACIFIC NORTHWEST

FUNGUS	NUMBER OF FUNGAL ISOLATES FROM POLES AIR SEASONED FOR VARYING LENGTH OF TIME (MONTHS)						
	UNPEELED	0-6	7-12	13-18	19-24	25+	TOTAL
Coriolus versicolor	3	4	3	17	1	11	39
Monokaryons	2	7	4	1	1	5	20
Fomitopsis cajanderi	27	10	16	-	7	-	60
Monokaryons	-	3	-	-	-	-	3
Gloeophyllum saepiarium	2	1	-	2	13	6	24
Haematostereum sanguirelentum	7	96	107	53	56	84	403
Peniophora spp.	-	3	44	14	14	2	77
Poria carbonica	-	6	21	18	35	66	146
Monokaryons	-	1	2	1	2	2	8
Poria placenta	2	-	10	6	7	7	32
Monokaryons	-	7	3	3	3	7	23
Poria xantha	-	-	-	-	-	1	1
Schizophyllum commune	1	8	2	8	2	2	23
Monokaryons	-	1	-	1	-	-	2
Sistotrema brinkmanii	1	53	92	18	20	27	211
Stereum hirsutum	2	3	4	1	3	5	18
NO. ISOLATES IDENTIFIED	47	213	302	143	164	225	1100
TOTAL ISOLATES[1]	102	345	503	244	324	375	1893
PERCENT ISOLATES IDENTIFIED	46	62	61	59	51	60	57

[1]"Total isolates" includes identified and unidentified Basidiomycetes as well as suspect fungi that have most of the morphological characteristics of Basidiomycetes but positive identification is pending.

B. DETERMINE THE ABILITY OF VARIOUS FUNGAL STRUCTURES TO INITIATE DECAY AND DETERMINE HOW AND WHEN POLES IN SERVICE ARE INFECTED WITH DECAY FUNGI.

Poria carbonica is the major decay fungus in Douglas-fir poles in the Northwest, but relatively little is known about its means of spread and infection in poles. Although infection can occur from soil-borne inoculum, air-borne inoculum in the form of basidiospores (from fruiting bodies) and small soil particles carrying hyphal fragments and chlamydospores (thick-walled survival spores) may be important. To assess the probability these various fungal structures (propagules) act as decay inoculum, the fungal units first must be isolated, their viability determined, and their ability to initiate decay studied.

In our earlier studies (Ann. Rpt. Aug. '81) we learned how to produce and isolate chlamydospores from other P. carbonica cells produced in culture. In our more recent studies, an aqueous suspension of chlamydospores was prepared and aliquots of the suspension were plated on malt agar. Individual chlamydospores aseptically picked from water-agar plates with the aid of a dissecting microscope were similarly plated to determine the ability of these spores to germinate. Germination averaged 60% when the spores were plated en masse and 50% when transfered individually to the growth medium.

Spore Infection of Wood

From the same batch of chlamydospores, single spores were aseptically placed on the end grain of small (1.5 x 2.5 x 2.5 cm), sterile, water soaked, Douglas-fir heartwood blocks and the blocks wre incubated in moist chambers at about 22°C. Infection was indicted by isolation of P. carbonica from the wood remote from where the single chlamydospores were placed.

Twenty blocks out of 32 inoculated with single chlamydospores became infected and all 20 had visible mycelium growing over the surface of the

blocks. P. carbonica was successfully isolated from each block. This 62% infection rate is similar to the germination percentage obtained for these spores on malt agar. Clearly, single chlamydospores are capable of infecting Douglas-fir poles under optimum conditions for fungal growth.

Poria carbonica is capable of growing through soil and surviving on organic matter in the soil. There is an infection potential from wind-borne soil particles containing fungal propagules, and our earlier experiments established that soil particles small enough to be wind-borne for short distances could contain viable fungal propagules. However, soil particles that become air-borne are relatively dry. Because chlamydospores are the only fungal structures that can stand desiccation, we delayed further studies on soil borne inoculum until we had completed our investigations of chlamydospores as infective units.

Basidiospores are an important source of air-borne decay fungus inoculum, and the relatively high frequency of monokaryons isolated in our infection studies tend to bear this out. Consequently, our efforts in laboratory studies have been concentrated on understanding the role of these spores in wood infection.

Effect of Light on Sporulation

Earlier experiments with P. carbonica suggested that light was important in inducing sporulation, that fungal nutrition was an influencing factor, and that addition of Douglas-fir wood to cultures growing on malt agar also stimulated sporulation. From these experiments, a standard fruiting medium containing 12.5 g malt extract per liter with Douglas-fir heartwood blocks added has been used in experiments to verify the influence of light on sporulation. Plates of the standard fruiting medium were inoculated with P. carbonica and incubated in the dark at 30°C until the colony diameters reached 4-5 cm. The

plates were then divided into three treatment groups: diffuse light, diffuse light sealed in plastic bags, and foil-wrapped to exclude light. The plates sealed in plastic provided a control for the foil-wrapped plates in which impeded gas exchange might have been a confounding factor. All three sets of plates were inverted and incubated at about 22°C in diffuse light. After 1 month, the cultures were examined and scored for fruiting body initials and sporulation.

Incubation in diffuse light was sufficient to stimulate basidiospore production by P. carbonica even when gas exchange to the cultures was limited by sealing the cultures in plastic (Table 15). This experiment demonstrates that on the standard fruiting medium adequate basidiospore production can be obained under essentially "room conditions" without elaborate light treatments and rigid temperature controls.

TABLE 15

THE INFLUENCE OF LIGHT ON BASIDIOSPORE PRODUCTION BY Poria carbonica IN CULTURE

	NUMBER OF PLATES		
TREATMENTS	FRUITING INITIALS	BASIDIOSPORES	TOTAL
DIFFUSE LIGHT	21	20	21
DIFFUSE LIGHT SEALED IN PLASTIC	15	15	15
DARK	0	0	15

Effect of Cold on Sporulation

Earlier we reported that a cold treatment (4 weeks at 5°C) appeared to stimulate basidiospore production, but further investigation has shown that cold treatments lead to production of abnormal fruiting bodies and relatively poor spore production. Consequently we have abandoned cold treatments to induce sporulation.

Effect of Wood Extractives on Sporulation

The standard fruiting medium contains several small Douglas-fir heartwood blocks per plate, but the fruiting bodies produced in these cultures generally occur near the edge of the plate, not on the wood blocks. This suggests there might be materials diffusing from the blocks that stimulate sporulation. A hot water extract of the wood was prepared by autoclaving 20 blocks (1.5 x 2.5 x 2.5 cm) in 1 liter of distilled water for 30 minutes. The blocks were each split into four pieces prior to extraction. The wood block extract was added to a culture medium at three concentrations: 500 ml, 250 ml and 125 ml per 500 ml of a medium containing 6.25 g of malt extract and 4.0 g of agar. In each case, the difference in medium volume was adjusted with distilled water, and a control with 500 ml of water was used. The extracted wood blocks were also used to make the standard fruiting medium. All plates were innoculated with P. carbonica and incubated in the dark at 30°C until the colonies reached 4-5 cm in diameter. Then the plates were inverted at room temperature (ie. about 22°C) with diffuse light and after 1 month the cultures were examined for fruiting initials and basidiospore production.

Addition of the wood extract failed to stimulate and may have inhibited basidiospore production (Table 16). Although the number of plates with fruiting initials and basidiospores were similar in the treatments receiving the extract, there were significantly less initials and spores per plate in the cultures growing on the highest extract concentrations. The cultures without extract or with extracted wood produced considerable basidiospores but the amount of spores was less than that obtained on the standard fruiting medium (Table 15). If there are diffusable substances in Douglas-fir heartwood that stimulate sporulation, they are either not removed by

hot water extraction, are destroyed during the extraction, or there may be inhibitory substances extracted that mask the stimulatory substances. In any event, addition of the actual wood blocks to the medium appears to be the simplest way to ensure adequate basidiospore production in culture.

TABLE 16

THE INFLUENCE OF A HOT-WATER WOOD EXTRACT FROM DOUGLAS-FIR HEARTWOOD ON BASIDIOSPORE PRODUCTION BY *Poria carbonica*

TREATMENTS ML OF WOOD EXTRACT	NUMBER OF PLATES		
	FRUITING INITIALS	BASIDIOSPORES	TOTAL
0	15	11	15
125	14	1	14
250	15	1	15
500	14	1	15
EXTRACTED WOOD BLOCKS	15	0	15
		10	15

Identifying Monokaryon Isolates

A single basidiospore germinates to form a monokaryon (n) mycelium and when two compatible monokaryons are placed in close proximity on a growth medium, the hyphae will fuse giving rise to a dikaryon (n + n) mycelium that forms clamp connections. Sixty monokaryons have been isolated from single basidiospores of a single dikaryon isolate of *P. carbonica* and we have mated these monokaryons in different combinations to determine the mating compatibility system in this fungus. In several experiments, the frequency of mating was much lower than would have been expected from randomly chosen basidiospores. In some Basidiomycetes, a special medium is needed to promote hyphal fusion and mating between monokaryons from the same parent dikaryon. We are currently investigating several of these media in attempts to enhance mating of *P. carbonica* monokaryons.

Dikaryon-monokaryon matings have been used by other investigators as a means of identifying unknown monokaryon isolates of decay fungi. Our experiments with matings between known dikaryons and monokaryons of P. placenta produced erratic results again suggesting that a special nutritional condition must be met for optimum fusion between compatible isolates. Consequently, with our present understanding of the genetics of these two Poria species, dikaryon-monokaryon matings are an unreliable means of identifying monokaryon isolates of these important decay fungi.

Infection study

To determine how and when poles are infected with decay fungi, pole sections placed upright and horizontally are being exposed at four pole yards in the Pacific Northwest. Initially poles were supplied at each yard, and cores were taken at the ends of each section to determine the initial fungal population in the wood. From these assays we discovered significant amounts of Basidiomycetes already present in the fresh poles and this posed the problem of susequent differentiation between expansion of these initial Basidiomycete colonies and new infections occurring during the exposure period. To overcome this problem beginning in August 1981 we obtained all pole sections from one source and they were heated in a kiln long enough to sterilize the wood. The kiln-dried pole sections were bioassayed to verify their sterility. Moisture determinations taken before and after heating indicated no significant moisture loss durng sterilization.

At each pole yard, 2 foot pole sections were exposed vertically and 4 foot sections were laid horizontal on treated studs. At 3-and 6-month intervals, pole sections were returned to Corvallis where they were extensively cored to determine the amount and identity of the Basidiomycetes that became established in the wood during each exposure period. The details of the experimental design were presented in our previous annual report (Aug. 1981).

Results from the first three 3-month exposure periods in 1981 indicate some significant trends in the establishment of Basidiomycetes and suspect fungi in the pole sections (Table 17). In the May-July period the infection level was relatively low in all yards averaging 4.3% of the cores with potential decay fungi. The percent infection increased slightly at all locations during the Aug.-Oct. period then increased dramatically during the Nov.-Jan. period except in the Arlington, WN location.

TABLE 17

INCIDENCE OF BASIDIOMYCETES AND SUSPECT FUNGI IN DOUGLAS-FIR POLE SECTIONS EXPOSED FOR 3 MONTH PERIODS AT FOUR LOCATIONS IN THE PACIFIC NORTHWEST

	FRACTION AND PERCENTAGE OF CORES WITH BASIDIOMYCETES SUSPECT FUNGI FROM 2 AND 4 FT SECTIONS EXPOSED FOR 3 MONTH INTERVALS DURING 1981-82.[1]					
PLOT LOCATION	MAY - JULY[2] FRACTION	%	AUG. - OCT. FRACTION	%	NOV. - JAN. FRACTION	%
ARLINGTON, WN	9/219	4.1	12/215	5.6	15/304	4.9
SCAPPOSSE, OR	17/335	5.1	15/211	7.1	48/288	17
EUGENE, OR	----------[3]		18/209	8.6	99/288	34
OROVILLE, CA	7/183	3.8	12/222	5.4	118/302	39

[1]Suspect fungi are isolates that have most of the morphological characteristics of basidiomycetes but positive identification is pending.

[2]Pole section in this group were not sterilized before they were exposed in the pole yards.

[3]Core culture lost due to extensive contamination of the cultures by non-basidiomycete fungi.

At this point in our investigation we have no totally viable explanation for the significant increase in infection during the Nov.-Jan. period nor for the infection increase in pole yards from north to south. Currently we are

preparing for computer analysis our data on fungal isolations from the pole sections, wood moisture content, and weather conditions during the exposure periods at each pole yard. When these analyses are completed we may have meaningful, potential explanations for the significant increase in infection with time and location.

Identification of the Basidiomycetes and suspect fungi isolated from the pole sections is currently underway but already significant numbers of monokaryons of Poria placenta and Fomitopsis cajanderi have been identified suggesting that in part infection was due to air-borne basidiospores of these fungi. Sampling at 3-month intervals will continue during the coming year, but because we obtained significant infection of the pole sections exposed for 3-month intervals we have discontinued the 6-month exposure portion of our study.

Decay Development Study

Rate of invasion of decay fungi. Fifteen 6-foot long pole sections, sealed on one end with three coats of Gaco A5400 to slow drying, were placed horizontally on treated skids. Groups of five pole sections will be added at 6-month intervals and other groups of five sections will be removed after 1, 2 and 3 years. Moisture content at time of removal will be measured at depths of 0.5, 1 and 2 inches at 1, 18 and 36-inch intervals from the ends along the top and bottom. Cores will be removed to the center at 6-inch intervals around the circumference 1 inch from each end and at 6-inch intervals from the ends.

After culturing, additional cores may be taken where decay fungi are detected and the pole sections will be dissected with a chain saw. This information will provide a three-dimensional view of decay distribution and an estimate of the volume of wood infested.

The 1-year group of pole sections has been returned to the Forest Research Laboratory and cores are being removed.

C. INVESTIGATE METHODS FOR PREVENTING INFECTION OF POLES BY DECAY FUNGI DURING AIR-SEASONING AND FOR ELIMINATING THE DECAY FUNGI PRIOR TO AND DURING PROCESSING . . . . . .

During Air Seasoning

Fifteen 6-foot long pole sections, sealed on one end with three coats of Gaco A5400 to slow drying, were placed horizontally on treated skids at each of the four test sites. The tops were flooded with a 32-percent solution of ammonium bifluoride known to prevent invasion of southern pine poles by decay fungi. Groups of five pole sections will be removed after 1, 2 and 3 years and distribution of moisture and decay fungi will be determined as described under Rate of Invasion.

Because of the presence of decay fungi in some sections when this study was initiated, another experiment was set up at the Northwest Forest Genetic Center, Corvallis. This study will evaluate the effectiveness of ammonium bifluoride for preventing infection of sterile pole sections and of fumigants (chloropicrin or methylisothiocyanate) for protecting non-sterile pole sections from decay fungi.