Early Sugar Maple Stem Discoloration and Microorganism Invasion in Simulated Wounds of Felling and Fire Scars

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ABSTRACT

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A total of 427 stem wounds were inflicted on 172 *Acer saccharum* trees, the majority by means of drawknives inserted at varying depths to simulate blazes and mild-to-severe felling scars. Heat applied from a propane torch simulated fire wounds. The wounds were of two basic sizes, and were inflicted in spring, summer, and autumn. The nature and rate of development of discolored wood that appeared beneath the wounds varied with the age, type, size, and severity of the wound, and the season in which they were inflicted. The most obvious histological features of discolored wood were assessed. The wound discoloration frequently was separated from clear wood by a pronounced dark greenish-brown zone. The most intense discoloration generally was associated with the most severe wound types. Few Hymenomycetes were isolated from the discolored wood in the 8 yr following wounding; however, non-hymenomycetes were frequently isolated, particularly *Phomopsis* sp. B and *Alternaria tenuis*. The identity and frequency of isolation of microorganisms varied widely with wound type, size, and severity, the time since infliction, and the season of infliction. All of these factors may be relevant in assessing potential quality reductions in sugar maple crop and amenity trees by the subsequent activity of decay-causing Hymenomycetes.

Recent investigations of the development of stem decay in second-growth sugar maple (*Acer saccharum* Marsh.) in Ontario suggest stem wounds as the major infection court (1). The most obvious types of stem wounds are vertical bands of dead tissue (with or without bark) caused primarily by the impact of neighboring trees falling from natural causes or as a result of felling and skidding operations (11). Occasionally these are caused by sunscald. In some locations stem wounds caused by fire also are common, but these wounds are more irregular in shape.

To initiate successful infections, Hymenomycetes usually responsible for stem decay must become established in a wood substrate which often has been considerably altered biochemically from that present before wounding. Furthermore, this tissue frequently is invaded soon after wounding by numerous non-hymenomycetous fungi and bacteria (8). Hymenomycetes must adapt to this substrate and interact successfully with the preliminary microfloral populations to develop and cause decay in the central stem regions. The nature of the early changes in tissue beneath fresh stem wounds and in the microfloral populations within that wood could therefore have a marked effect on the subsequent development of stem decay.

Many recent reports on the response to wounds in stem wood of living hardwood trees have been based on wounds made by boring holes or hammering chisels 1 cm or more into the sapwood (4, 5, 6, 7, 9, 10, 12). Although the results are useful to assess basic tissue changes and microorganism invasions in traumatically affected sapwood, their practical significance is tempered in that most natural stem wounds originate in an entirely different manner.

This paper reports visual and histological observations of the nature and development of wood discoloration associated with fresh wounds of different sizes and severity, made at different seasons of the year, to simulate felling wounds, blazes, and fire scars. The identity of microfloral populations isolated from discolored wood associated with the various types of wound, and at different intervals following wound infliction, is given.

MATERIALS AND METHODS

Four series of stem wounds were inflicted on healthy, second-growth sugar maple trees that had no external evidence of previous wounding. The trees averaged 57 years of age, and 15 cm in diameter 1.4 m above ground level. Each series was carried out in at least two of three sugar maple stands, all in the same region of south-central Ontario, but each on somewhat different soil moisture regimes and physiographic sites.

In the major experiment, series A, 306 wounds were inflicted on 102 trees to simulate felling wounds. One-third of the trees (34 trees) were wounded on each of three dates, viz., early May, early August, and early November. Three wounds were made on each tree by carefully removing the bark with drawknives and exposing the xylem in vertical bands. A single large wound about 5 cm wide was made at a height of roughly 114 to 160 cm on

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either the north or south aspect (Fig. 1), and two similar but smaller wounds roughly 2.5 cm wide were made on the opposite side of the tree at heights of 68 to 84 cm and 190 to 206 cm.

All wounds in the three other experiments (series B, C, and D) were inflicted in midsummer. In series B, 18 trees received the same-sized wounds as the trees in series A, and at similar locations on the stem, except that the lower small wound was omitted. The wounds were also inflicted with drawknives, but only the outermost bark was removed, and the inner bark was left intact over the cambium. This was done to simulate more nearly the large number of natural felling scars which appeared to originate as superficial bark scrapes or bruises rather than from complete bark removal. Because of the wide variation in bark thickness between the experimental trees, the depth to which bark was removed also varied widely.

Series C consisted of 33 sugar maples wounded with drawknives from heights of 68 to 114 cm on either the

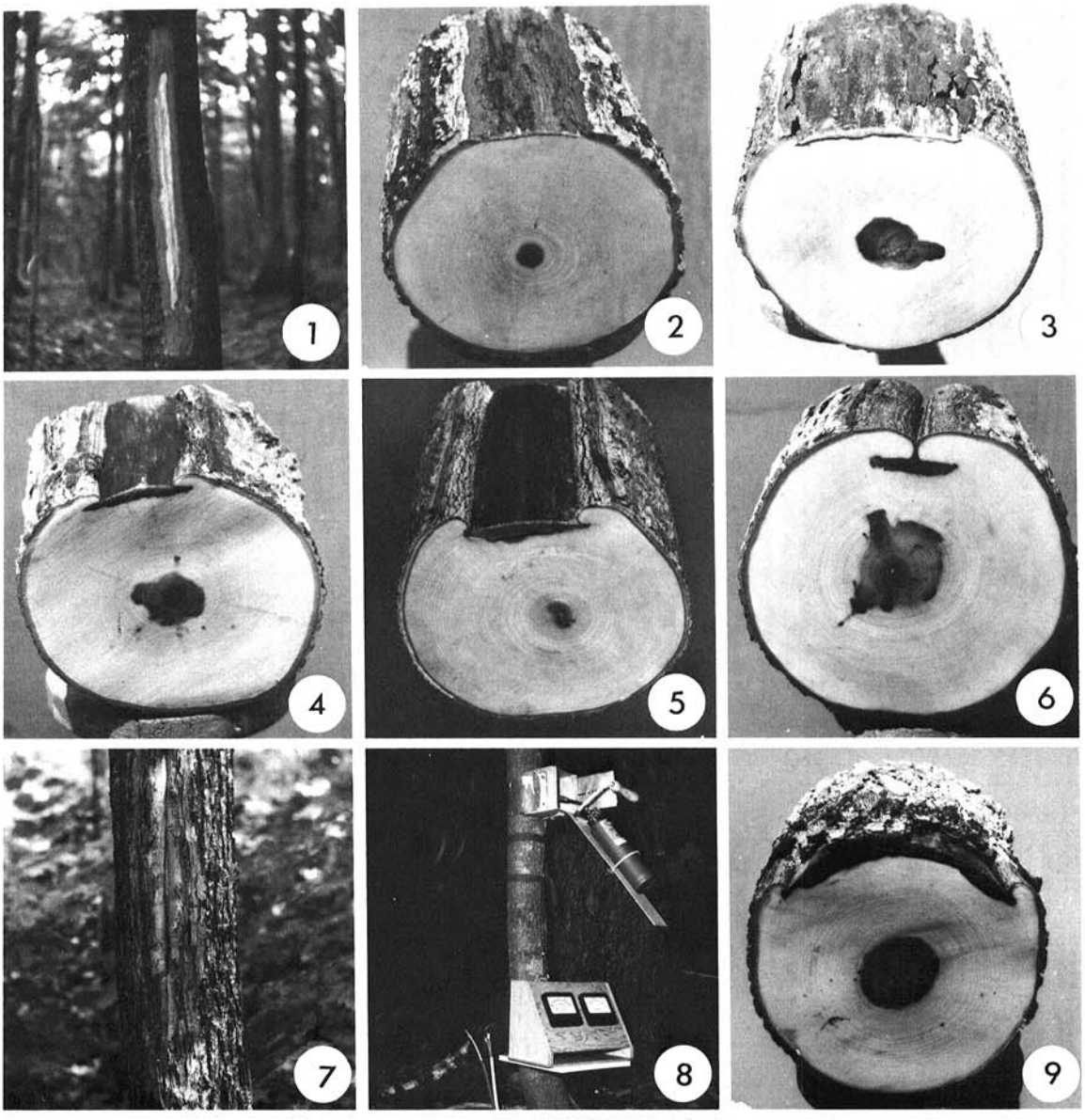

Fig. 1–9. Stem wounds and associated wood discoloration in sugar maple. **1)** Freshly made drawknife wound in which all of the bark was removed. 2-6) Cross sections of stems showing discoloration with drawknife wounds that had been inflicted for: **2)** 3 mo, **3)** 1 yr, **4)** 3 yr, **5)** 5 yr—which shows clearly the outermost band of relatively pale discoloration and the innermost very dark demarcation zone—and **6)** 7 yr. **7)** Seven-yr-old drawknife would almost completely healed by callus growth. **8)** Application of heat by propane torch to simulate fire wound. **9)** Cross-section of stem showing discoloration associated with heat wound inflicted 9 mo earlier.

south or north aspect, and from 144 to 190 cm on the opposite aspect. The 33 trees were divided into 11 sets of three trees. The six wounds in each set consisted of two with only the outer bark removed as in series B, two with all of the bark removed as in series A, and two with the phloem plus the outermost 3 or 4 mm of xylem tissue scraped off to simulate very severe felling scars and man-made blaze wounds. All wounds were approximately 5 cm in width, and the two wounds on a single tree were always of two different types. Within each set of three trees, each pair of wounds of similar type included an 'upper' and a 'lower' wound.

The fourth series (series D) consisted of 19 trees wounded once at a height of 1.2 m by the application of heat to a patch of bark, 17.5 cm high × 14 cm wide, to simulate fire wounds. A slightly curved copper plate of the same dimensions as the bark patch and approximately 6 mm thick was wired in position on the stems of the study trees. A propane torch was suspended on a bracket so that its flame played evenly on the plate for the period of time required to cause cambial death (Fig. 8). This time was determined from trials on other, nonsample trees by inserting needle-probe thermocouples in surface xylem tissue. The time required for cambial death generally depended on bark thickness and weather conditions, and was usually between 10 and 12 min.

The 172 wounded trees in the four series of experiments were selected at random and felled from 5 wk to 8 yr following wounding. Forty-one (almost 25%) were felled within 1 yr and 91 (over 50%) were felled within 3 yr of wounding. Each wound was carefully dissected and examined in the field laboratory, where the color and extent of stained wood were recorded. Radial sections of discolored tissue, including the cambium and some clear wood adjacent to the stain, were examined microscopically. From each of the large 46-cm wounds, four isolation attempts were made from tissue 2 to 3 mm within the cambium at predetermined locations, by aseptically removing the outermost millimeter or so with a flamed scalpel and inserting chips of wood roughly 2×2×6 mm, obtained from the newly exposed tissue, into test tubes containing 2% malt agar. Two similar isolation attempts were made from each of the small 16-cm wounds, and four to six attempts were made from each heat wound. From each wound, up to six additional isolation attempts were made at a depth of 5 mm, on the basis of whether or not stain was present at predetermined points at that depth. As many as eight additional attempts were made at a depth of 10 mm on the same basis. The test tubes were stored in the dark at room temperature and were examined at intervals of 2, 4, and 8 wk. When microorganisms were observed growing from the chips, the tubes were transferred to a cold room until it was convenient to initiate identification procedures. Chips from which no microorganisms could be detected after 8 wk at room temperature were considered sterile.

Nine sugar maple trees with no obvious stem wounds, selected in the three stands as being representative of the sample trees in size and vigor, were felled, dissected, and treated similarly to the wounded trees. The wood beneath the bark at locations in imaginary wounds where discoloration developed in the wounded trees, was examined microscopically and subjected to the same pattern of microorganism isolation attempts.

RESULTS

The nature and rate of development of stem discoloration.—Most of the trees felled and dissected within 6 mo of wound infliction possessed 1 to 2 mm of light- to medium-brown discolored wood beneath the cambium over the entire wounded region, limited by the dimensions of the wound (Fig. 2). This discoloration reached an average radial depth of approximately 2.25 mm in the next 6 mo, with the added stain generally a darker color. In many trees examined 1 yr or more after wounding, discolored tissue was separated from clear wood by a pronounced dark brown-green demarcation zone 1 to 2 mm wide (Fig. 3).

Three yr after wounding, the average depth of discoloration had increased to ≈5 mm (Fig. 4). It was difficult to judge the depth of this particular discoloration in the majority of wounds examined 4 yr or more after infliction because of the occurrence of an adjoining light- to medium-brown stain that extended radially from much or all of the wound area, tapering to merge with the inner core stain surrounding the stem pith. This wedge-shaped stain was quite different in color, histology, and microflora from the initial wound discoloration discussed in this paper, and will be the subject of a separate paper. In the few wounds examined that were 6 to 8 yr old and had not developed any wedge-shaped stain, the preliminary wound discoloration averaged 8 mm in depth, and ranged from 6 to 12 mm among wounds (Fig. 5).

Stem discoloration never extended into wood or callus formed after the wound was inflicted, or tangentially beyond the original wound borders. Vertical extensions of the discoloration beyond the wound limits were observed at about 18 mo, but these seldom extended more than 3 cm and were generally dark-green, tapered projections. The wounds were gradually covered by callus tissue (Fig. 6, 7), which developed mainly horizontally to close the wounds at an average rate of about 5 mm annually. As the wounds became covered by callus, the lateral edges of the discoloration darkened considerably to a very dark, red-green-black color. In the small minority of wounds which, when sampled, were completely healed by callus without the development of wedge-shaped stain to the stem core, the enclosed discoloration was extremely dark, almost black in some cases (Fig. 6). No discoloration of any kind was detected in the wood beneath the bark of the nine control trees that had not been wounded.

With the exception of wound height and aspect, variations in the wounding procedures altered appreciably the development of stem discoloration. The simulated fire wounds examined 5 wk after infliction were discolored to a depth of 5 to 8 mm; however, wounds examined thereafter showed little increase in stain depth. These stains were a relatively dark brown, generally with pronounced dark-green demarcation zones (Fig. 9). Similar demarcation zones were common in discolored wood associated with wounds where the inner bark was left intact; however, unlike the heat wounds, these wounds were associated with the slowest development of discoloration of all wound types, the average depth being only 2 mm and 3 mm 2 and 3 yr, respectively, after infliction. Wounds inflicted by the removal of both phloem and the outermost xylem tissue were associated with a

relatively rapid development of discoloration that averaged 6.75 mm in depth 3 yr after infliction. In these wounds, the inner demarcation zone was much less pronounced, and appeared to take longer to develop.

Wounds inflicted by removing all of the bark in early August had stain that averaged 1 mm in depth after 1 to 3 mo, 2.5 mm after 1 yr, 4 mm after 2 yr, and 5 mm after 3 yr. Similar wounds inflicted in early May had a noticeably slower development of discoloration that averaged only 3.75 mm at 3 yr. The early November wounds had less than 0.5 mm of stain when snow conditions first permitted access to them 4 mo after infliction; however, at 2 yr their discoloration had surpassed that of the August wounds, with an average depth of 6 mm. Regardless of season of infliction, the smaller (2.5 × 16 cm) wounds were associated with a consistently slower rate of development of stem discoloration than the larger (5 × 46 cm) wounds. This difference amounted to 1.5 to 2 mm in radial depth for wounds 3 yr old and older.

Histology.—Fresh, freehand radial sections of discolored stem wood, obtained from roughly the center of each wound, were examined microscopically. No chemical fixation or dehydration techniques were applied to the sections. Similarly located sections obtained from the nine nonwounded control trees showed that all cells were devoid of colored content. Sections prepared from wounds that had been inflicted only 1 mo earlier ranged in appearance from those with no discolored cells to those with yellow or amber masses in roughly 10 percent of the ray parenchyma cells in wood adjacent to the cambium, and scattered amber to dark amber granular vessel occlusions in the innermost discolored and adjacent clear wood. With longer intervals since wound infliction, and with increasing distance from the cambium within discolored tissue, the proportion of discolored to clear ray cells increased and the color of the ray cell deposits became darker and more pronounced, frequently dark amber or dark brown. However, in most relatively old wounds the outermost 1 mm or so still revealed only a small percentage of the ray cells discolored, and these were mostly pale amber or yellow. In discolored wood associated with wounds older than 2 or 3 yr, generally all ray cells inside this outer zone were discolored, often with very dark amber or occasionally reddish-brown deposits. Amber to dark-amber granular vessel occlusions occurred in some wounds. The dark brown-green demarcation zone usually possessed ray cells with dark reddish-brown cell contents, plus numerous dark amber vessel occlusions, both superimposed on a diffuse pale yellowish-green pigmentation. In clear wood adjacent to the discolored tissue all ray cells invariably were devoid of any colored content. However, dark amber to brown granular vessel occlusions and smaller brown vessel plugs were usually present for 1 mm or more within such tissue.

Dilute neutral red chloride was used as a vital stain on additional radial sections, prepared within 2 hr of tree felling. Ray cells were regarded as alive if they accumulated the stain and could be plasmolyzed and deplasmolyzed. No living cells were ever detected in discolored wood; however, they generally were found in the adjacent clear wood and often were in abundance very close to the stain border.

Variations in these histological characteristics generally reflected the variations in wood discoloration pattern associated with different wounding procedures. Thus, the demarcation zones in heat-induced wounds, and in drawknife wounds in which only the outer bark was removed, appeared as particularly vivid, bright, yellowish-green. The outermost 1 to 2 mm of discolored wood associated with complete bark removal in early November revealed very few stained (pale amber) ray parenchyma cells even in wounds 7 or 8 yr old. Similar wounds inflicted in early August had a high proportion of dark amber ray cells in this zone, often in wounds only 1 yr old. Wounds with phloem plus outer xylem removed had many ray parenchyma cells with reddish-amber deposits and numerous amber vessel occlusions in the outermost 1 to 2 mm of discolored wood, a unique feature of this wound type. Finally, the dark amber vessel occlusions observed in clear wood adjacent to the discolored tissue were most common, and occurred over a greater radial distance, in association with the wounds made by removing only the outer bark and leaving the inner bark intact.

Microorganism invasion.—A total of 360 isolation attempts were made from clear wood beneath the bark of the nine nonwounded control trees, at locations identical to those where similar attempts were made from discolored wood in the wounded trees. Six of these attempts yielded bacteria, one yielded *Phoma* sp., and the remaining 353, or 98.1%, yielded no microorganisms and therefore were considered to be sterile.

Table 1 shows the frequency with which various microorganisms were isolated, at different time intervals after wounding, from discolored wood associated with the 306 wounds of the major experiment, series A, in which the bark was removed, exposing the xylem. The two most frequently isolated microorganisms were the nonhymenomycetes *Phomopsis* sp. B and *Alternaria tenuis* Nees, which totally dominated the microflora associated with wounds inflicted within 3 yr of the time of sampling. Only two Hymenomycetes were isolated from this substrate, *Corticium laeve* Pers. ex Fr., which was fairly common but was not isolated from wounds inflicted less than 1 yr earlier, and *Peniophora cinerea* (Pers. ex Fr.) Cke., which was isolated only once, from an 8-yr-old wound. A few isolation attempts yielded sterile cultures in the discolored wood of recent wounds, mostly those inflicted in early November. However, the majority of sterile cultures from older wounds were from the dark green vertical projections beyond the wound limits, or from the brown-green demarcation zones. Practically all of the more common organisms showed either a positive or negative relationship with wound age, resulting in decidedly different microfloral populations at different periods after wound infliction (Table 1).

The association of the most frequently isolated of these microorganisms with wounds inflicted during different seasons is shown in Table 2. All of the microorganisms with the exception of *A. tenuis*, bacteria, and *Coniothyrium* sp., showed variation in frequency of occurrence with season of wounding. The five most common microorganisms, in order of frequency of occurrence, inhabiting the discolored wood associated with May wounds were *Phomopsis* sp. B, *A. tenuis*, *Phoma* spp., bacteria, and *C. laeve*; from August wounds *Phomopsis* sp. B, *A. tenuis*, *Phoma* spp.,

Cephalosporium spp., and *Libertella* spp.; and from November wounds *C. laeve, A. tenuis*, bacteria, *Phomopsis* sp. A, and *Phoma* spp. The most striking seasonal influences were displayed by *Phomopsis* sp. B which was isolated 171 times from the 102 August wounds, 139 times from the 102 May wounds, but only 35 times from the 102 November wounds; and by *C. laeve* which occurred 109 times in the November wounds, 39 times in the May wounds, but only five times in the August wounds.

Four microorganisms were markedly influenced by wound size. *Corticium laeve* and *Phomopsis* sp. A were from two to three times more abundant, and *Nodulisporium* sp. 5 was ten times more abundant, in the large 5 × 46 cm wounds than in the small 2.5 × 16 cm wounds, whereas *Libertella* sp. was almost four times more abundant in the smaller wounds. Sterile isolation attempts were almost four times more frequent, on a percentage basis, from the smaller than the larger wounds.

Whereas Tables 1 and 2 concern microorganisms isolated from series A wounds, Table 3 shows the frequency with which various microorganisms were isolated from discolored wood associated with wounds of the remaining series, B, C, and D. These series included four different types of wounds, all inflicted during midsummer. The first two columns represent the series B wounds and one-third of the series C wounds, both inflicted in the same manner; ie, by removing the outer bark while leaving the inner bark intact. For most microorganisms the results were similar; however, the relatively long delay (15 mo) before circumstances permitted sampling the series B wounds following their infliction very likely accounted for appreciable differences obtained for a few fungi such as *Phomopsis* sp. B and *C. laeve*. Both series within this wound type were frequently invaded by the two Hymenomycetes, *P. cinerea* and *C. laeve*, whereas they were relatively rare in the other three types of wound. On the other hand, *A. tenuis* and *Phomopsis* sp. A seldom were isolated from those wounds in which the inner bark had been left intact, but were fairly common in all of the other wound types as well as in series A wounds.

The microfloral populations obtained from discolored wood associated with wounds involving complete bark removal in series C were similar to those of the series A wounds; this was not surprising since the latter wounds also involved complete bark removal. However, where outer xylem tissue was excised in addition to the phloem in series C, *Phomopsis* sp. A was exceptionally common in discolored wood whereas *C. laeve* was relatively rare.

The discolored wood associated with heat-induced wounds revealed an almost completely different microflora from any of the foregoing wound types. Only *Phialophora* spp. and bacteria occurred in more than 8% of the isolation attempts, 16.5 and 15.1%, respectively. Only one isolation of *Phomopsis* sp. B was obtained, only two of *Phoma* spp., and none of *C. laeve*. Furthermore, six fungi were isolated from heat-induced wounds that never were associated with the three different drawknife wound types (Table 3).

TABLE 1. Microorganisms isolated from discolored sugar maple wood associated with 306 wounds at varying time intervals after infliction. Wounds made, by complete bark removal, on 102 trees (series A)

	Yield of isolation attempts, %[a]						
	Interval since wound infliction, years[b]						
Microorganism	<.4[c]	0.5-1.5	1.6-2.5	2.6-3.5	3.6-6.0	6.1-8.0	All
Phomopsis sp. B	56.3	43.7	33.7	21.3	29.6	9.7	28.3
Alternaria tenuis Nees	69.8	52.3	32.6	21.8	15.2	4.3	26.1
Phoma spp.	28.1	24.7	18.6	16.4	10.7	11.6	16.5
Bacteria	1.0	7.5	4.5	5.8	14.1	32.5	13.4
Corticum laeve Pers. ex Fr.	0	1.7	10.7	24.0	17.8	10.5	12.5
Phomopsis sp. A	0	17.2	14.0	15.1	5.9	4.0	9.5
Cephalosporium spp.	22.9	17.2	6.7	2.2	4.4	7.9	8.4
Coniothyrium sp.	0	4.0	8.4	5.8	8.5	7.9	6.6
Fusarium spp.	14.6	10.3	5.1	4.4	7.0	1.4	6.1
Libertella spp.	0	0	2.2	0	10.7	8.7	4.7
Epicoccum nigrum Lk. ex Fr.	15.6	10.3	5.1	3.1	2.6	0	4.6
Gliocladium roseum (Lk.) Thom.	4.2	8.0	2.2	2.2	5.9	2.5	4.1
Phialophora spp.	1.0	0.6	4.5	6.7	2.2	6.1	3.9
Actinomycetes	0	0	0	0.4	1.9	5.4	1.7
Nodulisporium sp. 5	0	1.1	1.7	2.7	0.7	2.5	1.6
Verticillium spp.	1.0	2.9	1.1	1.3	0.4	2.2	1.5
Cladosporium herbarum Lk. ex Fr.	1.0	1.7	3.4	0.4	1.1	1.1	1.4
Candida sp.	3.1	1.7	2.8	0	1.5	0	1.2
Cytospora spp.	0	0.6	0	0.4	1.1	0	0.4
Peniophora cinerea (Pers.) Cke.	0	0	0	0	0	0.4	0.1
Not identified	4.2	10.9	20.2	8.4	12.2	17.0	12.9
None, sterile	6.2	3.4	3.4	12.0	4.4	14.4	8.0

[a]The majority of isolation attempts yielded more than one microorganism.

[b]<0.4 yr, 96 attempts made from 36 wounds on 12 trees. 0.5-1.5, 174 attempts made from 60 wounds on 20 trees. 1.6-2.5, 178 attempts made from 54 wounds on 18 trees. 2.6-3.5, 225 attempts made from 48 wounds on 16 trees. 3.6-6.0, 270 attempts made from 60 wounds on 20 trees. 6.1-8.0, 277 attempts made from 48 wounds on 16 trees.

[c]The sampling interval <0.4 yr includes wounds sampled between 1 and 4 mo after infliction.

TABLE 2. Microorganisms isolated most frequently from discolored wood associated with 306 wounds inflicted during early May, early August, or early November on 102 sugar maple trees by complete bark removal (series A)

Microorganism	Yield of isolation attempts, %[a]		
	Season of wound infliction		
	Early May	Early August	Early November
Phomopsis sp. B	40.2	40.7	7.7
Alternaria tenuis Nees	29.2	27.1	22.7
Phoma spp.	17.6	22.4	10.1
Bacteria	13.6	12.6	13.9
Corticium laeve Pers. ex Fr.	11.3	1.2	24.0
Phomopsis sp. A	3.2	7.4	16.3
Cephalosporium spp.	2.3	14.5	7.5
Coniothyrium sp.	8.1	5.2	6.6
Fusarium spp.	4.6	9.3	4.2
Libertella spp.	0.3	12.6	0.7
Epicoccum nigrum Lk. ex Fr.	5.2	0.7	7.7
Gliocladium roseum (Lk.) Thom.	3.8	6.9	1.8
Phialophora spp.	2.6	0.5	8.1
Actinomycetes	3.5	1.2	0.9
Nodulisporium sp. 5	0	0.7	3.7
Not identified	15.3	6.9	16.7
None, sterile	5.5	8.1	9.7

[a]The majority of isolation attempts yielded more than one microorganism.

TABLE 3. Microorganisms isolated from discolored wood associated with four different types of stem wound inflicted during midsummer on sugar maple trees (series B, C, D)[a]

Microorganism	Yield of isolation attempts, %[b]					
	Removal of outer bark		Removal of all bark,	Removal of bark & outer xylem,	Heat,	Total no. of times isolated
	B[c]	C[d]	C[e]	C[f]	D[g]	
Phomopsis sp. B	16.3	52.2	58.0	42.7	0.7	206
Bacteria	22.2	18.6	14.3	15.3	15.1	112
Phomopsis sp. A	2.6	7.1	8.9	36.7	7.2	80
Peniophora cinerea (Pers.) Cke.	25.5	26.5	0.9	3.1	2.9	78
Corticium laeve Pers. ex Fr.	30.1	9.7	5.9	2.3	0	67
Alternaria tenuis Nees	1.3	2.7	25.9	20.6	4.3	66
Phoma spp.	17.6	9.7	10.7	8.4	1.4	63
Cephalosporium spp.	16.3	5.3	14.3	3.1	5.8	61
Coniothyrium sp.	1.3	1.8	13.4	6.1	3.6	32
Fusarium spp.	7.2	0.9	0.9	1.5	7.9	28
Phialophora spp.	2.6	0	0	0.8	16.5	26
Cytospora spp.	0.7	5.3	3.6	3.1	0	15
Candida sp.	1.3	1.8	0	3.8	0	9
Verticillium spp.	2.0	2.7	0.9	0	1.4	9
Gliocladium roseum (Lk.) Thom.	0.7	2.7	1.8	0	2.2	9
Epicoccum nigrum Lk. ex Fr.	0	0	0.9	1.5	4.3	9
Cylindrocarpon sp.	0	0	0	0	5.8	8
Diplodia sp.	0	0	0	0	5.0	7
Ceratocystis sp.	0	0	0	0	3.6	5
Phomopsis sp. 1	0	0	0	0	3.6	5
Pestalotia sp.	0	0	0	0	2.2	3
Hendersonia sp. 1	0	0	0	0	2.2	3
Not identified	12.4	18.6	13.4	13.7	13.7	92
None, sterile	11.8	0.9	0	1.5	9.4	38

[a]See text for full description of wound types.
[b]The majority of isolation attempts yielded more than one microorganism.
[c]Based on 36 wounds inflicted on 18 trees, from which 153 isolation attempts were made.
[d]Based on 22 wounds inflicted on 22 trees, from which 113 isolation attempts were made.
[e]Based on 22 wounds inflicted on 22 trees, from which 112 isolation attempts were made.
[f]Based on 22 wounds inflicted on 22 trees, from which 131 isolation attempts were made.
[g]Based on 19 wounds inflicted on 19 trees, from which 139 isolation attempts were made.

DISCUSSION

The initial rate of development and appearance of discolored stem tissue adjacent to fresh wounds on sugar maple was influenced by several factors, including the nature and severity of the wound, wound size, age, and the season of infliction. The most rapid initial rates of development of discoloration generally were associated with the more severe wound types, those that presumably caused the most rapid and extensive cell mortality in the underlying stem tissue. Hence heat wounds caused extensive cell mortality and discoloration at the time of treatment, and severe drawknife wounds, in which xylem as well as phloem was excised, also were associated with a relatively rapid development of discoloration. The least rapid initial development of discoloration was encountered in drawknife wounds in which only the outer bark was removed, and in similar wounds in which all of the bark was removed in early November when low temperatures very likely reduced the rate of cell mortality.

Wardell and Hart (12) and Shigo (9) showed that late autumn and winter wounding of deciduous tree stems resulted in a slower initial development of stem discoloration than did spring or summer wounding. The results from the present work confirm this, and because of the unusually long experimental period (up to 8 yr), they also shed light on subsequent discoloration development. Observations of wounds more than 2 yr old suggested that, despite the slower initial rate of stain development, late autumn wounds might eventually be more detrimental to tree quality than spring wounds. The spring wounds were associated with a much more rapid development of callus tissue, which after 2 yr appeared to reduce appreciably the rate of spread of discoloration. Discoloration did not appear to increase after the wounds were completely healed over. Apparently, once desiccation and aeration of the wood was stopped, the development of discoloration was inhibited, an idea already expressed by Houston (3).

The Hymenomycetes responsible for most of the decay in mature sugar maple were not isolated from the discolored tissue adjacent to stem wounds. The only two Hymenomycetes consistently isolated, *P. cinerea* and *C. laeve*, have been reported within the stems of living sugar maple, but only rarely and relatively sparsely.

In a study of fungal development in freshly cut pine bolts, Dowding (2) found that *Ceratocystis* spp., the sticky-spored fungi that commonly cause sap stain in coniferous trees and logs, were present in abundance only when bark beetle damage had occurred. The other fungi commonly found, many of which are reported herein on sugar maple, were all airborne saprophytes. Shigo (9) isolated *Ceratocystis* spp. more frequently than any other fungus from summer wounds, but never from autumn or winter wounds, inflicted on four deciduous species (not sugar maple). He attributed this largely to insect transmission at that time of year. In the present work, *Ceratocystis* spp. were isolated only five times, all from heat wounds (the only wound type that exhibited significant insect damage). Insects appeared to play a minor role, if any, in introducing microorganisms to sugar maple stems via the drawknife wounds.

The fact that the microflora inhabiting the discolored wood associated with heat-induced wounds were noticeably different from those associated with drawknife wounds (Table 3) revealed how much the nature of the wounding process influenced the microflora present in the adjoining tissue. Thus, it was not surprising that there was little similarity between the microflora reported herein and those obtained by Shigo from discolored tissue associated with holes drilled into healthy trees (9). However, the results of the present work agree with Shigo's in that the frequency of isolation and the species of microorganisms associated with stem wounds inflicted at different seasons and sampled at various intervals following infliction were different. Furthermore, the type, severity, and size of wounds also influenced appreciably the associated microfloral populations. This, coupled with the fact that the extent of xylem discoloration is also greatly affected, emphasizes the relevance of all of these factors in assessing potential quality reduction in sugar maple crop and amenity trees by the subsequent activity of decay-causing Hymenomycetes.

LITERATURE CITED

1. BASHAM, J. T., and H. W. ANDERSON. 1977. Defect development in second-growth sugar maple in Ontario. I. Microfloral infection relationships associated with dead branches. Can. J. Bot. 55:934-976.
2. DOWDING, P. 1970. Colonization of freshly bared pine sapwood surfaces by staining fungi. Trans. Br. Mycol. Soc. 55(3):399-412.
3. HOUSTON, D. R. 1970. Discoloration and decay in red maple and yellow birch; reduction through wound treatment. For. Sci. 17:402-406.
4. POTTLE, H. W., and A. L. SHIGO. 1975. Treatment of wounds on Acer rubrum with Trichoderma viride. Eur. J. For. Pathol. 5:274-279.
5. RIER, J. P., and A. L. SHIGO. 1972. Some changes in red maple, Acer rubrum, tissues within 34 days after wounding in July. Can. J. Bot. 50:1783-1784.
6. SHARON, E. M. 1973. Some histological features of Acer saccharum wood formed after wounding. Can. J. For. Res. 3:83-89.
7. SHARON, E. M., and A. L. SHIGO. 1974. A method for studying the relationship of wounding and microorganisms to the discoloration process in living trees. Can. J. For. Res. 4:146-148.
8. SHIGO, A. L. 1967. Succession of organisms in discoloration and decay of wood. Int. Rev. For. Res. 2:237-299.
9. SHIGO, A. L. 1976. Microorganisms isolated from wounds inflicted on red maple, paper birch, American beech, and red oak in winter, summer, and autumn. Phytopathology 66:559-563.
10. SUCOFF, E., H. RATSCH, and D. D. HOOK. 1967. Early development of wound-initiated discoloration in Populus tremuloides Michx. Can. J. Bot. 45:649-656.
11. VASILOFF, G. N., and J. T. BASHAM. 1963. Defect associated with trunk wounds in young second-growth sugar maple. Can. Dep. For., Bi-mon. Prog. Rep. 19, 2:1-2.
12. WARDELL, J. F., and J. H. HART. 1970. Early responses of sapwood of Quercus bicolor to mechanical injury. Can. J. Bot. 48:683-686.