EFFECT OF GAMMA RADIATION, WET-HEAT, AND ETHYLENE OXIDE STERILIZATION OF WOOD ON ITS SUBSEQUENT DECAY BY FOUR WOOD-DESTROYING FUNGI[1]

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ABSTRACT

The effects of gamma radiation, wet-heat, and ethylene oxide sterilization of red alder and ponderosa pine sapwood blocks on their subsequent decay by *Coniophora puteana*, *Poria monticola*, *Polyporus versicolor*, and *Poria weirii* were investigated.

After three months of decay, the mean weight losses of blocks sterilized by (1) an irradiation dosage of 2.5×10^6 and 5.0×10^6 rads, (2) wet-heat, and (3) ethylene oxide were not significantly different at the 5% level, with the exception of irradiated alder decayed by *C. puteana*. A radiation dosage of 10^7 rads caused a significant increase in decay of blocks of both wood species inoculated with *Poria weirii*.

Two direct effects of gamma radiation on wood occurred during the tests: (1) a small, but significant, decrease in the weight of wood substance, and (2) nonuniform moisture absorption by the wood.

INTRODUCTION

The two commonly used methods for sterilizing wood are wet-heat (121 C) and gaseous chemicals (ethylene oxide or propylene oxide). Unfortunately, heating wood drives off extractives already present as well as preservatives that may have been deliberately introduced into the wood for the purpose of toxicity test studies. Impregnation of wood with gaseous chemicals necessitates ventilation of the wood after treatment, to remove residues that may still remain in sufficient quantity to retard the subsequent growth of decaying fungi inoculated onto the wood. Smith (1965) has shown that growth of four-week cultures of *Lentinus lepideus* Fr. on wood sterilized with propylene oxide for 24 hr was severely retarded, although the wood had been ventilated for 24 hr previous to inoculation. Also, DaCosta and Osborne (1969) have shown that propylene oxide, when used to sterilize wood blocks impregnated with creosote, has the effect of increasing the toxicity of the creosote. Ethylene oxide is more readily removed from wood by ventilation than propylene oxide, but it is still unknown whether this process leaves residues in the wood that could retard growth of certain fungi. Unpublished results of Smith show that ethylene oxide reacts similarly to propylene oxide when used to sterilize creosote-treated wood, increasing its toxicity to *L. lepideus* (Madison isolate no. 534) but not to *Lenzites trabea* (Pers. ex Fries) Fries (Madison isolate no. 617).

The use of gamma radiation to sterilize wood subsequently to be used in decay tests has been investigated by Kenaga and Cowling (1959), Becker and Burmester (1962), Franz (1963), and Lutomski and Lawniczak (1967). With few exceptions, which are discussed later, these authors' results showed a tendency towards greater decay susceptibility in wood irradiated at dosages of 10^6 rads or higher. However, these observations were generally based on small and often very erratic differences in wood weight loss and, with the exception of the work of

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Kenaga and Cowling, the weight losses were not statistically analyzed.

Sharman and Smith (1970) have shown that a radiation dosage of 10^6 to 10^7 rads is sufficient to sterilize heavily contaminated ¾-inch cubes of ponderosa pine (*Pinus ponderosa* Laws.) and birch (*Betula* sp.) sapwood. The present experiment was designed to show whether gamma radiation could be used as an alternative method to wet-heat or ethylene oxide sterilization of blocks used for standard ASTM D-1413-61 soil block tests, without causing any changes in the susceptibility of the irradiated wood to four selected wood-destroying fungi.

MATERIALS AND METHODS

The following variables were considered:

1. Sapwood of two species—red alder (*Alnus rubra* Bong.) and ponderosa pine (*Pinus ponderosa* Laws.)—cut into ¾-inch cubes from boards air-dried for several months. Three hundred blocks of each wood species were selected by excluding the upper and lower 15% on a weight distribution curve. The final weight range for pine was 2.7–3.0 g and for alder 2.8–3.1 g. The blocks were numbered, oven-dried at 100 C overnight, and then weighed to the nearest milligram before sterilization.

2. Four wood-decaying fungi, two causing brown rot and two causing white rot:

   Coniophora puteana (Schum. ex Fr.) Karst (FPLV 9G);
   Poria monticola Murr. (FPLV 120B);
   Polyporus versicolor L. ex Fr. (FPLV 105C);
   Poria weirii Murr. (FPLV 129D).

3. Five sterilizing treatments:

   A. Ethylene oxide

   Sixty blocks of each wood species were impregnated with ethylene oxide under vacuum (Smith 1965) and ventilated for 24 hr on a horizontal laminar air-flow clean bench.

   B. Wet-heat

   Sixty blocks of each wood species were steam-sterilized in jars at 121 C for 30 min and cooled on a horizontal laminar air-flow clean bench.

   C, D, and E. Gamma radiation at three dosages—2.5×10^6, 5×10^6, and 10^7 rads

   Sixty blocks of each wood species were randomly stacked and sealed in double polyethylene bags, then irradiated at the three dosages for the appropriate times in a Gammacell 220 utilizing Co^{60} (Sharman and Smith 1970). The moisture content of the blocks at the time of irradiation was determined by oven-drying a group of 12 randomly selected blocks and found to be from 4.5 to 5.1%. After treatment, they were stored on the clean bench until planting.

The ASTM D1413-61 standard soil block test method employing a three-month incubation period was used with the following modifications:

(1) The size of the feeder strips used was $\frac{1}{16} \times 1 \times 2$ inches;
(2) The soil jars were of 16-oz capacity and were cylindrical in shape;
(3) One feeder strip supporting two blocks was used for each jar;
(4) A replication of 12 blocks was used for each variable condition;
(5) Twelve "check blocks" were used for each sterilizing treatment and for both wood species. These blocks were treated similarly to the experimental blocks but were placed in uninoculated soil jars.
(6) Approximately 200 g (dry weight) of soil was put into each soil jar and the moisture content of each jar was adjusted to 42 ± 1.5% by the addition of a calculated amount of water. A steam-sterilization time of 1 hr was used for these soil jars.
(7) A horizontal laminar air-flow clean bench was used to ensure complete

TABLE 1. *Average % weight loss of inoculated pine and alder sapwood after three months' incubation. (Replication of 12 blocks)*

Fungus	Treatment[a]	Ponderosa pine Average wt loss %	Ponderosa pine Standard deviation	Red alder Average wt loss %	Red alder Standard deviation
	A	63.0	2.2	55.3	3.1
	B	62.7	1.2	56.5	2.2
Coniophora puteana	C	63.0	2.8	52.0	1.7
	D	60.1	1.8	52.3	2.7
	E	62.9	2.6	53.6	2.3
	A	60.2	1.5	54.2	2.8
	B	61.2	1.8	56.7	2.5
Poria monticola	C	61.7	1.7	56.1	2.0
	D	60.6	2.3	53.9	3.7
	E	59.8	2.1	56.2	4.1
	A	14.9	1.3	30.3	4.1
	B	16.2	2.6	29.1	3.1
Polyporus versicolor	C	16.5	1.5	29.4	2.3
	D	14.4	1.2	30.4	3.0
	E	15.9	1.6	30.1	3.2
	A	45.5	2.3	41.9	2.2
	B	45.4	4.9	43.6	2.9
Poria weirii	C	44.9	4.3	43.3	3.6
	D	42.9	2.7	43.5	3.0
	E	48.9	3.3	46.1	1.6

[a] *Treatment*
A—Ethylene oxide
B—Wet-heat
C—Gamma radiation at a dosage of 2.5×10^6 rads
D—Gamma radiation at a dosage of 5×10^6 rads
E—Gamma radiation at a dosage of 10^7 rads.
These treatments refer also to Tables 2, 3 and 4.

sterility in all handling procedures for the soil jars, wood, and test fungi.

A statistical analysis of the results was made, using Duncan's multiple range test.

RESULTS AND DISCUSSION

Considering the four fungi and two wood species for treatments A, B, and C, there was no significant[2] difference between the percentage-weight-loss values after three months' incubation (Tables 1 and 4), with only one notable exception. These results extend the observation made by Smith (1965) for the fungus *L. lepideus* that ethylene oxide sterilization of wood is efficient and does not result in the retention of any toxic residues by the wood that may subsequently prevent the growth of some wood-destroying fungi. There was a significantly lower weight loss obtained with treatment C—*C. puteana* and red alder (Table 4) that must be considered an exception to the general trend.

At higher radiation dosages (treatments D and E), there was no significant increase in the amount of decay with the exception of both wood species irradiated at 10^7 rads and decayed by *P. weirii* (Tables 1 and 4). The results for ponderosa pine decayed by *C. puteana*, *P. monticola*, or *P. versicolor* at these treatments compare with part of the results obtained by Becker and Burmester (1962), who used *C. puteana* to decay *Pinus sylvestris* L., where radiation dosages from 3.75×10^5 to 1.50×10^7 r[3] failed to

[2] The use of "significant" throughout this paper refers to statistical terminology only.

[3] The quoted authors give no explanation for the abbreviation "r." However, in papers published before 1965, it is generally assumed that "r" refers to reps. (Paszner, personal communication.)

TABLE 2. *Average % moisture content of inoculated pine and alder sapwood blocks after three months' incubation. (Replication of 12 blocks)*

Fungus	Treatment	Ponderosa pine Average MC %	Ponderosa pine Standard deviation	Red alder Average MC %	Red alder Standard deviation
	A	80.0	6.5	70.0	6.1
	B	76.1	6.6	67.1	5.4
Coniophora puteana	C	84.9	9.2	64.7	3.6
	D	74.1	6.8	71.8	7.6
	E	71.9	7.5	68.7	4.6
	A	63.2	8.7	59.3	9.3
	B	72.2	17.0	60.9	9.6
Poria monticola	C	69.7	9.7	56.2	10.9
	D	65.8	11.3	52.2	6.9
	E	64.3	9.1	57.1	8.3
	A	43.9	1.4	48.3	3.7
	B	45.0	3.5	46.5	2.9
Polyporus versicolor	C	46.2	2.6	47.4	2.0
	D	42.2	2.5	47.5	2.4
	E	44.3	2.3	47.3	3.3
	A	108.4	10.5	105.8	3.8
	B	100.9	9.7	103.0	7.9
Poria weirii	C	101.4	9.6	112.2	11.4
	D	82.7	8.9	97.2	10.2
	E	109.9	15.0	105.8	6.2

make the wood more susceptible to decay; actually after eight weeks' incubation, the opposite reaction was indicated, since all irradiated wood was less susceptible to decay than the unirradiated controls. Lutomski and Lawniczak (1967), who used *C. cerebella* (Pers.) Duby to decay pine heartwood previously irradiated with radiation dosages from 1.0×10^4 to 1.11×10^6 rads, suggested that radiation dosages above 1.0×10^4 rads caused a slightly increased susceptibility to decay, although these authors stated that the differences in weight loss were so small that they could be disregarded.

TABLE 3. *Change in absolute weight and percentage moisture content of the uninoculated check blocks after 3 months in soil jars*

Wood	Treatment	Absolute wt loss (g)	Moisture content (%)
Pine	A	0.005	34.43
	B	0.009	33.48
	C	0.021	34.07
	D	0.020	32.56
	E	0.020	35.18
Alder	A	0.001	36.06
	B	0.008	33.62
	C	0.018	33.47
	D	0.015	31.69
	E	0.011	33.96

Kenaga and Cowling (1959), using *L. trabea* and ponderosa pine, showed that a radiation dosage of 10^7 reps[4] increased the susceptibility of the wood to decay, this being significant after nine weeks, but not after four weeks of incubation. In the present study, only *P. weirii* showed a significant increase in its ability to decay both pine and alder at the highest radiation dosage of 10^7 rads (Table 4). It appears that at this radiation dosage, the wood is being made more available to fungal attack and decay, although it is obvious that not all fungi respond similarly.

Weight-loss results for the decayed pine, previously irradiated at the three radiation dosages, indicate some heterogeneity, with

[4] 1 rep = 0.93 rads.

TABLE 4. *Statistical analyses of results*

Means not underscored by the same line may be regarded as different at the 5% level or better (Duncan's multiple range test).

* sig. at 5%; ** sig. at 1%.

1. Analyses of variance of percentage weight loss (grams) for the decayed blocks.

	Source	*df*	*M.S.*	*F*	
a) Ponderosa pine *Coniophora puteana*	Treatments	4	18.624	3.90**	
	Error	55	4.772		
	D	B	E	A	C
	60.14	62.74	62.89	63.01	63.02
Poria monticola	Treatments	4	7.333	2.03 n.s.	
	Error	55	3.621		
	E	A	D	B	C
	59.76	60.21	60.56	61.21	61.72
Polyporus versicolor	Treatments	4	9.716	3.27*	
	Error	55	2.968		
	D	A	E	B	C
	14.35	14.90	15.87	16.14	16.51
Poria weirii	Treatments	4	56.600	4.34**	
	Error	55	13.040		
	D	C	B	A	E
	42.88	44.93	45.37	45.52	48.91
b) Alder *Coniophora puteana*	Treatments	4	44.420	7.43**	
	Error	55	5.980		
	C	D	E	A	B
	52.02	52.34	53.64	55.34	56.49
Poria monticola	Treatments	4	18.788	1.94 n.s.	
	Error	55	9.679		
	D	A	C	E	B
	53.91	54.23	56.05	56.20	56.67
Polyporus versicolor	Treatments	4	4.037	0.40 n.s.	
	Error	55	10.088		
	B	C	E	A	D
	29.10	29.42	30.09	30.32	30.42
Poria weirii	Treatments	4	27.524	3.72**	
	Error	55	7.404		
	A	C	D	B	E
	41.86	43.30	43.54	43.60	46.06

2. Analyses of mean absolute weight loss (grams) for the undecayed control blocks.

	Source	*df*	*M.S.*	*F*	
a) Ponderosa pine	Treatments	4	0.00065	33.2**	
	Error	55	0.00002		
	A	B	D	E	C
	0.005	0.009	0.020	0.020	0.021
b) Alder	Treatments	4	0.00050	18.7**	
	Error	55	0.00003		
	A	B	E	D	C
	0.001	0.008	0.011	0.015	0.018

TABLE 4. Continued.

3. Analyses of percentage moisture content results for the undecayed control blocks.

a) Pine	*Source*		*df*	*M.S.*	*F*
	Treatments		4	11.777	3.81**
	Error		55	3.089	
	D	B	C	A	E
	32.56	33.48	34.07	34.43	35.18
b) Alder					
	Treatments		4	29.084	9.45**
	Error		55	3.076	
	D	C	B	E	A
	31.69	33.47	33.62	33.96	36.06

the middle dosage of 5×10^6 rads tending to result in lower weight losses than either the lower or higher radiation dosages (Tables 1 and 4). A similar result was reported by Becker and Burmester (1962), after decay of pine and spruce by *C. puteana* and *L. lepideus* at a dosage of 7.6×10^6 rads, although their observation would probably not be substantiated by a statistical analysis. Also, their results were evident only after two weeks of decay, when test variability is known to be high. The four-week decay results of Kenaga and Cowling (1959) showed a significantly reduced rate of decay for saturated wood irradiated at 0.6×10^6 reps per hour with a total dosage of 10^6 reps when compared with lower and higher dosages of radiation. However, their results for this and lower dosage rates after a nine-week decay period showed no similar significant trends.

At the conclusion of the incubation period, more moisture was present in wood decayed by the white-rot fungus *P. weirii* than in wood decayed by the other three fungi (Table 2). It is also interesting to note the high variability of the moisture-content values, which do not necessarily reflect high variability in the weight-loss values. This would indicate the relative insensitivity of the decay process to minor fluctuations in moisture content of the wood substrate.

Gamma radiation of both ponderosa pine and red alder wood had two direct effects apart from any possible secondary effects that it may have had on susceptibility of wood to decay. First, all three radiation dosages resulted in a small but significant decrease in weight of wood substance (Tables 3 and 4), this decrease in most cases being about twice that normally experienced with oven-drying and wet-heat or ethylene oxide sterilizing techniques. The decrease in dry weight of wood following irradiation and oven-drying was about 0.8%, this being at least 0.4% higher than that expected because of the oven-drying process. This extra 0.4% corresponds closely to the 0.3% weight-loss value obtained by Seifert (1964) using *Pinus sylvestris* irradiated with $10^{6.5}$ r. Seifert proposed that most of this weight loss is due to decarboxylation, which was evidenced by carbon monoxide and carbon dioxide release.

The second direct effect of gamma radiation was to cause the moisture absorption of the wood to be nonuniform. Thus, the percentage moisture content for blocks irradiated at both 2.5×10^6 and 10^7 rads was significantly higher than that for blocks irradiated at 5×10^6 rads (Tables 3 and 4). Again this agrees with the hygroscopicity results of Kenaga and Cowling (1959), who showed a higher absorption of water vapor for ponderosa pine following irradiation at dosages both below and above 10^6 reps. However, Paton and Hearmon (1957), using irradiated Sitka spruce, showed a decrease in hygroscopicity at doses of 10^7 and 10^8

rads; therefore the effect of gamma radiation on the ability of wood to absorb moisture appears to vary with different species of wood.

CONCLUSIONS

1. In conventional three-month incubation tests of fungal decay of wood, for the two wood species and four fungi studied, gamma-radiation sterilization at 2.5×10^6 rads is a suitable alternative to either wet-heat or ethylene oxide methods.
2. At all three radiation dosages tested, some very small loss of wood substance occurs, but this does not affect the final decay-weight loss pattern.
3. At radiation dosage of 10^7 rads, with *P. weirii* on both pine and alder, some increase in decay is evident over that occurring in wood sterilized by either wet-heat or ethylene oxide.
4. Any changes in hygroscopicity of wood following gamma radiation at the tested dosages are small, variable, and could be ignored in any decay-weight loss studies.

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