

# GAMMA RADIATION STERILIZATION OF PONDEROSA PINE AND BIRCH SAPWOOD<sup>1</sup>

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## ABSTRACT

The sterilizing effects of gamma radiation on microbiologically contaminated ponderosa pine and birch sapwood (beams and cubes) conditioned to moisture contents ranging from 0.5% to 150% were examined.

The lethal radiation dosage required depended on the moisture content of the wood and also, perhaps, on the initial concentration of microorganisms within the wood. Fungi were more sensitive to radiation than bacteria, but a radiation dose between 1 and 5 M rads was sufficient to kill all microorganisms in  $\frac{3}{4}$ -inch cubes of both woods. Smaller radiation dosages were required to sterilize wood at either very low or very high moisture contents than at intermediate moisture contents. Both woods had similar lethal dosage requirements for sterilization.

Seven fungal species isolated after a radiation dosage of  $5 \times 10^5$  rads were found to be Fungi Imperfecti. Following gamma irradiation of wood samples, there was no evidence of an aftereffect modifying the growth of microorganisms in the wood.

## INTRODUCTION

Gamma radiation has been used to sterilize various forms of biological material, and although some workers have successfully used gamma radiation to sterilize wood, many of the controlling factors still remain obscure.

One of these factors, that of the necessary radiation dosage for sterilization of microorganisms, has been investigated by Bellamy and Lawton (1954), McLaren et al. (1957), Pan et al. (1959), and Kashkina and Abaturv (1967). They generally concluded that fungi are killed by a dosage of 1 M rad and bacteria are sterilized by dosages ranging from 1 M rad to 4 M rads.

The effect of moisture content of the substrate on the gamma radiation sterilization process was noticed by Jackson et al. (1967) in their study of soil microbiological populations. Soil samples containing 30% moisture by weight were sterilized at lower radiation dosages than air-dry soil samples. Bellamy and Lawton (1954), working with

*Staphylococcus aureus* 209, found that the mean lethal radiation dosage for frozen cells at  $-78$  C increased fivefold over the mean lethal dosage for cells irradiated in suspension at room temperature, and that the mean lethal dosage for dry cells irradiated at room temperature was slightly higher than that for the cells frozen at  $-78$  C. Other workers (Friedin 1958; Popenoe and Eno 1962; Johnson and Osborne 1964; Kashkina and Abaturv 1967) have mentioned moisture content in their radiation studies, but have not considered the effects of different specific moisture contents on the required lethal radiation dosage.

An aftereffect of irradiation has been mentioned by several workers (Daniels et al. 1953; Glegg and Kertesz 1956; Davis et al. 1956; Glegg 1957; Friedin 1958; and Iizuka and Ito 1968). With irradiated cellulose, Glegg (1957) suggested that the aftereffect was the result of further degradation of the cellulose occurring after irradiation had ceased, and was caused by the interaction of free radicals with absorbed oxygen. This effect, persisting as long as 30 days after irradiation, was initiated by oxygen and terminated in the presence of water. However, Daniels et al. (1953) found that the aftereffect of irradiation of aqueous solutions of DNA occurred *in*

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*vacuo* and was enhanced by the presence of oxygen. Izuka and Ito (1968) showed that an aftereffect could influence the results of irradiation on a microbiological population. They stored rice samples at 10 C and 30 C for 30 days after irradiation between 0.2 and 1.2 M rads and found a slight decrease with time in the number of surviving microorganisms in unpolished Japanese rice and Spanish rice stored at 30 C. This effect was not noticed in samples stored at 10 C.

Gamma radiation of wood could be an effective means of obtaining sterile samples, necessary for further biological deterioration studies, without causing the changes associated with normal heat sterilization at 121 C.

This paper describes experiments to examine the effect of different moisture contents on the lethal dosage requirement for the sterilization of two woods, a viability test on fungi isolated from these experiments, and also experiments to determine if, following irradiation of the wood, any aftereffect will influence this lethal dosage requirement. Other reported variables, such as temperature, presence of oxygen, and dosage rate dependence, have not been examined because of apparatus limitations.

#### MATERIALS AND METHODS

##### *Experiment 1. Sterilization of wood beams*

In this experiment, the following variables were considered:

- 1) Two wood species, ponderosa pine (*Pinus ponderosa* Laws.) and birch (*Betula* sp.) sapwood, cut into experimental beams measuring 5 × 5 × 60 mm;
- 2) Five approximate moisture contents based on oven-dry weight. One hundred and eighty beams of each wood were buried in moist, unsterilized soil in perforated polyethylene bags. They were mixed occasionally and left to become colonized by fungi and bacteria for four months, before being wiped free of soil and brought to the desired moisture contents, as follows:

0%—beams were put into double

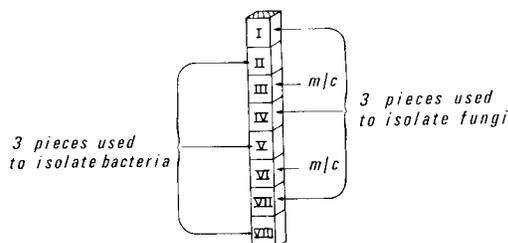


FIG. 1. Beam, divided into eight pieces; six pieces were used for the culture of fungi and bacteria and two pieces for moisture-content (m.c.) determination.

polyethylene bags, frozen overnight, and then freeze-dried;

7%—beams were conditioned to approximately constant weight at normal room conditions;

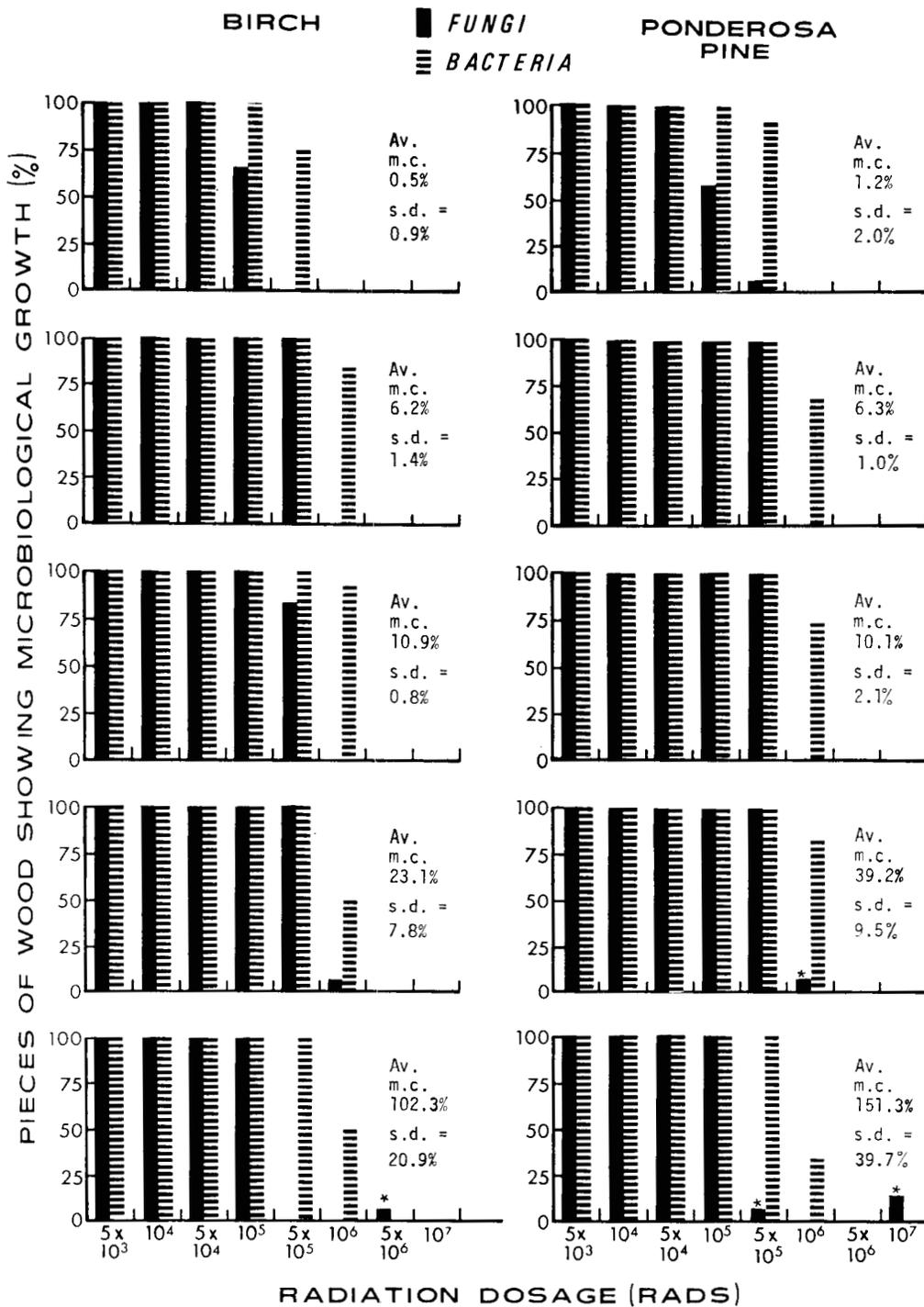
10%—beams were conditioned to approximately constant weight in a controlled-environment cabinet;

30%—beams were conditioned to approximately constant weight in a controlled-environment cabinet;

100%—beams were used immediately after removal from damp soil;

- 3) Eight radiation dosages,  $5 \times 10^3$ ,  $10^4$ ,  $5 \times 10^4$ ,  $10^5$ ,  $5 \times 10^5$ ,  $10^6$ ,  $5 \times 10^6$ ,  $10^7$  rads. The irradiation source was a Gammacell 220 utilizing  $\text{Co}^{60}$  with a dosage rate of  $1.1169 \times 10^6$  rads/hr.

Four replicate beams for each test condition were sealed in double polyethylene bags, and the bags for each radiation dosage were irradiated separately. The effect of the polyethylene bags used in these experiments, as to alteration in the radiation dosage absorbed by the wood, was considered negligible. The polyethylene bags were stacked at random in the Gammacell, with the beams in a vertical position to insure uniform penetration of gamma rays. Following irradiation, the bags were transferred to a horizontal, laminar air-flow clean bench, and the beams were cut into eight approximately equal pieces, using bone-cutting forceps. Three pieces from each beam (Fig. 1: I, IV, VII) were placed in



\*probable contamination  
 (see table 1)

petri dishes on an acid-malt medium containing 2% agar, 2% malt, and 0.5% malic acid to isolate the fungi. Three other pieces, (Fig. 1: II, V, VIII) were placed in petri dishes containing a nutrient medium of 2% agar and 0.8% dehydrated beef-extract peptone broth to isolate the bacteria. Each petri dish contained six pieces of wood from two beams. The moisture contents of two pieces from each beam (Fig. 1: III and VI) were calculated and considered to be representative of the moisture content of that beam. The petri dishes were incubated at 25 C, and the presence or absence of microbiological growth was noted daily. Pieces of wood showing no microbiological growth after four weeks were recorded as negative.

#### *Experiment 2. Sterilization of wood cubes*

In this experiment the following variables were considered:

- 1) Ponderosa pine sapwood, cut into  $\frac{3}{4}$ -inch cubes;
- 2) Two approximate moisture contents based on oven-dry weight, 7% and 100%, the cubes being brought to these moisture contents as in Experiment 1;
- 3) Three radiation dosages:  $10^5$ ,  $5 \times 10^5$ , and  $10^6$  rads. The cubes were left to become colonized by microorganisms as were the beams in Experiment 1. Fifteen cubes were used at each moisture content and sealed in replicates of five in double polyethylene bags for irradiation. Following irradiation, each cube was split longitudinally into four pieces. Two pieces from opposite corners were used for moisture-content determinations. The remaining two pieces were placed on suitable media in petri dishes, one containing acid malt and the other nutrient agar, and incubated at 25 C. Growth was recorded as in Experiment 1.

#### *Experiment 3. Viability test*

Fourteen fungi, which were isolated at dosages of  $10^5$  rads or more in Experiment 1, were inoculated onto 1.2 ml of malt-agar medium contained in  $1\frac{1}{2}$ -dram glass vials with screw caps and incubated at 25 C until the media were overgrown. Four vials of each fungus were prepared and treated in the following manner: one vial was used as a control, one was irradiated at the dosage at which the fungus was originally isolated, and the remaining two vials were irradiated at two consecutive dosage levels below this. The dosage levels used were the same as those in Experiment 1. Following irradiation, two samples of each fungus were inoculated onto malt-agar tubes and incubated at 25 C, and any fungal growth was recorded. All fungal isolations that showed no sign of growth after four weeks were recorded as negative. Wherever possible, the fungi were identified as to their genus.

#### *Experiment 4. Aftereffect*

In this experiment the following variables were considered:

- 1) Two woods, ponderosa pine and birch sapwood, cut into experimental beams measuring  $5 \times 5 \times 60$  mm;
- 2) Two approximate moisture contents, 0% and 7%, were obtained as in Experiment 1;
- 3) Two radiation dosages,  $10^5$  and  $5 \times 10^5$  rads;
- 4) Six post-irradiation storage-time periods of 0, 3, 6, 9, 15 and 21 days.

Forty-eight beams of each wood were left to become colonized by microorganisms as in Experiment 1. The beams were wiped free of soil and each wood was treated as follows: 12 beams for each moisture content tested and 12 beams for each irradiation dosage were cut into seven approximately equal pieces with bone-cutting forceps and distributed into seven double polyethylene

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FIG. 2. Effect of increasing radiation dosage on microbiological growth in ponderosa pine and birch sapwood beams at different moisture contents. (Replication number, 12 pieces of beam = 100%.)

TABLE 1. Viability of the 14 fungi most resistant to radiation isolated from Experiment 1, when irradiated at the highest dosage to which they were originally resistant and two lower dosages

Specimen	Isolation dosage (rads)	Control	Radiation dosage							Fungus
			$10^4$	$5 \times 10^4$	$10^5$	$5 \times 10^5$	$10^6$	$5 \times 10^6$	$10^7$	
a	$10^5$	+ <sup>1</sup> +	+	+	+					<i>Fusarium</i> sp. 1
b	$10^5$	+	+	+	+					Unknown-encrusting hyphae, no evidence of spores
c	$5 \times 10^5$	+		+	+	+				Unknown
d	$5 \times 10^5$	+		+	+	+				<i>Fusarium</i> sp. 2 red staining
e	$5 \times 10^5$	+		+	+	+				<i>Fusarium</i> sp. 3
f	$5 \times 10^5$	+		+	+	+	+	+	+	<i>Fusarium</i> sp. 4
g	$5 \times 10^5$	+		+	+	+				<i>Fusarium</i> sp. 5
h	$5 \times 10^5$	+		+	+	+				<i>Aureobasidium pullulans</i>
i	$5 \times 10^5$	+		+	+	+				Phycomycete sp.
j	$5 \times 10^5$	+		+	+	—				<i>Aspergillus</i> sp. rough spores
k	$10^6$	+			+	—	—			Basidiomycete sp.
l	$5 \times 10^6$	+				—	—	—		<i>Penicillium</i> sp. rough spores
m	$10^7$	+					—	—	—	Unknown
n	$10^7$	+					—	—	—	<i>Penicillium</i> sp.

<sup>1</sup>+ = growth and — = no growth.<sup>2</sup>Viable but different in color.

TABLE 2. Effect of postirradiation storage on microbiological viability in pieces of ponderosa pine and birch sapwood beams. (Replication no. = 6 pieces of beam)

Wood	Moisture content %	Radiation dosage (rads $\times 10^5$ )	Storage time after irradiation (days)											
			0		3		6		9		15		21	
			F*	B**	F	B	F	B	F	B	F	B	F	B
Ponderosa pine	1.2	1	0	6	0	6	0	6	0	6	0	6	0	6
	10.6	1	6	6	6	6	6	6	6	6	6	6	6	6
	1.2	5	0	0	0	4	1	4	0	3	0	3	0	3
	10.6	5	6	6	6	6	6	6	6	6	4	6	3	6
Birch	0.7	1	0	6	1	6	0	6	0	6	0	6	0	6
	10.3	1	6	6	6	6	6	6	6	6	6	6	6	6
	0.7	5	1	1	0	3	0	1	0	1	0	0	0	1
	10.3	5	6	6	5	6	2	6	6	6	6	6	5	6

F\* = Number of pieces of beam showing fungal growth. Replication = 6 pieces of beam.

B\*\* = Number of pieces of beam showing bacterial growth. Replication = 6 pieces of beam.

bags in rotation so that each bag contained 12 random pieces. The pieces of wood were brought to the desired moisture contents as in Experiment 1. Moisture-content determinations were made on one bag containing 12 random pieces of wood for both woods and both moisture contents. The remaining bags were sealed and placed at random into two larger polyethylene bags for irradiation at the stated dosages. Following irradiation, the bags were placed in a Dewar flask at room temperature for the required storage period, after which the pieces of wood were placed on either acid malt or nutrient agar. Six wood pieces were put in each petri dish, which were then incubated at 25 C and examined daily for fungal growth.

#### RESULTS

##### *Experiment 1. Wood beams*

Although the required lethal irradiation dosage for fungi is less than that for bacteria (Fig. 2), the results obtained with ponderosa pine and birch were similar for either fungi or bacteria. The lethal radiation dosage for fungi growing within wood having a moisture content from 6% to 40% was between  $5 \times 10^5$  and  $10^6$  rads, whereas fungi within wood having either a very low (0.5 to 1.0%) or very high (100 to 150%) moisture content required a lethal radiation dosage of between  $10^5$  and  $5 \times 10^5$  rads.

Bacteria within wood at moisture contents from 6% to 40% required a lethal radiation dosage between  $10^6$  and  $5 \times 10^6$  rads; however, with ponderosa pine at very low and high moisture contents, the required radiation dosage was lower, being close to  $10^6$  rads. Birch at a low moisture content behaved similarly to ponderosa pine, whereas at a high moisture content a decrease in the required lethal irradiation dosage was less evident.

##### *Experiment 2. Wood cubes*

As in Experiment 1, the required lethal radiation dosage for fungi was lower than for bacteria (Fig. 3). Fungi growing within wood at a moisture content of 7.6% required a lethal radiation dosage close to  $10^6$  rads, whereas fungi within wood at a moisture

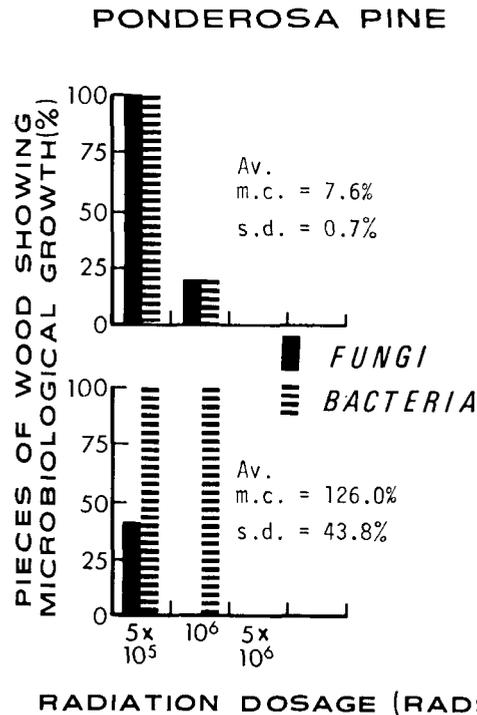


FIG. 3. Effect of increasing radiation dosage on microbiological growth in ponderosa pine sapwood cubes at different moisture contents. (Replication number, 5 pieces of cube = 100%.)

content of 126% required a lethal radiation dosage of between  $5 \times 10^5$  and  $10^6$  rads.

The results were reversed for the bacteria, where the lethal radiation dosage within wood at a moisture content of 7.6% was closer to  $10^6$  rads; whereas in wood at a moisture content of 126% the required lethal radiation dosage was higher (Fig. 3).

##### *Experiment 3. Viability test*

The control fungi and those irradiated at a dosage of  $10^5$  rads or less were all viable when reinoculated onto malt agar (Table 1). Fungi *d* and *g* grew in only one tube after being irradiated at a dosage of  $5 \times 10^5$  rads. Fungi *j* and *k* were not viable after being subjected to the radiation dosage at which they were originally isolated, although they grew at lower radiation dosages. Fungi *l*, *m* and *n*, which were isolated at high radiation dosages, were not

viable when subjected to these radiation dosages again, nor at lower dosages.

All the fungi irradiated resembled their original controls except fungus *f*, in which the mycelium turned from white-grey to black on irradiation at the dosage at which it was originally isolated.

Microscopic examination of the isolated fungi revealed the presence of five *Fusarium* species, two *Penicillium* species, one *Aspergillus* species, *Aureobasidium pullulans*, an unknown species of a Basidiomycete and a Phycomycete, and three fungi that were not identified.

#### Experiment 4. Aftereffect

There was no apparent increase in lethal effectiveness of the original radiation dosage after storing the wood pieces for 21 days (Table 2).

#### DISCUSSION

The lethal dosage requirements for fungi and bacteria occurring in wood were found in Experiments 1 and 2 to be  $10^6$  rads and  $10^6$  to  $5 \times 10^6$  rads, in agreement with the values reported in the literature (Bellamy and Lawton 1954; McLaren et al. 1957; Pan et al. 1959; McLaren et al. 1962; Johnson and Osborne 1964; Jackson et al. 1967, and Kashkina and Abaturvov 1967). These dosages are effective on mixed populations of microorganisms such as are found in soil. The fact that bacteria are more resistant to radiation than fungi has been reported by McLaren et al. (1957), Stotzky and Mortenson (1959), Jackson et al. (1967) and Kashkina and Abaturvov (1967). Certain spore forms of bacteria are most resistant to irradiation (Sykes 1965; Kashkina and Abaturvov 1967).

Examination of the data on individual pieces of the beams in Experiment 1 failed to show evidence of a position effect, so it may be concluded that the effect of radiation, using this Gammacell, was relatively uniform along the length of the beams.

The microbiological populations in both woods were more sensitive to radiation when the wood was either very dry (moisture content 0.5 to 1.2%) or very wet (moisture content 102 to 150%), and there was

some indication that sensitivity was greater in the drier than in the wetter state (Experiment 1, Fig. 2). This increased sensitivity of microorganisms to gamma radiation at high moisture contents was observed by Jackson et al. (1967) and Kashkina and Abaturvov (1967) using moist and dry soils. Franz (1963) also noticed this effect of moisture on irradiation, using ponderosa pine wood inoculated with *Lenzites trabea*, where fungus growth in irradiated green wood was inhibited more than in wood with a moisture content of 8%. There is no proven mechanism to explain this observation, but a number of factors may be contributory. It is known that gamma irradiation of water results in the formation of peroxides. These compounds are not strong germicides themselves, but they have a deleterious effect on cell proteins resulting in harmful oxidation reactions. It could be expected that the wettest wood samples would produce the most peroxides, which would result in greater sensitivity to a given radiation dosage.

It is also known that gamma irradiation of wood results in molecular oxidative cleavage of the cellulose and lignin, thereby producing a large increase in the numbers of carboxyl and carbonyl groups. In untreated cotton fiber, this increase in carboxyl group formation has been shown to be from about 0.5% to 8% by weight at a radiation dosage between  $5 \times 10^6$  and  $5 \times 10^7$  rads (Paszner 1968). This increase in carboxyl groups could result in a decrease in pH within the wood. Where the quantity of available water is very low (freeze-dried wood), the concentration of carboxyl groups could perhaps cause the pH to fall to such a level as to be inhibitory for fungal or bacterial growth. The acid-malt agar used to isolate the fungi is already adjusted to pH 4.0, and any further decrease due to carboxyl group formation could prevent fungal growth. It is well known that media with a low pH are inhibitory to the growth of bacteria. Therefore the mechanism of sterilization by irradiation may not be the same at the two ends of the moisture scale.

If this pH effect were taking place within the wood, it is obvious that the apparent

increase in sensitivity of very dry wood to irradiation might not be a true increase in effective irradiation, but rather a failure to culture microorganisms from the wood owing to unfavorable growth conditions. Another explanation for the apparently increased sensitivity of microorganisms in freeze-dried wood to radiation could be that the freeze-drying treatment may have reduced the microbiological population in the wood, which would then require a lower radiation dosage for sterilization (Bellamy and Lawton 1954; Jackson et al. 1967). However, Smith (1968) found no evidence to suggest a decrease in microbiological population in contaminated sapwood that had been freeze-dried, and therefore this possibility seems unlikely. Whatever the reason, there is an obvious practical advantage in pretreating wood to either very low or very high moisture contents for sterilization by gamma radiation.

Since the lethal irradiation dosage for microorganisms in the  $\frac{3}{4}$ -inch cubes (Experiment 2) was essentially the same as that required for the beams, it can be concluded that any radiation dosages derived from Experiment 1 can also apply to standard ASTM D-1413-61  $\frac{3}{4}$ -inch cubes (ASTM D-1413, 1961). From the results for fungi within wood at a moisture content of 7.6%, there is a slight indication that the cubes may be more difficult to sterilize than the beams, which, if significant, could support the contentions of Bellamy and Lawton (1954) and Jackson et al. (1967) that the lethal radiation dosage is dependent on the initial concentration of the microbiological population. In this case, the cubes would obviously contain many more organisms than the beams.

Comparing the two woods reveals no obvious consistent difference in their lethal dosage requirement. Barton (1966) reported that different wood species are affected differently by similar radiation conditions because of the different quantitative and qualitative composition of their extractives, phenolic compounds giving greater radiation protection. Smith and Mixer (1959) also reported this in redwood. The extractive content of ponderosa pine sapwood is

greater than that of birch sapwood (Isenberg 1951), and it would be expected that ponderosa pine would require greater radiation dosages for sterilization. However, this was not observed. Possibly the extractives in ponderosa pine sapwood are not high in phenolic material, or perhaps the magnitude of difference between the radiation dosages that were used was too great to enable any effect to show.

In Experiment 3, fungi  $j$  to  $n$  were not viable after being irradiated at the dosage from which they were originally isolated. They may have suffered sufficient damage during the original irradiation process so that a second dose proved lethal; however, this seems unlikely, since the only fungi affected were those occurring at the higher radiation dosages. The fact that the wood may have offered greater protection to the fungi than malt agar during the irradiation process cannot be overlooked, although Bors and Glubrecht (1967) found that the growth of *Merulius lacrymans* and *Coniophora cerebella* in wood was inhibited by lower radiation dosages than in Biomalz agar. It seems most probable from the results that these fungi were contaminants, especially since their occurrence was so limited. Very few observations have been reported of fungi able to withstand irradiation dosages in excess of 1 M rad, although Iizuka and Ito (1968) isolated some radio-resistant yeasts in unpolished Japanese rice at dosages of 1 M rad or more, and Johnson and Osborne (1964) isolated imperfect yeasts and several members of the Fungi Imperfecti at dosages of 1 M rad.

The fungal identifications generally agree with those of Johnson and Osborne (1964), most of the fungi isolated belonging to the Fungi Imperfecti. Several *Fusarium* species were isolated at a dosage of  $5 \times 10^5$  rads, this group appearing to be relatively radiation resistant. *Aureobasidium pullulans* was also isolated and, although not reported by Johnson and Osborne, this fungus has been mentioned by Skou (1969) as being very radiation-resistant.

The results of Experiment 4 failed to provide any evidence for the existence of an aftereffect in wood following gamma

radiation. There were very few changes in the effect of radiation after 21 days of storage, and there was nothing to suggest that this effect was heightened with time. If the aftereffect is terminated in the presence of water, the results should have shown an increased radiation effect in the freeze-dried wood. Since wood is not degraded as easily as pure cellulose because of the protective influence of lignin (Smith and Mixer 1959; Paszner 1968), any aftereffect in wood could be negligible when compared with that in pure cellulose.

#### CONCLUSIONS

Gamma radiation is a reliable and effective sterilizing agent for wood and could be used for the sterilization of the standard 3/4-inch cubes used in ASTM D-1413-61 tests. The degrading effects of gamma radiation on wood, which are reported to become severe at dosages exceeding 1 M rad, could be kept to a minimum by pretreating the wood so that the moisture content was either very low (freeze-dried) or very high, thereby reducing the required lethal irradiation dosage.

Ponderosa pine and birch require comparable dosages of radiation for sterilization and there is no obvious aftereffect, resulting in an increased efficiency of killing of microorganisms with time.

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