Microorganisms Isolated From Wounds Inflicted on Red Maple, Paper Birch, American Beech, and Red Oak in Winter, Summer, and Autumn

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ABSTRACT

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Microorganisms were isolated from wood adjacent to 459 wounds inflicted during winter, summer, and autumn on 42 trees: 27 *Acer rubrum,* five *Betula papyrifera,* five *Fagus grandifolia,* and five *Quercus rubra*. The fungi isolated most frequently from 1- to 4-week-old winter wounds were *Cytospora* spp.; from the same age summer wounds, *Ceratocystis* spp.; and from the same age autumn wounds, *Phialophora* spp. Bacteria were isolated frequently from 1- to 4-week-old summer and autumn wounds, but seldom from 1- to 4-week-old winter wounds. Bacteria and nonhymenomycetous fungi were the only microorganisms isolated from recently inflicted wounds. Hymenomycetes were isolated from a few 7-, 8-, 9-, and 10-week-old summer wounds, but not from winter wounds until after 5 months. These data indicate that the first microorganisms to invade wounds inflicted at different seasons in the northeastern USA are different and that the time before Hymenomycetes are isolated also differs for wounds inflicted at different times of the year.

Additional key words: wood discoloration and decay, succession of microorganisms.

Seasonal variations in temperature affect the growth of microorganisms and the activities of insects that disseminate microorganisms. Therefore, it might be expected that different microorganisms would be isolated from wounds inflicted on trees at different times of the year. The first microorganisms to grow in fresh wounds inflicted at different times of the year could greatly affect the subsequent development of discolorations and decays. This is a report of studies designed to determine the first microorganisms to grow in fresh wounds made in winter, summer, and autumn. Emphasis was on the microorganisms isolated from the injured tissues, not on how they got there.

MATERIALS AND METHODS

Wounds were inflicted on boles of 27 red maple (*Acer rubrum* L.), five paper birch (*Betula papyrifera* Marsh.), five American beech (*Fagus grandifolia* Ehrh.), and five red oak (*Quercus rubra* L.) on the Massabesic Experimental Forest, Alfred, Maine. Wounds on all but four red maple trees were made by drilling a hole 1.3 cm in diameter and 5 cm deep into the trees. On those four the wounds were made by pounding a chisel with a 1.3-cm tip 5 cm into the wood. Three to 48 wounds were made on each tree at 0.5, 1, 1.5, and 2 m above ground. When several wounds were made on one tree at one time, each was made at a different height. When additional wounds were made later on the same tree, they were made at the same height as the previous wounds. Wounds were spaced so that no two were vertically aligned.

In one experiment, two trees of each species each received four wounds on 13, 20, 27 January, and 3 February (total, 16 wounds per tree). On 10 February, one tree of each species was felled, and each wound was dissected in the laboratory. The remaining trees were felled 5 months later. The same wounding practice was used in other experiments. Eight trees were wounded on 17, 24, 31 July, and 7 August; and four trees were harvested on 14 August and four on 26 October. Four trees were wounded the same way on 19 October and 2, 9, and 16 November, then were harvested on 24 November.

The isolation procedure was as used previously (5, 6). Chips of wood, $3 \times 3 \times 10$ mm, were cut in a systematic pattern from wood above and below the wound immediately after the billet with the wound was split longitudinally through the wound (Fig. 1) with a flamed ax. The chips were placed in a medium consisting of 10 g malt extract, 2 g yeast extract, 20 g agar, and 1 liter distilled water. Microorganisms growing from the chips were examined several times after 10 days incubation at 25 C.

The same harvest and isolation procedures were carried out on two vigorous nonwounded red maples from the study area, and 180 chips of clear wood were incubated in the agar medium in August.

RESULTS AND DISCUSSION

The frequency of isolation and the species of microorganisms associated with 1-, 2-, 3-, and 4-week-old wounds made in winter, summer, and autumn were different (Table 1). Few bacteria were isolated from the winter wounds. *Cytospora* sp. was isolated frequently. No discolored wood was associated with the winter wounds. Most of the chips did not yield microorganisms. No

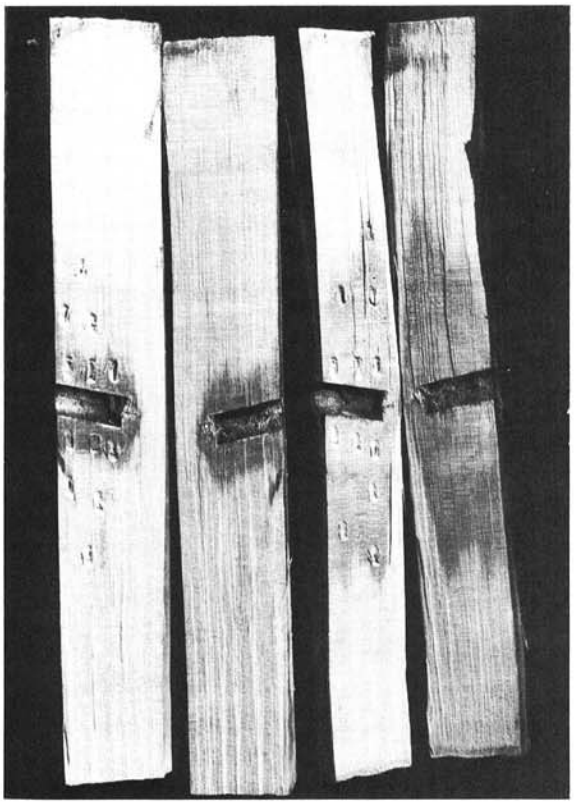

Fig. 1. Discolored wood associated with wounds in red maple. Billets were split longitudinally and chips of wood were cut from discolored wood and placed in an agar medium.

isolation chips from the two nonwounded red maple trees yielded microorganisms. Microorganisms are usually not associated with clear wood (2).

From 1-, 2-, 3-, and 4-week-old summer wounds, bacteria and *Ceratocystis* spp. were isolated most frequently (Table 1). Discolored wood was associated with all wounds. The 1-, 2-, and 3-week-old wounds in birch had small margins of discoloration around the wounds, but the 4-week-old wounds had columns of discoloration ranging from 6 to 15 cm both above and below the wounds. In beech, the discolored wood was a narrow band around the wound. In oak, there was a faint color change in the sapwood around the wound. In maple, some wounds had discolored wood 3 cm both above and below. These results indicate that the discoloration processes develop much faster in summer than in winter.

From 1-, 2-, 3-, and 4-week-old autumn wounds, *Phialophora* spp. and bacteria were isolated frequently. Only a few chips did not yield microorganisms. The columns of discolored wood were similar to those associated with summer wounds. One chip from a 2-week-old wound in birch yielded a Hymenomycete.

The results indicate that bacteria and nonhymenomycetous fungi infected summer and autumn wounds soon after they were inflicted.

After 5 months, isolations were made from four more trees, one of each species, that were wounded in winter (Table 2). Almost all chips yielded bacteria, but few fungi were isolated. *Cytospora* sp. was isolated from many oak chips, but not from chips from the other trees. No Hymenomycetes were isolated.

Five red maples were wounded on 18 February and were harvested 5.5 to 17 months later (Table 3). Bacteria were isolated frequently from wood associated with the

TABLE 1. Microorganisms isolated most frequently from wood adjacent to 1-, 2-, 3-, and 4-week-old wounds inflicted during winter, summer, and autumn on red maple, paper birch, American beech, and red oak

Microorganism	Wounding period	Chips that yielded microorganisms (%)			
		Red maple	Paper birch	American beech	Red oak
Bacteria:	Winter[a]	6	14	17	6
	Summer[b]	64	97	63	65
	Autumn[c]	56	72	97	64
Phialophora spp.:	Winter	0.5	0	1	0
	Summer	16	10	0	5
	Autumn	15	35	22	0
Cytospora spp.:	Winter	17	19	30	3
	Summer	3	0	0	0
	Autumn	26	15	7	1
Ceratocystis spp.:	Winter	0	0	0	0
	Summer	11	61	31	73
	Autumn	0	0	0	0
Chip sterile, no microorganism isolated	Winter	69	69	61	70
	Summer	35	3	26	5
	Autumn	1	4	2	13

[a]Winter wounds inflicted on 13, 20, and 27 January and 3 February, harvested 10 February; four trees, each 16 wounds, 48 chips per wound of each age, 192 chips total each tree.

[b]Summer wounds: inflicted on 17, 24, and 31 July and 7 August; four trees harvested 14 August; four trees, each 12 wounds, 36 chips per wound of each age, 144 chips total each tree.

[c]Autumn wounds: inflicted on 19 October and 2, 9, and 16 November; harvested 24 November; beech, birch, and oak received three wounds on each date; 36 chips per wound, 144 chips each tree.

TABLE 2. Microorganisms isolated most frequently from wood adjacent to 5-month-old wounds inflicted in winter on red maple, paper birch, American beech, and red oak[a]

Microorganism[b]	Chips that yielded microorganisms (%) from:			
	Red maple	Paper birch	American beech	Red oak
Bacteria	100	93	94	89
Phialophora spp.	0	8	1	0
Cytospora spp.	0.5	4	0	27
Fusarium sp.	18	0.8	0	8
Not identified	5	9	7	0
No microorganism	0	7	6	11

[a]Wounds inflicted on 13, 20, and 27 January, and 3 February; all harvested 10 July. From the paper birch, 240 chips from 20 wounds were taken and the other trees each yielded 192 chips from 16 wounds. Total chips are given for all wounding periods because after 5 months there were no differences among the four wounding periods in winter.

[b]Other microorganisms that were isolated from a few chips were *Graphium* spp., *Pyrenochaeta* sp., *Cephalosporium* sp., and *Trichoderma* sp. No Hymenomycetes were isolated.

TABLE 3. Microorganisms isolated most frequently from wood adjacent to wounds inflicted on red maple trees on 18 February and harvested 5.5 to 17 months later

	Chips that yielded each microorganism (%) from tree No.:				
	1	2	3	4	5
	Wound age, months				
Microorganism	5.5[a]	6	16	16	17
Bacteria	93[b]	90	67	60	42
Phialophora spp.	66	26	46	32	27
Cytospora spp.	1	0.4	4	4	7
Fusarium sp.	25	26	4	0.10	0
Gliocladium sp.	5	2	3	0	0
Trichoderma sp.	2	0	2	0	11
Ceratocystis spp.	0	0	0	0	0
Graphium spp.	0.3	0	0	0	0
Mucor spp.	0	8	2	2	6
Actinomycetes	0.3	8	0	2	4
Not identified	2	7	6	2	0
Hymenomycetes	0.7	23	10	8	28
	(2)[c]	(11)	(4)	(7)	(6)
No microorganism	2	0	11	0	5

[a]The 5.5-month-old wound was drilled; all others were made with a chisel.

[b]Number of wounds and chips respectively from each tree were: 1, 24-288; 2, 20-240; 3, 21-252; 4, 17-204; 5, 9-108.

[c]Number in parentheses indicates number of wounds that yielded Hymenomycetes.

TABLE 4. Microorganisms isolated most frequently from wood adjacent to 7-, 8-, 9-, and 10-week-old wounds inflicted during summer on red maple, paper birch, American beech, and red oak[a]

	Chips that yielded microorganisms (%) at wound age (weeks):															
	Red maple				Paper birch				American beech				Red oak			
Microorganism	7	8	9	10	7	8	9	10	7	8	9	10	7	8	9	10
Bacteria	69	81	94	53	78	81	86	83	72	53	69	47	100	100	100	100
Phialophora spp.	31	11	11	8	19	17	17	17	11	0	14	22	0	0	0	0
Ceratocystis spp.	19	17	44	22	42	42	39	50	44	19	22	11	75	100	83	89
Graphium spp.	28	47	58	28	25	33	47	36	19	19	3	0	61	36	14	31
Cytospora spp.	28	6	3	25	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium sp.	0	17	3	8	0	0	0	0	0	6	3	3	0	17	0	0
Hymenomycete	11	0	0	8	0	3	3	0	6	14	8	0	0	0	3	3
No microorganism	8	14	0	17	19	14	6	11	14	33	22	36	0	0	0	0

[a]Each tree received three wounds on 17, 24, and 31 July, and 7 August 1972; 12 wounds per tree; all trees harvested 26 October 1972; 36 isolation chips from around each wound.

5.5- and 6-month-old wounds. *Phialophora* spp. and *Fusarium* spp. were isolated frequently from the 5.5- and 6-month-old wounds. No chips yielded *Ceratocystis* spp. This suggests that *Ceratocystis* spp. require fresh wounds that have not been infected by other microorganisms. Hymenomycetes were isolated from some wounds on all trees (Table 3).

One maple, birch, beech, and oak tree each received three wounds on 17, 24, 31 July, and 7 August; and the trees were harvested on 26 October (Table 4). The wounds were 7, 8, 9, and 10 weeks old. Bacteria, *Ceratocystis* spp., and *Graphium* sp. were isolated with high frequency from all wounds. Two wounds on maple, birch, and oak, and three wounds on beech yielded Hymenomycetes. The four other trees that were wounded on 17, 24, 31 July, and 7 August, but were harvested on 14 August (Table 1), did not yield Hymenomycetes. These data show that Hymenomycetes were isolated from summer wounds after 7 weeks (Table 4), but not from winter wounds until after 5 months (Tables 2 and 3). These results suggest that, as wounds are inflicted from winter to summer, the time that it takes for Hymenomycetes to infect the wound decreases.

A longer-term experiment with red maple was begun with wounds made on 11 March. Trees were harvested over a 26-month period (Table 5). Bacteria and *Phialophora* spp. were the first microorganisms isolated from the youngest wounds. After 5 months, Hymenomycetes were isolated from wounds on all except three trees that had 9-, 12-, and 23-month-old wounds. Results from other experiments showed that *Ceratocystis* spp. were isolated frequently from recently inflicted summer wounds (Table 4). These results indicate that *Ceratocystis* spp. infect recently inflicted wounds not infected by other microorganisms, while Hymenomycetes infect wounds that are infected by other microorganisms.

The Hymenomycetes that were isolated came from relatively close to the wound and 1-5 cm above or below. These tissues were injured severely by the types of wounds inflicted.

These results agree with those of Sproston and Scott (8), who isolated *Cytospora* sp. (*Valsa leucostomoides* Pk.) frequently in March from sugar maple tapping wounds. Also, Houston (4) isolated *Graphium* spp. frequently from wounds that were inflicted during summer on *Acer rubrum* and *Betula alleghaniensis* Brit.

Hepting and Shigo (3) showed that decay associated with fire wounds on oaks in the South developed much more rapidly than on oaks with similar wounds in the North. The warmer temperatures in the South would not only favor the growth of microorganisms over a longer period, but the temperatures would also favor the activities of insects over a longer period. The insects could be important vectors for certain fungi, especially those with moist spores, such as *Ceratocystis* spp. It is well known that *Ceratocystis* spp. are disseminated by insects. This may be why so many summer wounds were infected with *Ceratocystis* spp. in these studies.

Emphasis in these studies was on the identification and time of detection of microorganisms isolated from fresh wounds and not how they got there. For example, it is possible that temperature was an important factor because it affected the activity of insects that, in turn, acted as vectors. Also, it is possible that some of the microorganisms isolated from the recently injured tissues were already present in small compartments of defects in the tree, as described by Shortle (7) and Etheridge et al. (1). But, regardless of how these microorganisms got there, there were differences in the species and frequencies of isolations at different times of the year. These results suggest that the events that follow wounding at different times of the year in the northeastern United States will be

TABLE 5. Microorganisms isolated from 96 wounds inflicted on 17 red maple trees in March and harvested 1.5 to 26 months later

Microorganism[a]	Chips that yielded microorganisms (%) from tree No.:																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Wound age, months:																
	1.5	1.5	2	2.5	4	4.5	5.5	5.5	9	12	15	16	21	21	23	25	26
Bacteria	99	86	97	100	99	51	64	74	93	97	37	68	97	62	100	94	100
Phialophora spp.	6	8	29	20	46	11	40	40	48	17	17	13	38	10	79	51	47
Cytospora spp.			14	9			3		2	10				6	1		
Ceratocystis spp.					4				1								
Fusarium spp.					1	3	3	11	4	33			28	10	3	8	
Gliocladium sp.					3	4	4							3	3		
Mucor sp.						54	11	8									
Hypoxylon spp.											3		8				
Slime molds						3	7			88			1	4			
Actinomycetes								1	1	7	2		6	5		14	18
Hymenomycete							19	10			40	33	6	56		4	11
							(2)[b]	(2)			(5)	(3)	(2)	(5)		(1)	(2)
Not identified		3	9		6		6	7	8	7					21	4	
Nematodes						14	8					10					
No microorganism	1	13					1		1		19			7	17		
No. chips from all wounds[c]	72	72	126	108	78	72	72	72	84	60	144	60	144	144	72	72	72

[a]Other microorganisms isolated from a few chips were *Graphium* sp., *Pyrenochaeta* sp., *Mortierella* spp., *Penicillium* spp., and *Cephalosporium* sp.

[b]Numbers in parentheses indicate number of wounds that yielded Hymenomycetes.

[c]Six wounds per tree, except three wounds on tree 2 and five wounds on trees 4 and 10.

different because of the different numbers and species of microorganisms that will be interacting with the tree.

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