FORMATION OF HEARTWOOD AND DISCOLORED SAPWOOD IN WHITE OAK AND WHITE SPRUCE

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This is to certify that the

thesis entitled

Formation of Heartwood and Discolored Sapwood in White Oak and White Spruce

presented by

John Frederick Wardell

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Botany & Plant Pathology

Gary R. Hooper
Major professor

Date July 27, 1971

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ABSTRACT

FORMATION OF HEARTWOOD AND DISCOLORED SAPWOOD IN WHITE OAK AND WHITE SPRUCE

By

John Frederick Wardell

Formation of heartwood and discolored sapwood was studied in white oak (Quercus alba L.) and white spruce (Picea glauca (Moench) Voss). An increment borer was used to induce formation of discolored sapwood.

Qualitative differences in ether and butanol-soluble compounds were detected between sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white oak and white spruce with paper chromatography. Few qualitative and quantitative differences in ether-soluble compounds were detected between DS and HW of white oak with thin-layer chromatography. Many qualitative and quantitative differences were detected between these tissues and SW. DS of white spruce was quantitatively different from SW and HW which were similar to each other.

The degree of similarity of chemical constituents between SW, HW and DS was determined for ether and butanol-soluble compounds from both tree species. Every

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compound detected in a tissue was considered as a separate character in constructing a similarity index. Calculation of similarity indexes between the 3 tissues of white oak and white spruce showed that they were different in chemical composition from each other.

Changes in levels of inorganic elements were detected during formation of DS and HW in both tree species. Potassium and P decreased in DS and HW of white spruce, but Mg and Mn decreased only in DS. Calcium and Mn were highest in HW. Few changes, however, were significantly different from levels of the same element in SW. Potassium increased in DS of white oak, but decreased in HW. Phosphorous, Mg and Mn decreased in HW while Cu, B and Al increased in DS. Calcium sometimes increased or decreased in DS and decreased in HW.

Ash content was highest in HW, intermediate in SW and lowest in DS of white spruce. Differences between tissues were not always significant. The pH of the cold water extract was similar from SW and HW and lowest for DS. Ash content was highest in DS, intermediate from SW and lowest in HW of white oak. The pH of the cold water extract was highest from DS, intermediate in SW and lowest from HW.

An electron microprobe x-ray analyzer - scanning microscope showed that changes in levels of elements occurred at different radial positions in SW and HW of

both tree species. Differences in levels of elements were detected between ray and non-ray cells. The distribution of elements in DS was different from that in SW. Phosphorous, Ca and Mg were studied in both tree species while Mn, K, Cl, S and O were also studied in white oak.

Chlorine, S, O (white oak) and Mg (white spruce) were not affected by changes in radial positions in SW or HW. Phosphorous decreased from outer to inner SW and Ca was lowest in outer SW of both tree species. Magnesium and Mn decreased between inner SW and outer HW. Potassium increased from outer to inner SW, but decreased from inner SW to outer HW. Calcium, Mg, K and Mn increased from outer to inner HW of white oak. In white oak, more P, Mg (DS, SW), Ca, S (DS, SW, HW), Mn, K and Cl (SW, HW) were detected in ray cells than in vascular elements. In white spruce, more P (SW), Ca (SW, HW) and Mg (DS) were detected in ray cells than in vascular elements.

The distribution of elements in ray and non-ray cells at different radial positions in DS of white spruce injured in spring and white oak injured in winter was not very different from that observed in SW. Phosphorous decreased between middle and inner DS while Ca was highest in inner DS of white spruce. Calcium and Mn were not changed at different radial positions in DS of white oak. More drastic changes were observed in DS of white oak injured in spring. In ray cells, more P was detected in middle

than in outer DS which had more P than inner DS. No difference in P was detected at different positions in non-ray cells. Differences in K were not detected at radial positions in DS.

In both tree species, DS was more resistant than SW (both) and heartwood (spruce) to decay by Poria monticola and Polyporous versicolor 4 months after mechanical injury when trees were wounded in late April. Ellagitannins were not responsible for the greater durability of DS of white oak to P. monticola.

Physiological condition of white oak at the time of mechanical injury affected development of DS. Increases in K and ash content and development of decay-resistant discolored sapwood were not observed until 7 months after mechanical injury when trees were wounded in December, but increases in K and ash content and development of decay-resistant discolored sapwood were observed within 4 months after mechanical injury when trees were wounded in late April.

DS and HW are distinct tissues in both tree species. Formation of DS and HW of white spruce is much different from that in white oak.

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John Frederick Wardell

A THESIS

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ACKNOWLEDGMENTS

Deep appreciation is expressed to Dr. John H. Hart for his counsel and assistance throughout the period of this investigation and during the preparation of the manuscript. Thanks are also due to Dr. James W. Hanover, Dr. Eldon A. Behr, Dr. Gary R. Hooper and Dr. H. Paul Rasmussen for their critical evaluation of the thesis.

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To my wife, for her encouragement, help and patience, I am indebted.

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INTRODUCTION

Heartwood is composed of dead cells that originate from physiological processes. The factor(s) responsible for initiating the series of events which leads to heartwood formation is unknown. Several factors have been suggested which initiate the transformation process, but none have conclusive support.

Stewart (1966) stated that the heartwood was a depository for excretions from living cells in the sapwood. The extraneous materials, toxic to these cells, were translocated to the center of the tree, accumulated to lethal concentrations and caused the death of living cells. The continued translocation of excretions resulted in outward movement of the heartwood boundary.

The isolation of fungi from heartwood of Nothofagus cunninghamii Oerst., myrtle beech, and Sloanea woollsii F. Muel., yellow carbeen, led Chattaway (1952) to believe that the primary stimulus in heartwood formation was pathological. Prior to cell death, a period of increased cell metabolism resulted in utilization of surplus starch and subsequently the formation of tyloses and gum plugs. After cell death, the breakdown of cellular membranes allowed the extractives to

escape from the cells. Changes in the air-moisture relationships within the cells solidified the extractives.

Loss of water and entry of air have been considered responsible for heartwood formation. Abnormal withdrawals of moisture reserves resulted in the inflow of atmospheric oxygen into the tissue. The death of parenchyma cells and formation of tyloses resulted from continued withdrawal of moisture and accumulation of atmospheric oxygen in vessel elements. Characteristic discolorations were caused by oxidative processes (Zycha, 1948).

Heartwood formation occurs in Fagus sylvatica, European beech, trees when their age is between 80 and 100 years. Investigators have suggested that, for this and other species of trees, inner cells of sapwood died without external stimuli when they reached a certain age (Zycha, 1948).

Mechanical injury to sapwood may result in discoloration (discolored sapwood). The damaged cells darken prematurely and resemble heartwood in color. The nature of the wounding stimulus appears immaterial (Hart, 1965). Some authors considered microorganisms were responsible for some discolorations in sapwood. More than one-fourth of the isolations from discolored sapwood of Liriodendron tulipifera yielded bacteria, but very few yielded fungi (Roth, 1950). Microorganisms,

however, are not a prerequisite for discoloration but greatly enhance the discoloration processes (Shigo, 1965; 1968).

Formation of discolored sapwood may result from the action of certain enzymes produced by wounded parenchymatous cells. The enzymes are translocated various distances and produce physiological reactions which result in local necrosis (Hart, 1965).

The purpose of this investigation was to compare heartwood with discolored sapwood in forest tree species. Phenolic compounds were used to evaluate the chemical relationship between discolored sapwood and heartwood (Part I). Changes in mineral content were studied during formation of heartwood and discolored sapwood (Part II) and the durability of heartwood and discolored sapwood to wood-decay organisms was investigated (Part III).

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LITERATURE CITED

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PART I

PART I

PHENOLIC COMPOUNDS AS CRITERIA FOR EVALUATION OF THE CHEMICAL RELATIONSHIP BETWEEN DISCOLORED SAPWOOD, HEARTWOOD AND SAPWOOD

Qualitative and quantitative chemical differences have been reported between discolored sapwood, heartwood and sapwood. Hillis and Inoue (1968) have shown that the composition of phenolic compounds in Sirex noctilo-affected wood of Pinus radiata is different from that of sapwood and heartwood. Pinobanksin and pinocembrin were detected in heartwood, but not in S. noctilo-affected wood. Pinosylvin was detected in S. noctilo-affected wood, but not in sapwood. Damaged sapwood (cause unknown) contained small amounts of unidentified phenols not detected in other tissues.

Fungal heartwood formed in Prunus domestica var. Victoria after attack by Stereum purpureum contained significant quantities of scopoletin, a coumarin absent from heartwood (Hillis and Swain, 1959). A similar distribution for the lignan, isoolivil, occured in Prunus jamasakura attacked by Polyporous versicolor (Hasegawa and Shirato, 1959).

The reaction zone of Fomes annosus-infected Pinus taeda and Picea abies is characterized by the accumulation of phenolic compounds present in the heartwood of both species of trees (Shain and Hillis, 1971). Damaged sapwood of P. radiata has less pinosylvin than its monomethyl ether; the reverse is true for S. noctilo-affected (Hillis and Inoue, 1968).

Cells surrounding kino veins of Eucalyptus spp. contained polyphenols different from those in uninjured wood (Hillis, 1958; Skene, 1965). In cankers or galls induced by Cronartium fusiforme on P. taeda. 16 compounds appeared and 13 compounds disappeared compared with healthy tissue (Rowan, 1970). Lignans detected in sapwood of P. abies were fewer in number and quantity than those detected in the reaction zone (Shain and Hillis, 1971).

In my study, the distribution of phenolic compounds was used to investigate the chemical relationships between discolored sapwood and heartwood or sapwood in white oak (Quercus alba L.) and white spruce (Picea glauca (Moench) Voss).

MATERIALS AND METHODS

The white oak were located 4 miles northwest of White Cloud, Michigan and were co-dominant in the forest stand. The white spruce were located at W. K. Kellogg Forest, Augusta, Michigan and were intermediate in a closed, pure stand of the species.

Trees were mechanically damaged with an increment borer. Twelve holes in 3 rows or 20 holes in 4 rows were bored into each tree approximately 1.5 m above ground level. Two white oak were injured in late December, 1969 and felled in early January, 1971. Two white oak and 2 white spruce were wounded in late April, 1970. The oak were felled in early November, 1970 and the spruce in early January, 1971. White oak were 13-17 cm in diameter (DBH) and contained 10-18 growth rings of sapwood and 25-30 growth rings of heartwood. White spruce were 13 cm in diameter (DBH) and contained 24-27 growth rings.

Trees were felled and a bolt was removed with the rows of borer holes located in the middle. To prevent desiccation the ends of the bolt and borer holes were covered with alumninum foil. The bolts were cut, in a longitudinal plane, into sections 1-2 cm in thickness and stored at -5 C.

Bulked samples of sapwood, heartwood and discolored sapwood were used from oak wounded in spring (S), in winter (W) and spruce wounded in spring. Both fresh and oven-dried material were used.

Fifty g of fresh material were extracted for 48 hours on a rotary shaker with 500 ml of hot water. The initial 500 ml of water was removed and extraction was continued another 48 hours with 500 ml of cold water.

Both extracts were combined and reduced to 250 ml in a rotary evaporator at 45 C.

Fresh material was dried 72 hours on a laboratory bench and ground to pass through a 2 mm mesh screen in a Wiley mill. The Wiley mill did not overheat during the grinding process. The material was dried to a constant weight in a 40 C oven. The method of Hanover and Hoff (1966), with modifications, was used to extract phenolic compounds from the oven-dried material.

Five g of oven-dried material were extracted 5 minutes with 100 ml of hot water. The mixture was homogenized 4 minutes and filtered. The residue was washed with 50ml of hot water and all filtrates were combined.

Extracts from both methods were washed with five 50 ml portions of ethyl ether followed with five 50 ml portions of butanol. The ether fraction was evaporated to near-dryness under forced air and dissolved in ethanol. Fractions were reduced to near-dryness in a rotary evaporator at 45 C and brought to 1 ml with ethanol or butanol, respectively.

Two-dimensional paper chromatography was used to separate the phenolic compounds. Fifty microliters of each extract were spotted with a micropipette onto Whatman 3 MM chromatography paper. Papers were irrigated in the first direction with 6% acetic acid and in the second direction with benzene, acetic acid, water (6:7:3)

or butanol, acetic acid, water (4:1:5) for the ether and butanol fractions, respectively.

Dried chromatograms were examined under ultraviolet light, exposed to ammonia fumes and reexamined in the ultraviolet. Chromatograms were sprayed with a solution of 0.4 g of diazotized sulfanilic acid in 100 ml of water and oversprayed with 2N NaOH. Several authentic compounds were chromatographed separately or in combination with the extracts. Compounds which appeared on the chromatograms were numbered and R_f values were determined and characterized by their responses to the treatments.

Thin-layer chromatography was also used to examine phenolic compounds in the ether fraction from oven-dried material. Twelve and 25 microliters of each extract were applied with a micropipette onto Silica gel GF 254 thin-layer plates (Analtech, Inc., Wilmington, Del.) and plates were developed to a height of 19 cm with benzene, methanol, acetic acid (45:8:4). Dried plates were viewed under short and longwave ultraviolet light.

The distribution of sugars in fresh tissue was studied in both white oak injured in winter. The water fraction, material left after washing the water extract with ether and butanol, was reduced to near-dryness in a rotary evaporator at 45 C and brought to 3 ml with water.

Twenty microliters of an extract were applied with a micropipette onto Whatman No. 1 chromatography paper and the papers were irrigated in one direction with butanol, acetic acid, water (6:1:2). Glucose and sucrose (5 mg/ml) were used for standards. Dried chromatograms were sprayed with para-anisidine (2.5 g in 10 ml conc HCl and 100 ml glacial acetic acid) and heated 3-5 minutes at 105 C. Spots which appeared were outlined under ultraviolet light.

RESULTS AND DISCUSSION

Fourteen compounds were detected in the ether fraction and 46 compounds were found in the butanol fraction of fresh tissue from white oak. Nineteen compounds were detected in the ether fraction and 46 compounds were found in the butanol fraction of dried tissue from white oak. Twenty-seven compounds were found in the ether fraction and 20 compounds were detected in the butanol fraction of fresh tissue of white spruce. Sixteen compounds were detected in the ether fraction and 11 compounds were found in the butanol fraction of dried tissue from white spruce.

R_f value and color reactions of each compound are presented in Tables 7-10 of Appendix I. Composite chromatograms of the ether and butanol fractions from both tree species are shown in Figures 7-14 of Appendix I.

Other investigators have shown that compounds in discolored sapwood were qualitatively and quantitatively different from compounds in heartwood and sapwood (Hillis and Inoue, 1968; Shain, 1967; Shain and Hillis, 1971). Such differences have been used to support claims that the processes leading up to death of living cells affect the composition of extractives (Hart, 1968; Hillis, 1968). In both tree species, I found differences in phenolic compounds between discolored sapwood and heartwood or sapwood (Tables 1-4). In addition to qualitative differences, quantitative changes in phenolic compounds were detected between the 3 tissues using paper chromatography (Figures 1-3). Compounds which accumulated during formation of discolored sapwood did not always accumulate during heartwood formation (Figures 2,3).

Thin-layer chromatography was also used to study the ether fraction from dried material of white oak and white spruce. Discolored sapwood of white spruce was quantitatively different from both sapwood and heartwood. Color intensity was strongest for most compounds detected in the discolored sapwood (Figure 4). Few quantitative or qualitative differences were detected between heartwood and discolored sapwood of white oak. Qualitative and quantitative differences were observed between these tissues and the sapwood (Figures 5, 6).

TABLE 1. Compounds in fresh tissue of white oak which separate discolored sapwood (DS) from heartwood (HW) and sapwood (SW).

Compound	Fraction	SW	HW	DS (W)[1]	DS (S)
12	ether	A[2]	A	A	P
20	"	P	P	A	A
21	"	A	P	A	A
22	"	A	P	A	A
23	"	A	P	A	A
1	butanol	A	P	P	P
3	"	A	P	P	P
5	"	A	P	P	A
6	"	A	P	A	A
9	"	P	A	A	A
11	"	P	P	A	A
14	"	A	P	A	A
15	"	P	A	A	A
18	"	A	P	A	A
20	"	A	A	P	A
23	"	A	P	A	A
26	"	A	P	P	A
27	"	P	P	A	A
28	"	P	A	A	A
29	"	A	A	P	A
30	"	P	A	A	A
32	"	A	A	P	A
34	"	P	A	A	A
35	"	A	P	A	A
37	"	A	P	A	A
42	"	A	P	A	A
43	"	A	P	A	A
44	"	A	P	A	A
46	"	A	P	P	A
47	"	A	P	A	A
50	"	P	A	A	A
51	"	A	A	A	P
52	"	P	A	P	A
53	"	A	P	A	A
54	"	A	P	P	P
57	"	A	A	P	A
58	"	A	A	P	A
61	"	A	P	A	A

[1](W) - injured in winter; (S) - injured in spring

[2]A - not detected; P - detected

TABLE 2.--Compounds in dried tissue of white oak which separate discolored sapwood (DS) from heartwood (HW) and sapwood (SW).

Compound	Fraction	SW	HW	DS (W)[1]	DS (S)
2	ether	A[2]	P	A	A
3	"	A	A	A	P
4	"	A	P	P	P
7	"	A	P	P	A
8	"	A	P	P	P
10	"	A	A	A	P
11	"	A	P	A	A
14	"	A	P	A	A
15	"	A	P	A	P
16	"	A	A	P	P
18	"	A	P	P	P
19	"	A	P	P	P
1	butanol	A	P	P	P
2	"	A	P	P	P
3	"	A	P	P	P
4	"	A	P	A	A
7	"	A	P	A	P
8	"	A	A	P	P
10	"	P	A	P	P
15	"	A	P	A	P
16	"	P	P	A	A
20	"	P	P	A	A
21	"	A	A	P	A
22	"	A	A	P	A
24	"	A	P	A	A
26	"	A	P	P	A
28	"	P	A	A	A
31	"	A	A	P	A
32	"	P	A	P	P
42	"	A	P	P	P
44	"	A	P	A	A
45	"	A	P	A	A
46	"	A	P	A	A
48	"	A	P	A	A
49	"	A	P	A	A
55	"	A	P	A	A
56	"	A	P	A	A
60	"	A	P	P	P
61	"	A	P	A	A

[1](W) - injured in winter; (S) - injured in spring

[2]A - not detected; P - detected

TABLE 3.--Compounds in fresh tissue of white spruce which separate discolored sapwood (DS) from heartwood (HW) and sapwood (SW).

Compound	Fraction	SW	HW	DS
1	ether	A[1]	A	P
4	"	A	A	P
5	"	A	A	P
6	"	A	A	P
8	"	A	A	P
9	"	A	A	P
10	"	A	A	P
11	"	A	A	P
12	"	A	P	A
13	"	A	A	P
15	"	A	A	P
16	"	A	A	P
17	"	A	A	P
19	"	A	P	P
21	"	A	P	P
22	"	A	A	P
23	"	A	P	P
24	"	A	A	P
25	"	A	A	P
26	"	A	A	P
27	"	A	P	P
28	"	A	P	P
1	butanol	A	A	P
3	"	A	A	P
5	"	A	P	A
6	"	A	A	P
7	"	A	A	P
8	"	A	A	P
9	"	A	A	P
11	"	A	A	P
12	"	A	A	P
13	"	A	A	P
14	"	A	A	P
15	"	A	A	P
17	"	A	A	P
18	"	A	A	P
19	"	A	A	P
20	"	A	A	P
23	"	A	A	P

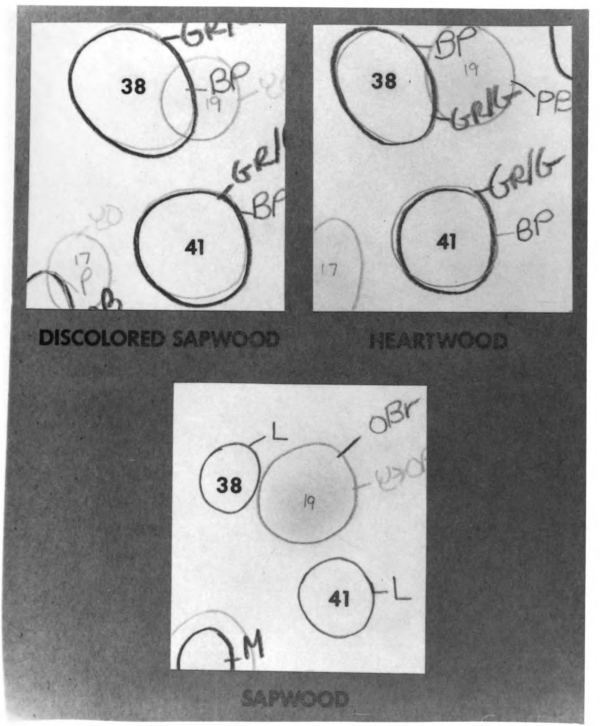
[1] A - not detected; P - detected

TABLE 4.--Compounds in dried tissue of white spruce which separate discolored sapwood (DS) from heartwood (HW) and sapwood (SW).

Compound	Fraction	SW	HW	DS
4	ether	P[1]	A	P
5	"	P	A	P
6	"	A	A	P
8	"	P	A	P
12	"	A	P	P
16	"	A	A	P
17	"	A	A	P
18	"	A	A	P
19	"	A	A	P
22	"	A	A	P
25	"	A	A	P
3	butanol	P	A	P
7	"	P	A	P
10	"	P	A	A
18	"	A	A	P
19	"	A	A	P
21	"	A	A	P
22	"	A	A	P

[1] A - not detected; P - detected

FIGURE 1.--Chromatographic evidence for quantitative changes in phenolic compounds after mechanical injury to the sapwood. Compounds 38 and 41 from the butanol fraction of dried tissue of white oak.


38
BP
19
41
17
DISCOLORED SAPWOOD
38
BP
19
41
BP
17
HEARTWOOD
38
L
OBr
19
41
L
M
SAPWOOD

FIG

FIGURE 2.--Chromatographic evidence for quantitative changes in phenolic compounds after mechanical injury to the sapwood. Compound 54 from the butanol fraction of dried tissue of white oak.

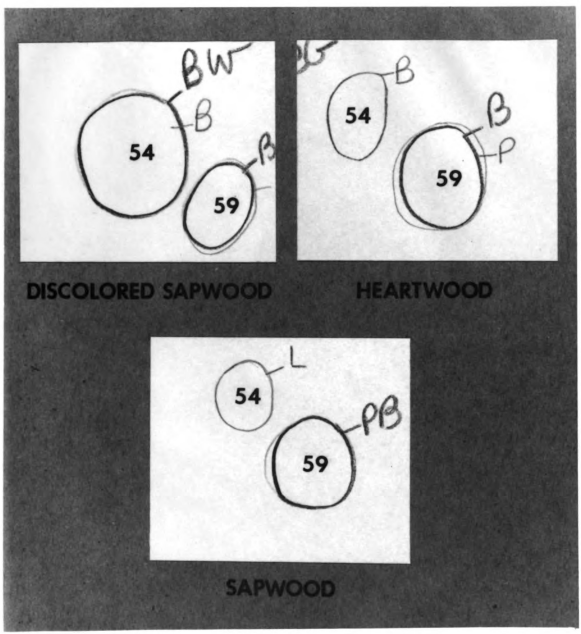

BW
B
54
B
59
DISCOLORED SAPWOOD
B
54
B
P
59
HEARTWOOD
L
54
PB
59
SAPWOOD

FIGURE 3.--Chromatographic evidence for quantitative changes in phenolic compounds after mechanical injury to the sapwood. Compound 9 from the ether fraction of dried tissue of white oak.

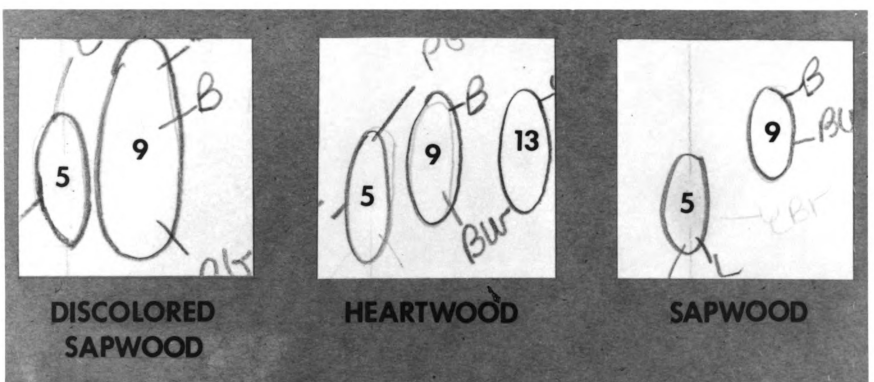

5
9
B
5
9
13
B
BW
5
9
B
DISCOLORED SAPWOOD
HEARTWOOD
SAPWOOD

FIGURE 4.--Chromatographic evidence for quantitative differences in phenolic compounds of the ether fraction from discolored sapwood (D), heartwood (H) and sapwood (S) of dried tissue of white spruce. Greater color intensity is shown by darkened spots. Twenty-five (left) and 12 (right) microliters of each extract were used.

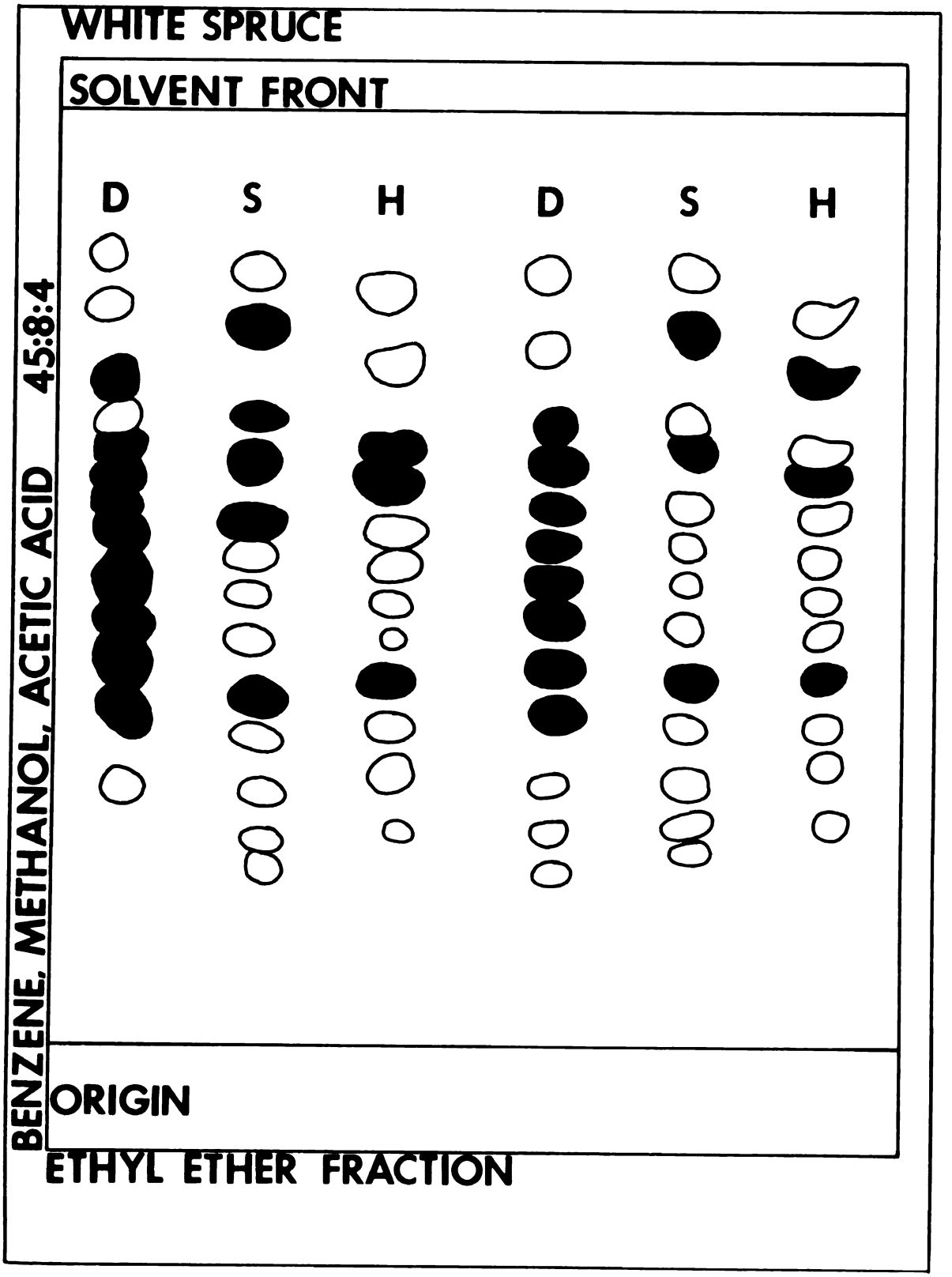

WHITE SPRUCE
SOLVENT FRONT
D
S
H
D
S
H
BENZENE, METHANOL, ACETIC ACID 45:8:4
ORIGIN
ETHYL ETHER FRACTION

FIGURE 5.--Chromatographic evidence for similarities and differences in phenolic compounds of the ether fraction from discolored sapwood (D), heartwood (H) and sapwood (S) of dried tissue of white oak (injured in spring). Arrows show possible differences between tissues. Twenty-five (left) and 12 (right) microliters of each extract were used.

[1]Color abbreviations: B - blue; P - purple; f - fluorescence; p - pale; • - trace.

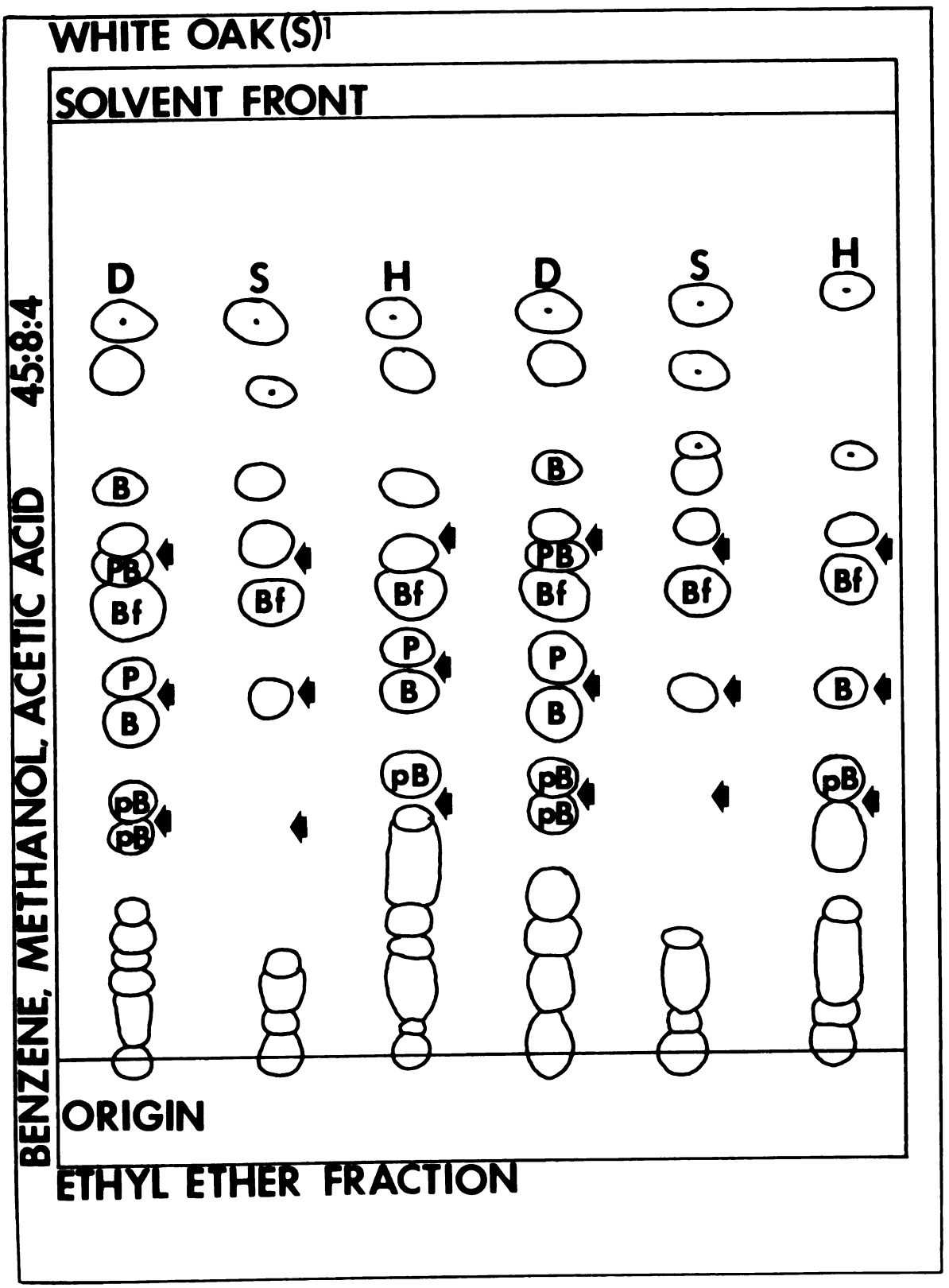
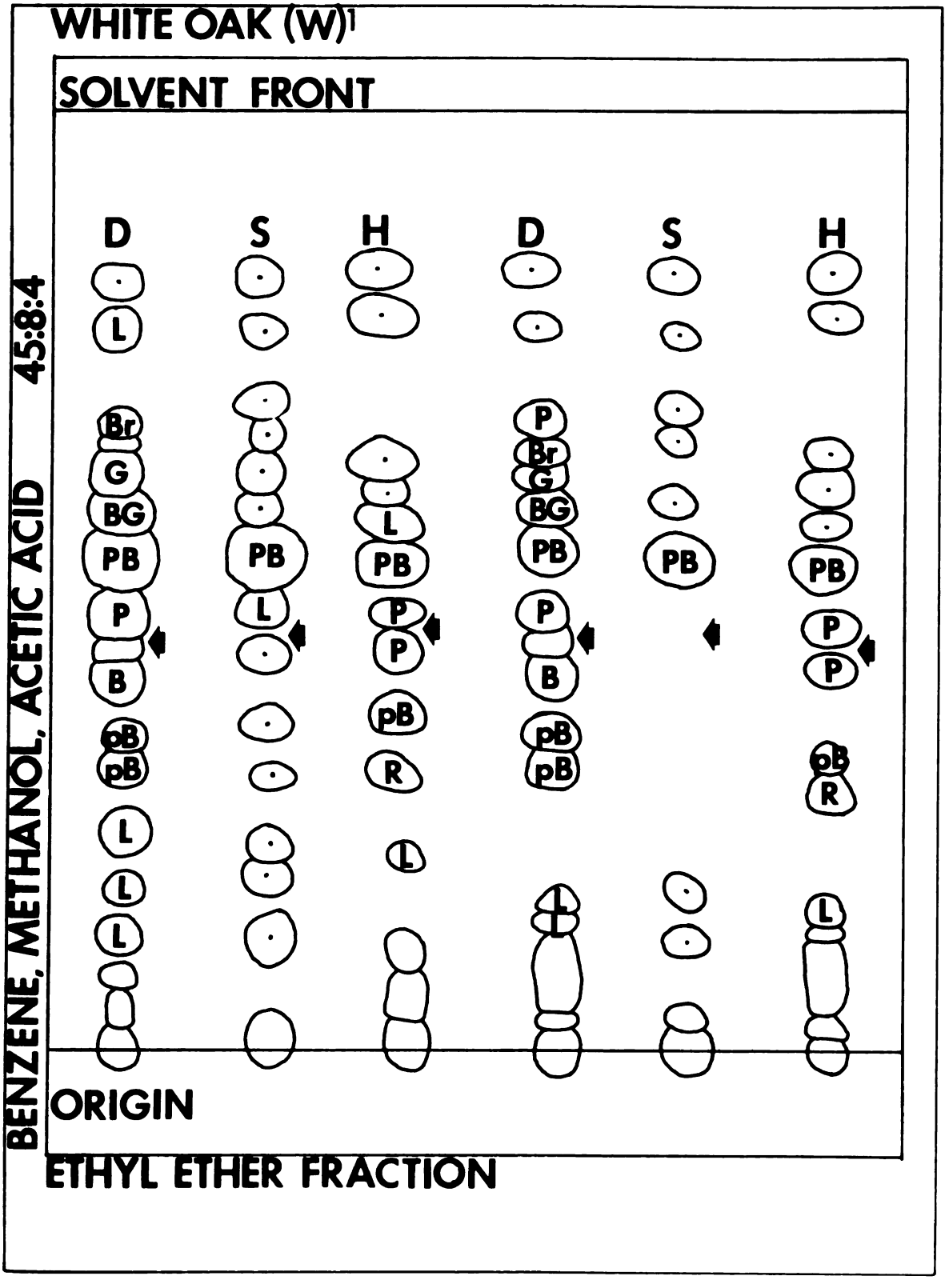

WHITE OAK(S)[1]
SOLVENT FRONT
D
S
H
D
S
H
B
PB
Bf
P
B
pB
pB
B
PB
Bf
P
B
pB
pB
Bf
B
Bf
B
pB
BENZENE, METHANOL, ACETIC ACID 45:8:4
ORIGIN
ETHYL ETHER FRACTION

FIGURE 6.--Chromatographic evidence for similarities and differences in phenolic compounds in the ether fraction from discolored sapwood (D), heartwood (H) and sapwood (S) of dried tissue of white oak (injured in winter). Arrows show possible differences between tissues. Twenty-five (left) and 12 (right) microliters of each extract were used.

[1]Color abbreviations: L - light; Br - brown; G - green; B - blue; P - purple; R - red; p - pale; • - trace.


WHITE OAK (W)1
SOLVENT FRONT
D
S
H
D
S
H
L
Br
G
BG
PB
P
B
pB
pB
L
L
L
L
PB
L
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P
pB
R
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L
BENZENE, METHANOL, ACETIC ACID 45:8:4
ORIGIN
ETHYL ETHER FRACTION

Paper chromatography showed that both fractions of phenols had compounds which separated discolored sapwood, heartwood and sapwood from each other in oak and spruce. The butanol fraction from white oak was more useful than the ether fraction for separating the 3 tissues. Both fractions from white spruce were equally useful for this purpose. Compounds detected in discolored sapwood of white oak which separated it from sapwood or heartwood, were also detected in heartwood and sapwood, respectively. Compounds which distinguished discolored sapwood from other tissues in white spruce were often detected only in this tissue. Compounds detected in sapwood and heartwood of oak were not detected in discolored sapwood and helped to separate these tissues from discolored sapwood. Few compounds in sapwood and heartwood of white spruce separated these tissues from discolored sapwood. These observations indicate that formation of discolored sapwood might be different in the 2 species of trees. Other differences were observed between discolored sapwood of white oak and white spruce (Part II, III).

Thin-layer chromatography indicated that differences in the ether fraction between sapwood, heartwood and discolored sapwood were largely quantitative for both species of trees. Thin-layer plates were developed with a different solvent system and in only one direction. Direct comparisons between results obtained by

both techniques are not easily made. Whether a similar situation also exists with the butanol fraction is unknown. Previous investigators (Hillis and Inoue, 1968; Shain, 1967) have used quantitative differences between tissues as evidence that discolored sapwood was different from sapwood and heartwood. The quantitative differences observed between the 3 tissues support the conclusion that they are chemically distinct from each other.

No previous literature was found concerning the relationship between discolored sapwood, heartwood and sapwood in both tree species. Some recent work with Picea abies will be published soon (Shain and Hillis, 1971). This work describes qualitative and quantitative differences between lignans in the reaction zone and sapwood and quantitative differences between the reaction zone and heartwood. The reaction zone of P. abies was produced by fungus infection and mechanical injury to the sapwood.

I, however, found qualitative differences between discolored sapwood and both sapwood and heartwood of white spruce. The number of lignans detected by Shain and Hillis was much less than the number of phenolic compounds I detected. The chance for variability in chemical constituents between tissues would be much greater with phenolic compounds. Different conclusions

reached by their and my studies might be due to this reason. The reaction zone of P. abies was much different from discolored sapwood of white spruce in other characteristics (Part II, III).

Other evidence used to evaluate the chemical relationships between discolored sapwood, heartwood and sapwood was the degree of similarity of chemical constituents in both fractions for the 3 tissues. Every compound detected in a tissue was considered as a separate character in constructing a similarity index. The results were expressed as the per cent of compounds in common between the tissues using the formula (Wilkinson, 1970):

$$\frac{\text{Compounds in common for tissue A+B}}{\text{Total compounds in tissue A+B}} \times 1000$$

With fresh tissue, the ether fraction of discolored sapwood from oaks wounded in spring and winter was more like sapwood than heartwood while the butanol fraction was similar to both tissues. When discolored sapwood was combined, both fractions were similar to those from sapwood and heartwood. Differences detected in phenolic compounds between discolored sapwood of oaks wounded in winter and spring were nearly as great as that between heartwood and sapwood. Formation of discolored sapwood usually caused fewer or similar changes

in phenolic compounds as heartwood formation. Most pronounced changes occurred in the butanol fraction during formation of discolored sapwood and heartwood (Table 5).

With dried tissue, the ether fraction of discolored sapwood from oaks was more like heartwood than sapwood while the butanol fraction was similar to both tissues. Differences detected in phenolic compounds between discolored sapwood of oaks wounded in winter and spring were much less than between sapwood and heartwood. Formation of discolored sapwood caused fewer or similar changes in phenolic compounds as heartwood formation. Most pronounced changes usually occurred in the ether fraction during formation of heartwood and discolored sapwood (Table 5).

With fresh tissue, the ether fraction of discolored sapwood from spruces was more like heartwood than sapwood while the butanol fraction was similar to both tissues. Formation of discolored sapwood caused much greater changes in phenolic compounds than heartwood formation. Most pronounced changes occurred in the ether fraction during heartwood formation, but in the butanol fraction during formation of discolored sapwood (Table 6).

TABL

D

D

H

S

T

D

D

H

S

colo
Wood

TABLE 5.--Similarity between phenolic compounds from fresh and dried tissue of white oak.

Fresh Tissue[1]								
Tissue	DS (S)		DS (W)		DS		HW	
	Percent of Compounds in Common[2]							
DS (W)	82	63						
DS	--	--	--	--				
HW	73	49	76	63	78	62		
SW	84	51	89	60	80	58	78	56

Dried Tissue[1]								
Tissue	DS (S)		DS (W)		DS		HW	
	Percent of Compounds in Common[2]							
DS (W)	77	82						
DS	--	--	--	--				
HW	77	75	81	66	81	75		
SW	55	77	66	65	61	74	52	67

[1]DS (S) - discolored sapwood; injured in spring; DS (W) - discolored sapwood; injured in winter; SW - sapwood; DS - discolored sapwood; HW - heartwood.

[2]First number - ether fraction; second number - butanol fraction

TABLE 6.--Similarity between phenolic compounds from fresh and dried tissue of white spruce.

Fresh Tissue[1]				
Tissue	DS		HW	
	Percent of Compounds in Common[2]			
HW	54	26		
SW	32	27	63	86

Dried Tissue[1]				
Tissue	DS		HW	
	Percent of Compounds in Common[2]			
HW	55	46		
SW	67	71	71	60

[1]DS - discolored sapwood; HW - heartwood; SW - sapwood

[2]First number - ether fraction; second number - butanol fraction

With dried tissue from spruce, both fractions were more like sapwood than heartwood. Formation of discolored sapwood caused similar changes in phenolic compounds in the ether fraction and fewer changes in the butanol fraction than heartwood formation. Most pronounced changes occurred in the butanol fraction during heartwood formation. Both fractions were similarly affected during formation of discolored sapwood (Table 6).

Calculation of chemical similarity between tissues was an attempt to give a quantitative value to relationships between the tissues. In addition, the effect of mechanical injury to sapwood could be compared with heartwood formation. Fewer chemical similarities between heartwood or discolored sapwood compared with sapwood would indicate greater changes in phenolic compounds during formation of either tissue.

Formation of discolored sapwood in white oak was generally accompanied by similar or fewer changes in phenolic compounds than heartwood formation. Formation of discolored sapwood in fresh tissue of white spruce was accompanied by greater changes in phenolic compounds than heartwood formation. Similar or fewer changes in phenolic compounds occurred during formation of discolored sapwood compared with heartwood using oven-dried material.

Different conclusions about whether discolored sapwood was more like heartwood or sapwood for both species of trees were reached depending on whether fresh or dried tissue was used. Because of the small sample size used in this study, the effect of technique upon results is not completely understood. Further work in this area should be encouraged. Calculation of similarity of phenolic compounds between the 3 tissues showed them to be different in composition from each other. This observation supports the conclusion that discolored sapwood is chemically different from sapwood and heartwood.

Analysis of the distribution of sugars showed that sapwood and discolored sapwood were very similar to each other in white oak. Glucose, fructose, sucrose and 1 or 2 additional sugars (Rg values less than 1) were detected in both tissues. Glucose, fructose and 1 additional sugar (Rg value greater than 1) were detected in heartwood.

The distribution of sugars in oak followed a different pattern than that of phenolic compounds. Sapwood and discolored sapwood were very similar to each other. The number of sugars detected in woody tissue was much less than the number of phenolic compounds. The chance for variability would be much greater with phenolic

compounds. The chance for variability would be much greater with phenolic compounds and might better reflect the actual relationship between the 3 tissues. Hillis and Inoue (1968) detected differences in phenolic compounds, but not sugars in different tissues of P. radiata.

Because of the objectives of this study, it did not appear necessary to identify the phenolic compounds detected in tissues from both tree species. However, several authentic compounds were chromatographed separately and in combination with extracts in an attempt to tentatively identify a few of the phenolic compounds. Caffeic acid co-chromatographed with compound 9 in the ether fraction (oak) and compound 54 in the butanol fraction (oak). In addition, the color reactions of caffeic acid, 9 and 54 were identical. Compounds 9 and 54 were tentatively identified as caffeic acid. Paper chromatography indicated that large increases in compounds 9 and 54 occurred during formation of discolored sapwood, but not heartwood. Compounds such as caffeic acid have been suggested as being important in biosynthesis of lignin, flavanoids and related compounds (Neish, 1964).

Two-dimensional chromatograms of the ether fraction were developed with butanol, acetic acid, water (4:1:5), in addition to benzene, acetic acid, water (6:7:3).

Compounds 5, 9, 17 and 18 in the ether fraction were the same as compounds 40, 54, 59 and 60 in the butanol fraction of oak, respectively. In addition, compounds 38 and 41, detected in the butanol fraction, were also detected in the ether fraction. Few compounds detected in the ether fraction of white spruce were also detected in the butanol fraction. Because of the large amounts of these compounds which were detected in the tissue, their identification seems desirable.

SUMMARY

The chemical relationship between discolored sapwood, heartwood and sapwood was studied in white oak and white spruce. Fresh and oven-dried material was used. A different water extraction procedure was used with fresh than with oven-dried material. Two-dimensional paper chromatography was used to separate phenolic compounds in the ether and butanol fractions of each tissue. Thin-layer chromatography was also used to separate phenolic compounds in the ether fraction of oven-dried material.

Some compounds were detected in the ether or butanol fractions of fresh material, but not detected in these fractions from oven-dried material. The opposite situation also occurred. Other investigators (Wilkinson, 1970) have observed the same phenomenon. Similar

conclusions, however, were obtained whether fresh or oven-dried material was used.

Phenolic compounds from both fractions were detected in discolored sapwood, heartwood and sapwood of both tree species which separated these tissues from each other. In white oak, few qualitative and quantitative differences in phenolic compounds of the ether fraction were observed between discolored sapwood and heartwood with thin-layer chromatography. Qualitative and quantitative differences were detected between these tissues and sapwood. In white spruce, discolored sapwood was quantitatively different from sapwood and heartwood which were similar to each other. Color intensity of compounds was greatest from discolored sapwood.

A similarity index was constructed with phenolic compounds from both fractions. Every compound detected in a tissue was considered as a separate character. Calculation of similarity indexes between the 3 tissues of white oak and white spruce showed that they were different in chemical composition from each other.

The results presented here support the conclusion that discolored sapwood is chemically different from heartwood and sapwood in white oak and white spruce.

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LITERATURE CITED

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APPENDIX I

[1]Color abbreviations: B - blue; P - purple; W - white; Gr - grey; G - green; Y - yellow; R - red; O - orange; L - light; NV - not visible; Br - brown; V - violet; Pk - pink; b - bright; d - deep.

TABLE 7.--R_f values and color reactions of the ether-soluble compounds from woody tissue of white oak.[1]

Compound	R_f		UV		Diazotized Sulfanilic Acid	2N NaOH
	Benzene, Acetic Acid, Water (6:7:3)	6% Acetic Acid	Untreated	NH_3		
Caffeic Acid	.06	.40	B	-	CBr	PG
Para-hydroxy-Benzoic Acid	.29	.73	-	-	YBr	dYO
Ferulic Acid	.64	.43	P	B	CBr	RV
Vanillic Acid	.64	.68	-	-	YBr	O
Vanillin	.74	.79	dP	-	CBr	OPV
1	.00	.02	PW	YW	CBr	Br
2	.00	.08	-	W	-	-
3	.02	.00	G	BW	-	-
4	.02	.05	P	NV	-	-
5	.00	.46	P	W	Y	CBr, PG
6	.00	.59	P	W	-	-
7	.00	.84	Y	-	-	-
8	.02	.61	G	BG	-	-
9	.04	.42	B	BW	CBr	PG
10	.05	.00	P	BW	-	-
11	.02	.08	P	-	-	-
12	.03	.03	RO	-	-	-
13	.08	.41	-	YG	-	-
14	.15	.60	L	BP	-	-
15	.27	.58	-	P	-	-
16	.25	.00	L	P	-	-
17	.33	.48	P	bB	-	-
18	.43	.63	P	G	-	-
19	.48	.52	PB	W	-	-
20	.28	.65	L	P	-	-

TABLE 7.--Continued.

Compound	R_f		UV		Diazotized Sulfanilic Acid	2N NaOH
	Benzene, Acetic Acid, Water (6:7:3)	6% Acetic Acid	Untreated	NH_3		
21	.50	.55	-	YG	-	-
22	.62	.55	-	YG	-	-
23	.65	.66	-	YG	-	-
24	.94	.00	B	-	-	-

TABLE 8.--R_f values and color reactions of the butanol-soluble compounds from woody tissue of white oak.[1]

Compound	R_f		UV			
	Butanol, Acetic Acid, Water (4:1:5)	6% Acetic Acid	Untreated	NH_3	Diazotized Sulfanilic Acid	2N NaOH
Catechin	.55	.51	-	-	YBr	-
Quercetin	.60	.01	Y	-	Y, CBr	Br
Ferulic Acid	.82	.44	P	B	CBr	RV
Caffeic Acid	.74	.39	B	BW	CBr	PG
Para-hydroxy-Benzoic Acid	.86	.73	-	-	CBr	dYC
1	.00	.03	P	Y	-	-
2	.00	.04-.44	L	-	-	-
3	.00	.58	L	-	-	-
4	.00	.58	-	-	YBr	Br
5	.06	.45	-	-	YBr	Br
6	.11	.01	-	Y	-	-
7	.17	.01	P	Y	-	-
8	.17	.54	-	-	YBr	Br
9	.18	.06	P	Y	-	-
10	.19	.42	-	-	YBr	Br
11	.25	.60	-	P	YBr	Br
12	.28	.04	P	Y	CBr	Br
13	.29	.20	P	Y	CBr	Br
14	.27	.18	L	Y	-	-
15	.31	.08	L	Y	-	-
16	.29	.33	-	-	CY	-
17	.30	.43	-	L	CY	-
18	.30	.84	-	B	-	-
19	.32	.66	-	-	YBr	-
20	.34	.72	-	P	-	-

TABLE 8.--Continued.

Compound	R_f		UV		Diazotized Sulfanilic Acid	2N NaCH
	Butanol, Acetic Acid, Water (4:1:5)	6% Acetic Acid	Untreated	NH_3		
21	.35	.38	-	-	YBr	-
22	.27	.47	-	-	YBR	-
23	.38	.49	-	-	C	-
24	.38	.56	-	-	CY	-
25	.38	.72	P	BG	-	-
26	.39	.34	L	P	-	-
27	.40	.30	P	YG	-	-
28	.43	.83	-	-	CY	BrR
29	.42	.54	L	-	-	-
30	.43	.78	PB	P	-	-
31	.44	.70	P	PB	-	-
32	.43	.72	BG	Br	-	-
33	.46	.64	P	B	Y	CBr
34	.45	.22	-	-	Y	-
35	.45	.39	YG	-	-	-
36	.50	.70	P	-	YBr	CBr
37	.51	.82	-	-	-	Pk
38	.54	.45	BG	GrG	-	-
39	.54	.88	-	-	CBr	Pk
40	.57	.47	-	-	Y	CBr, PBr
41	.57	.59	BG	GrG	-	-
42	.58	.81	-	-	Y	CBr
43	.60	.49	-	-	-	YBr
44	.62	.70	-	P	-	-
45	.58	.88	-	-	-	CBr
46	.63	.73	-	P	-	-
47	.64	.76	-	-	C	Br

TABLE 8.--Continued.

Compound	R_f		UV		Diazotized Sulfanilic Acid	2N NaCH
	Butanol, Acetic Acid, Water (4:1:5)	6% Acetic Acid	Untreated	NH_3		
48	.64	.84	YW	Y	-	-
49	.66	.42	-	YG	-	-
50	.66	.75	G	-	-	-
51	.68	.03	RC	-	-	-
52	.68	.37	-	G	-	-
53	.68	.79	-	-	-	CBr
54	.74	.41	B	BW	CBr	PG
55	.77	.86	-	-	CBr	Pk
56	.77	.84	YW	Y	-	-
57	.70	.24	G	-	-	-
58	.79	.49	B	-	-	-
59	.80	.44	P	bB	-	-
60	.81	.63	P	G	-	-
61	.85	.63	YG	B	-	-
62	.93	.00	B	-	-	-

TABLE 9.--R_f values and color reactions of the ether-soluble compounds from woody tissue of white spruce.[1]

Compound	R_f		UV			
	Benzene, Acetic Acid, Water (6:7:3)	6% Acetic Acid	Untreated	NH_3	Diazotized Sulfanilic Acid	2N NaOH
1	.00	.00	P	-	-	-
2	.00	.05	P	B	-	-
3	.00	.10	-	P	-	-
4	.06	.00	P	PW	C	Br
5	.05	.03	P	B	-	-
6	.00	.34	-	-	C	-
7	.00	.62	P	-	-	-
8	.00	.42	B	BW	C	-
9	.05	.61	G	W	-	-
10	.08	.33	PB	-	-	-
11	.11	.00	P	BW	-	-
12	.13	.53	-	BG	-	-
13	.16	.00	-	BW	-	-
14	.22	.00	-	-	C	RBr
15	.25	.00	-	P	-	-
16	.34	.00	-	-	C	RBr
17	.62	.00	-	-	C	Br
18	.77	.00	-	-	C	Br
19	.16	.54	-	L	-	-
20	.20	.69	-	-	C	RBr
21	.21	.54	-	P	-	-
22	.36	.45	-	P	CY	RBr
23	.36	.67	-	-	C	-
24	.58	.76	-	-	C	RBr
25	.63	.38	P	-	-	-
26	.65	.37-.61	-	-	-	RBr
27	.67	.51	YG	-	-	-
28	.71	.66	-	P	-	-

TABLE 10.--R_f values and color reactions of the butanol-soluble compounds from woody tissue of white spruce.[1]

Compound	R_f		UV		Diazotized Sulfanilic Acid	2N NaOH
	Butanol, Acetic Acid, Water (4:1:5)	6% Acetic Acid	Untreated	NH_3		
1	.00	.05	P	-	-	-
2	.00	.67	-	-	-	RBr
3	.20	.04	P	-	-	-
4	.28	.04	P	-	-	-
5	.29	.09	-	P	-	-
6	.31	.06	P	B	BrC	-
7	.39	.08	P	-	-	-
8	.43	.56	L	-	-	-
9	.45	.10	P	-	-	-
10	.52	.55	-	-	Y	-
11	.52	.56	L	W	-	-
12	.57	.42	-	-	C	-
13	.75	.30	B	W	-	-
14	.75	.42	B	W	-	-
15	.81	.53	-	P	-	-
16	.81	.68	-	-	Y	RBr
17	.82	.34	P	-	-	-
18	.84	.74	-	YG	C	RBr
19	.86	.52	PW	YG	-	-
20	.85	.65	-	P	-	-
21	.86	.43	-	-	Y	-
22	.87	.40	P	-	-	-
23	.88	.71	-	YG	-	-
24	.93	.00	-	L	-	-

FIGURE 7.--Composite chromatogram of the ether-soluble compounds from fresh woody tissue of white oak.

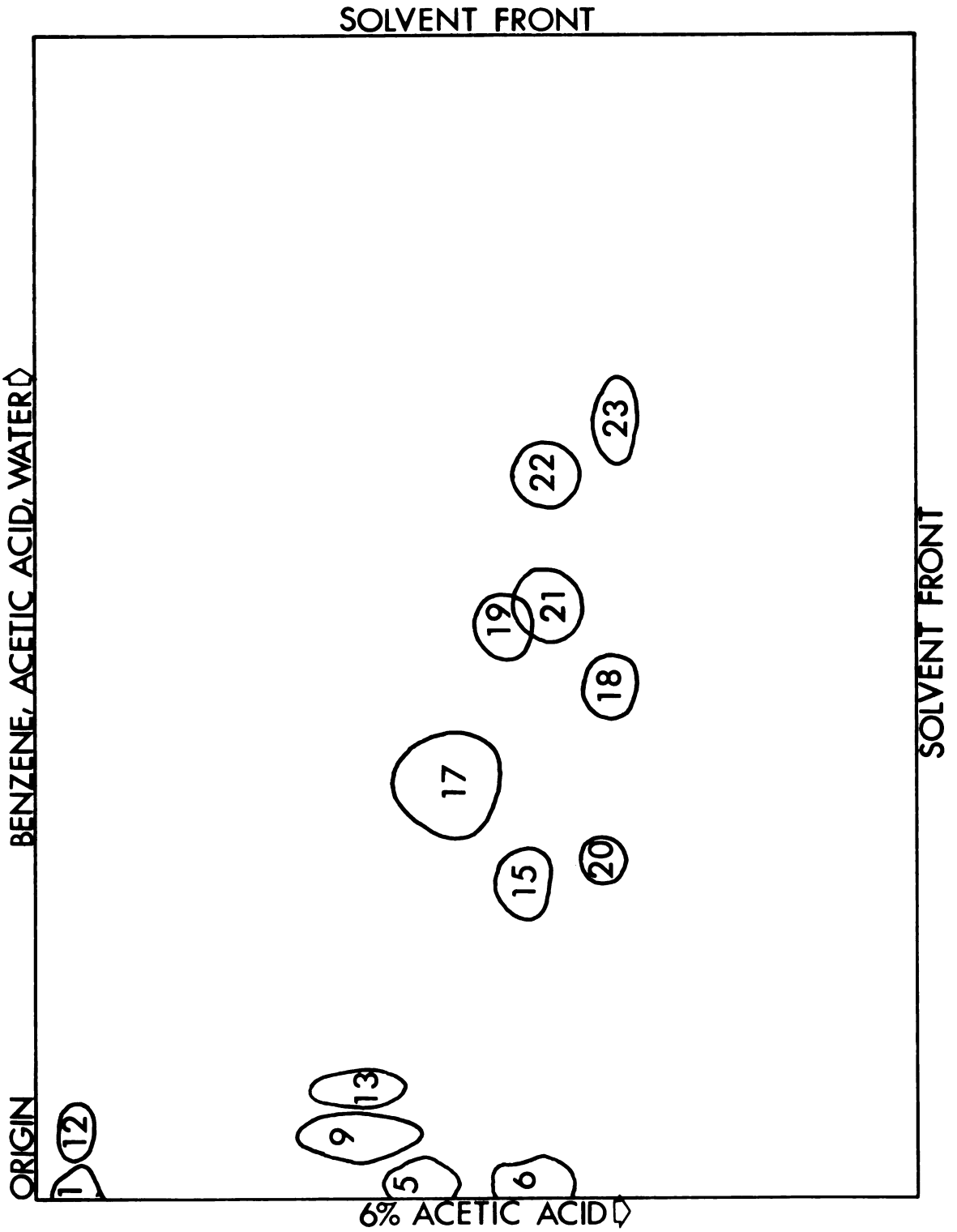
SOLVENT FRONT
BENZENE, ACETIC ACID, WATER
SOLVENT FRONT
ORIGIN
6% ACETIC ACID
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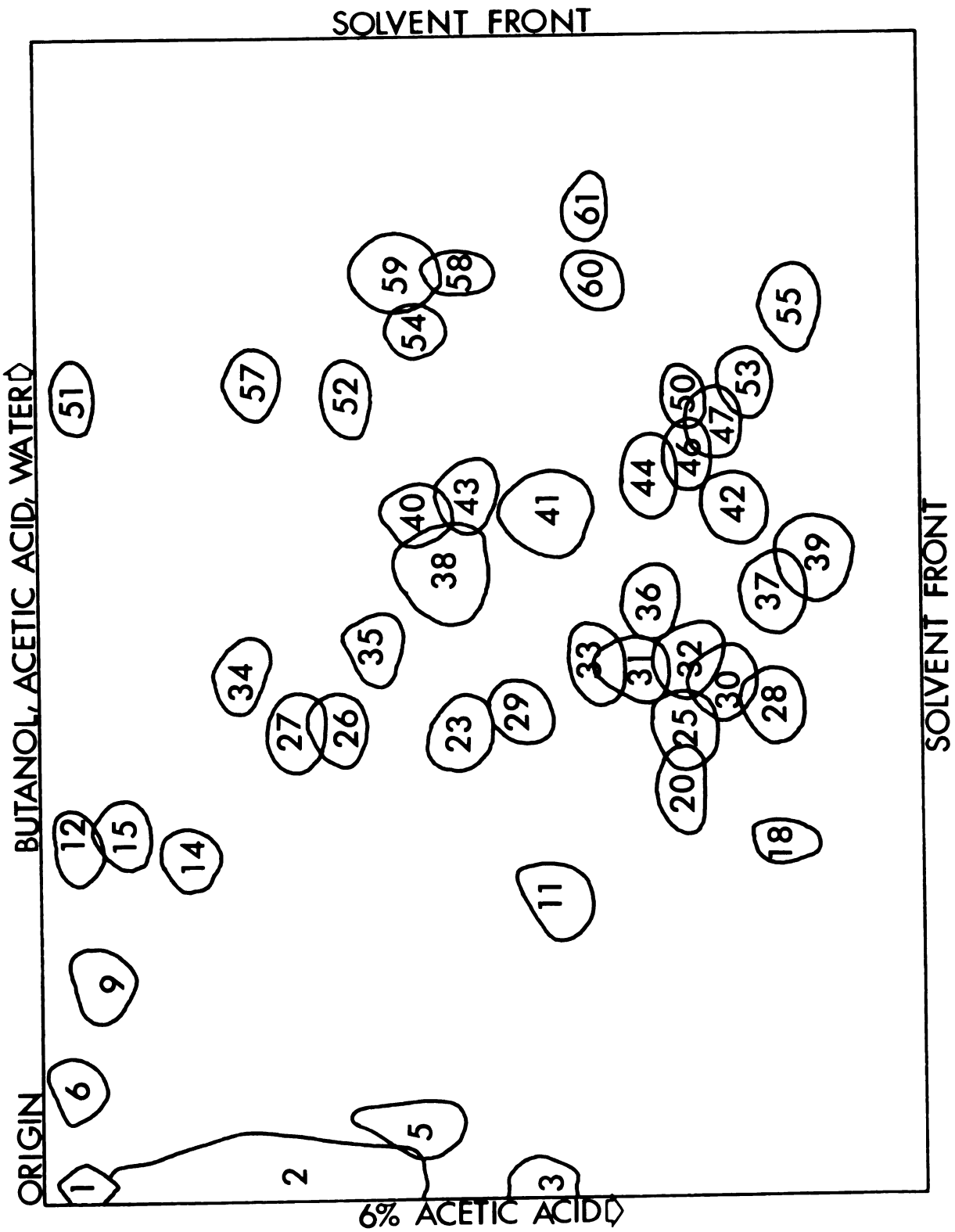
FIGURE 8.--Composite chromatogram of the butanol-soluble compounds from fresh woody tissue of white oak.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BUTANOL, ACETIC ACID, WATER
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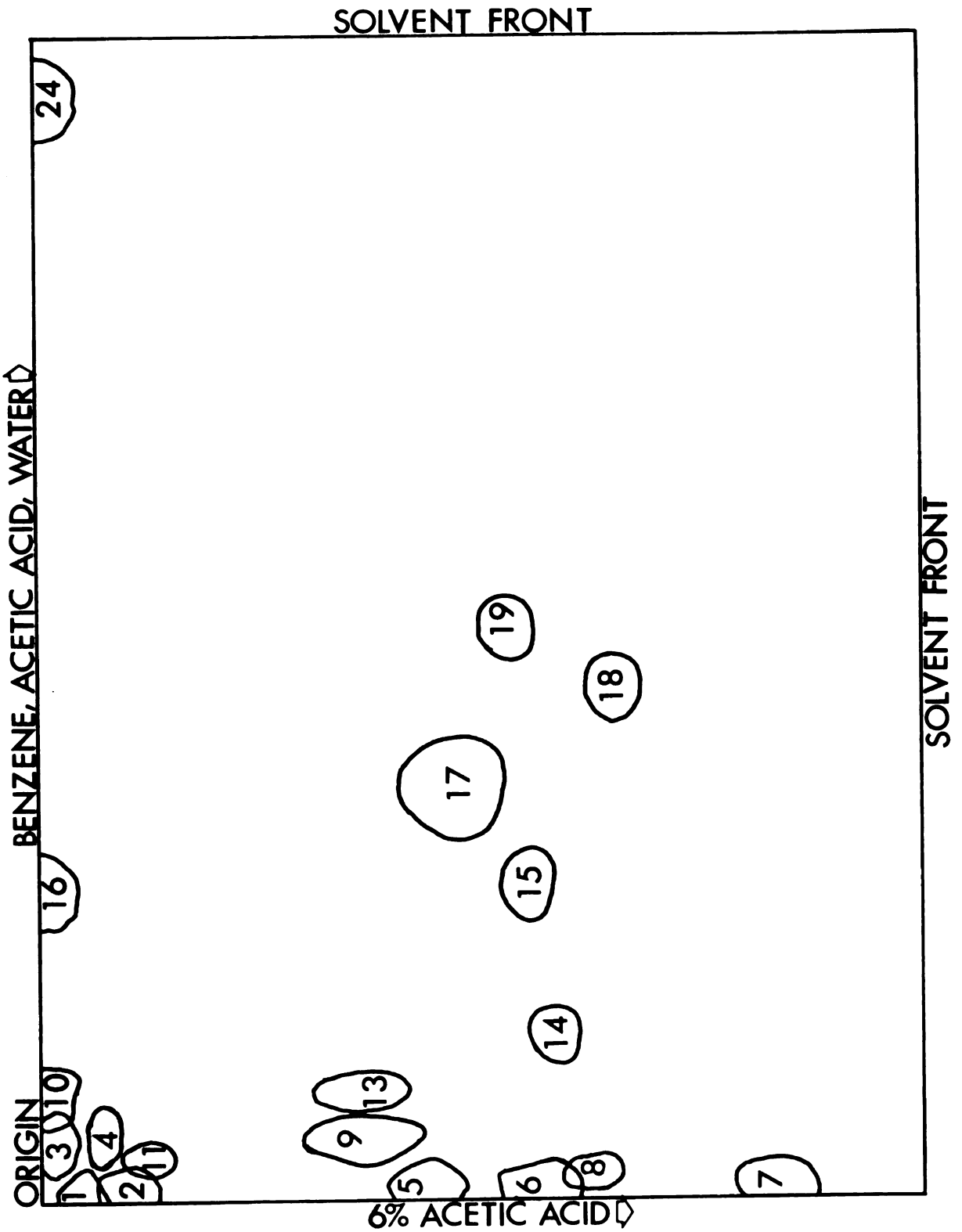
FIGURE 9.--Composite chromatogram of the ether-soluble compounds from dried woody tissue of white oak.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BENZENE, ACETIC ACID, WATER
SOLVENT FRONT
ORIGIN
6% ACETIC ACID
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FIGURE 10.--Composite chromatogram of the butanol-soluble compounds from dried woody tissue of white oak.

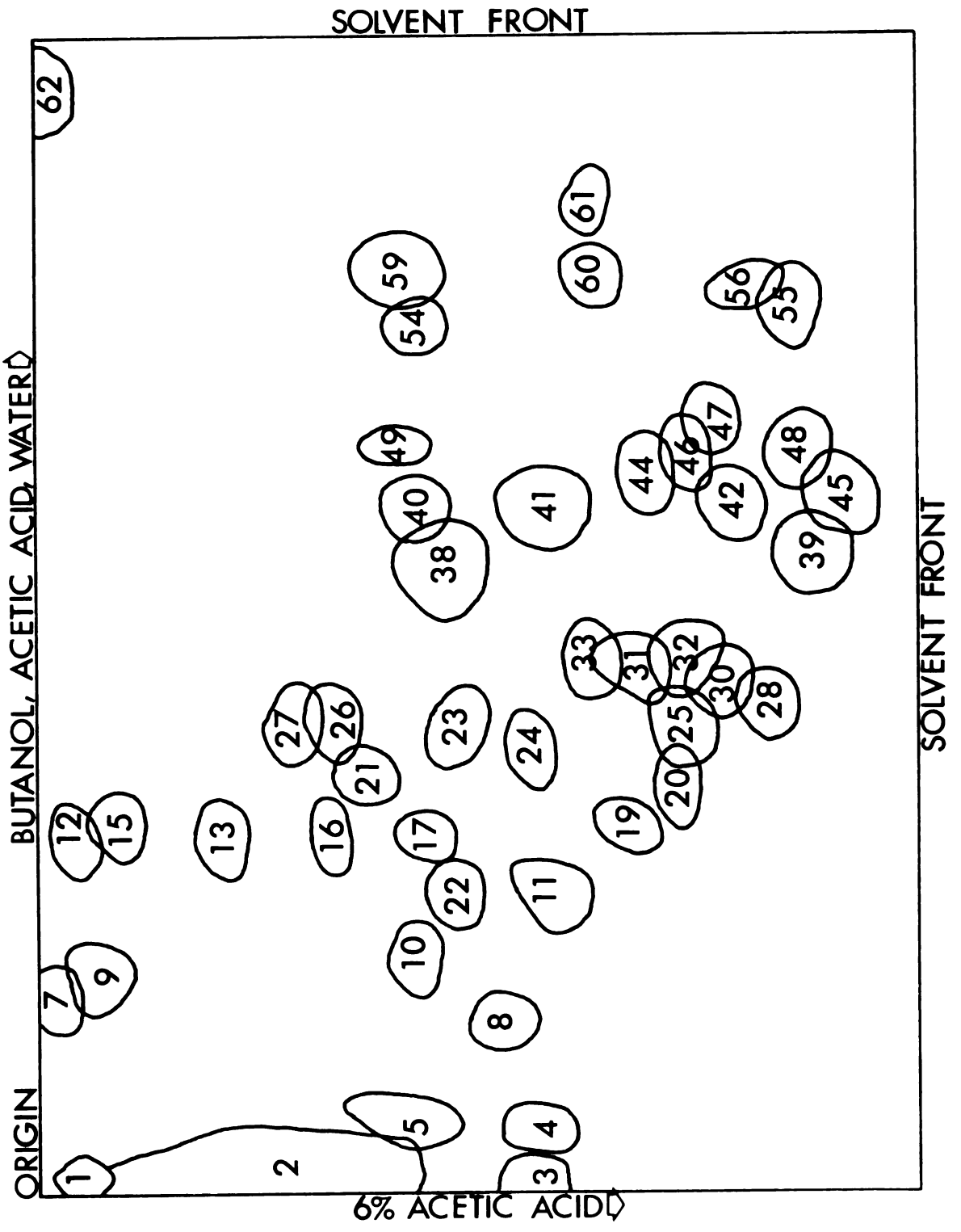
SOLVENT FRONT
BUTANOL, ACETIC ACID, WATER
ORIGIN
6% ACETIC ACID
SOLVENT FRONT
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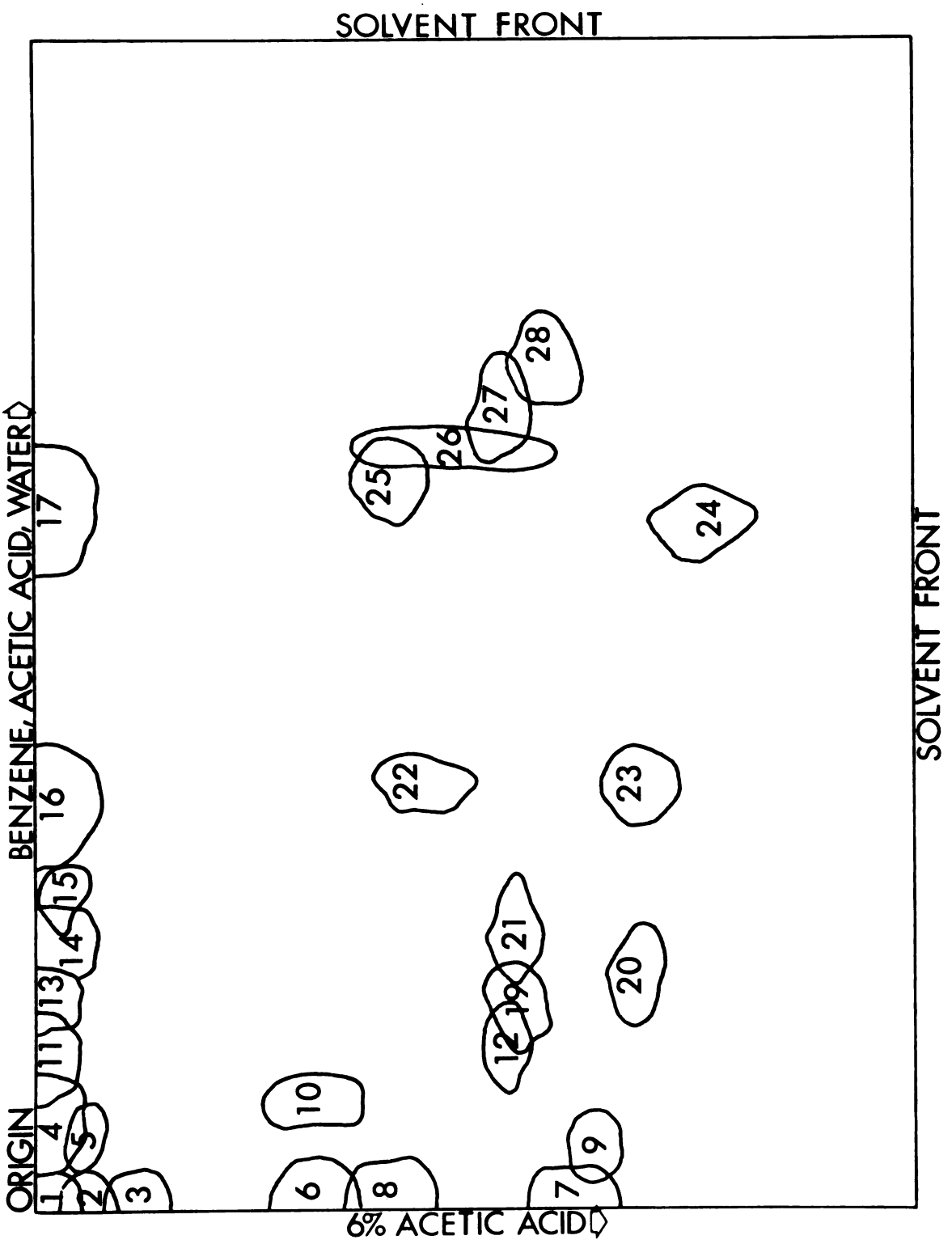
FIGURE 11.--Composite chromatogram of the ether-soluble compounds from fresh woody tissue of white spruce.


SOLVENT FRONT
BENZENE, ACETIC ACID, WATER
SOLVENT FRONT
ORIGIN
6% ACETIC ACID
1
2
3
4
5
6
7
8
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28

FIGURE 12.--Composite chromatogram of the butanol-soluble compounds from fresh woody tissue of white spruce.

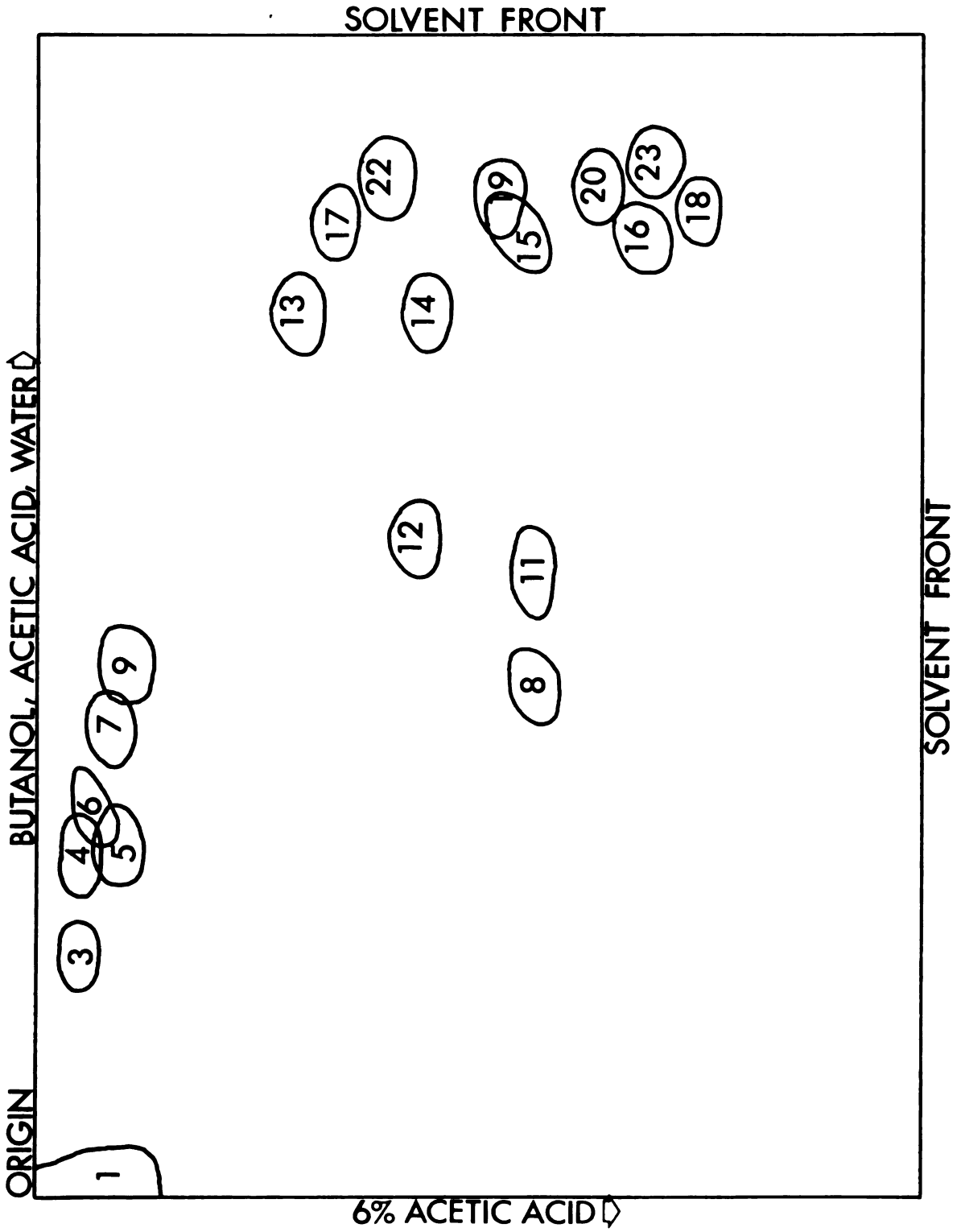
SOLVENT FRONT
BUTANOL, ACETIC ACID, WATER
SOLVENT FRONT
ORIGIN
6% ACETIC ACID
1
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FIGURE 13.--Composite chromatogram of the ether-soluble compounds from dried woody tissue of white spruce.

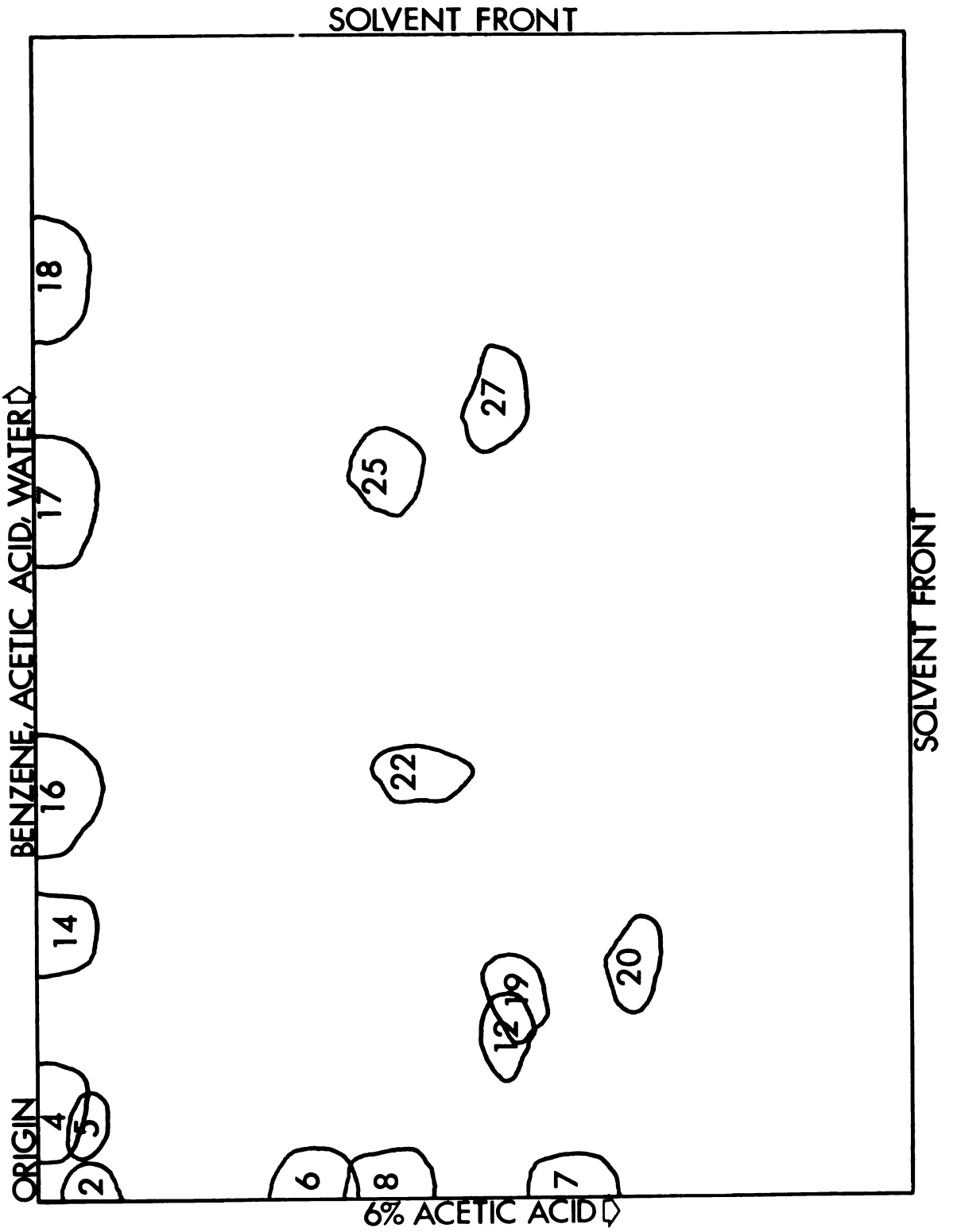
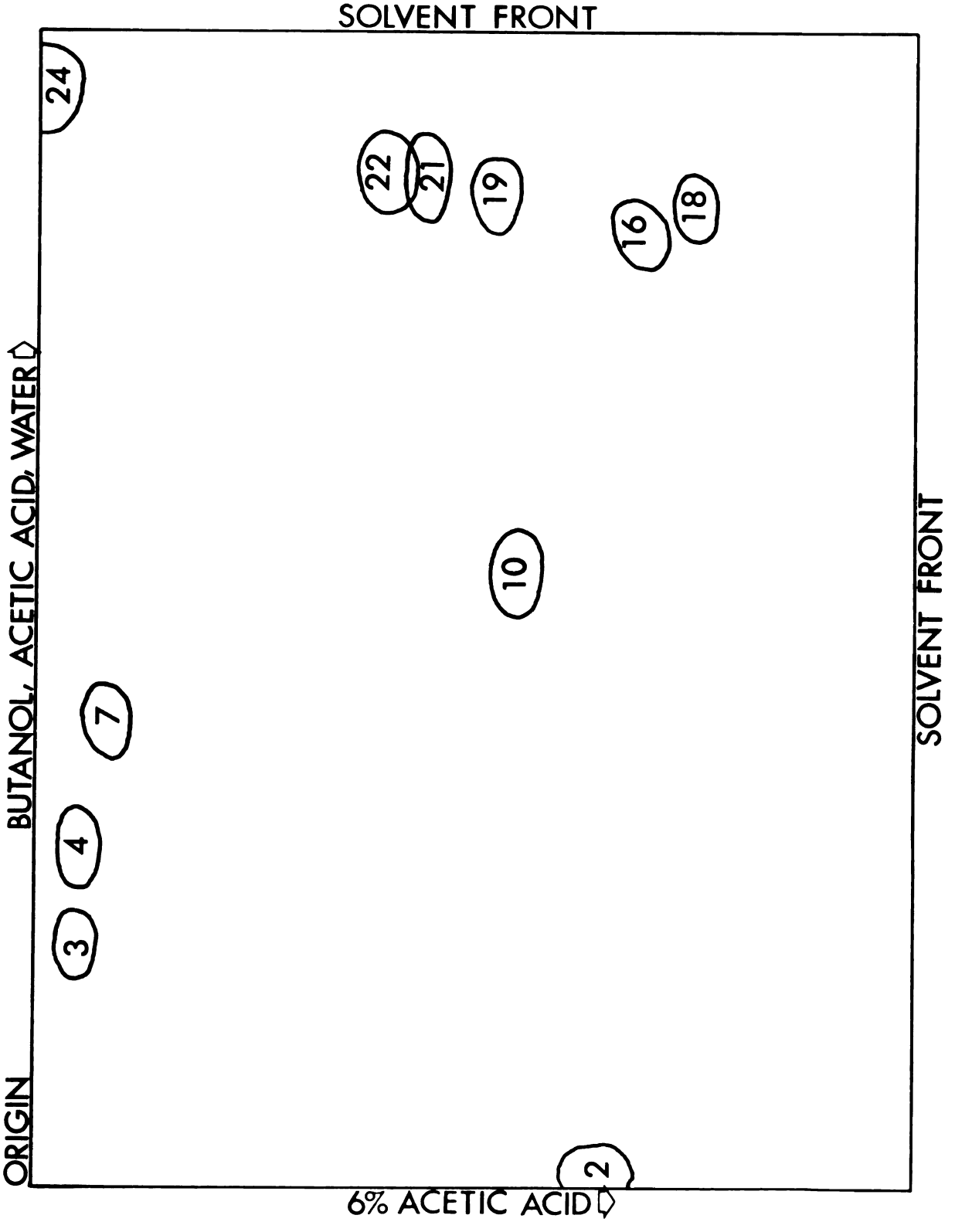

SOLVENT FRONT
BENZENE, ACETIC ACID, WATER
SOLVENT FRONT
ORIGIN
6% ACETIC ACID
2
4
5
6
8
7
14
16
17
18
20
22
25
27
12
19

FIGURE 14.--Composite chromatogram of the butanol-soluble compounds from dried woody tissue of white spruce.


SOLVENT FRONT
24
22
21
19
16
18
BUTANOL, ACETIC ACID, WATER
10
SOLVENT FRONT
7
4
3
ORIGIN
2
6% ACETIC ACID

APPENDIX II

APPENDIX II

EFFECT OF POLYPOROUS VERSICOLOR AND PORIA MONTICOLA ON PHENOLIC COMPOUNDS FROM WHITE OAK AND WHITE SPRUCE

Cowling (1961) studied decay of sapwood blocks of Liquidambar styraciflua by Polyporous versicolor (white-rot fungus) and Poria monticola (brown-rot fungus). In all stages of decay, P. versicolor depolymerized lignin and carbohydrates only as rapidly as the depolymerized products were used by the fungus. In initial stages of decay, P. monticola degraded lignin and carbohydrates more rapidly than the degraded products were used. After a 20-30 per cent weight loss, carbohydrates were depolymerized less rapidly than the depolymerized products were used by the fungus.

In my study, I investigated the effect of P. versicolor and P. monticola on the number of compounds detected in discolored sapwood, heartwood and sapwood of white oak (Quercus alba L.) and white spruce (Picea glauca (Moench) Voss).

Location of both species of trees, methods of mechanical injury and initial preparation of wood samples

is described in Part I. White oak injured in winter and white spruce injured in spring which were used for chromatographic investigations (Part I) were used for this study.

The resistance of discolored sapwood, sapwood and heartwood to decay was measured using the agar-block method (McNabb, 1958). Three blocks of discolored sapwood and 5 blocks of heartwood and sapwood were exposed to each wood-decay organism. Polyporous versicolor L. ex Fr. (USDA isolate Madison 697), a white-rot fungus, and Poria monticola Murr. (USDA isolate Madison 698), a brown-rot fungus, were the test organisms used.

Blocks were dried 14 days at 40 C and weighed to determine the initial dry weight of the blocks. After incubation with the fungus, blocks were removed from the decay chambers and the fungus was carefully scraped from the blocks. Blocks were immediately weighed to determine their moist weight. The final dry weight was determined after drying the blocks 14 days at 40 C. Weight loss was expressed as percent of the initial dry weight of the block. Blocks which were not exposed to the decay fungi served as controls.

Blocks of each tissue, approximately 15 x 8 x 8 mm in size, were cut with the longest dimension parallel to the radial axis of the tree. The blocks were steamed 20 minutes at 99 C and placed aseptically into the decay

chambers 14 days after the chambers had been inoculated with the test organism. Preparation of decay chambers is described in Part III. The decay chambers were incubated at 26 C for 6 weeks.

Two g (oven-dried weight) of each tissue from control blocks and blocks decayed by each fungus were extracted for phenolic compounds. Preparation of the wood sample, extraction of oven-dried material and two-dimensional paper chromatography of the ether and butanol fractions is described in Part I.

Heartwood from both white oak was more resistant to decay than sapwood, but only discolored sapwood from 1 white oak was more resistant to decay than sapwood and heartwood. The discolored sapwood of white spruce was more resistant to decay than sapwood and heartwood. Sapwood and heartwood were not different from each other in their resistance to either decay fungus (Table 11).

More compounds were detected in the ether and butanol fractions from sapwood, heartwood and discolored sapwood of white oak exposed to P. monticola than to P. versicolor (Table 12). Except for discolored sapwood, similar results were obtained with white spruce. Only 1 or 2 compounds were detected from blocks of discolored sapwood exposed to both decay organisms (Table 13).

Cowling (1961) showed that P. versicolor rapidly assimilated depolymerized products, but initially P. monticola did not. Similar results observed with my chemical constituents might be for the same reason. Polyporous versicolor is able to rapidly use lignin, but P. monticola only affects lignin to a minor extent (Cowling, 1965). Increases in the number of compounds detected in P. montocola-decayed material might result from the ability of the fungus to degrade, but not assimilate that portion of the lignin molecule. The phenomenon observed by Cowling (1961) for sapwood was also observed for heartwood and probably discolored sapwood of white oaks. Resistant and susceptible tissues of this tree species were affected in a similar fashion by both wood-decay organisms.

The R_f value and color reactions of each compound are given in Tables 14-17. Composite chromatograms of the ether and butanol fractions from both tree species are shown in Figures 15-18 while the distribution of compounds in control and fungus-decayed tissue of oak and spruce is given in Tables 18-21.

TABLE 11.--Weight loss (% dry weight) of sapwood (SW), heartwood (HW) and discolored sapwood (DS) after 6 weeks exposure to Poria monticola and Polyporous versicolor.

			Poria monticola			Polyporous versicolor		
Species	No. Trees	Injured Harvested	SW	HW	DS	SW	HW	DS
White oak	1	12-69 1-71	25.2 a	6.9 b	22.3 a	33.5 a	7.8 b	20.9 b[1]
White oak	1	12-69 1-71	27.8 a	10.3 b	0.3 c	35.4 a	11.9 b	15.5 b
White spruce	2	4-70 1-71	38.2 a	41.2 a	17.3 b	6.9 a	7.2 a	1.3 b

[1]For each set of values, averages followed by different letters differ significantly (0.05 level).

TABLE 12.--Number of compounds detected in blocks of control and fungus-decayed tissue of white oak.

Fraction	Tissue[2]	Treatment[1]		
		Control	Pv-decayed	Pm-decayed
ether	SW	7	4	17
	HW	13	10	18
	DS	17	12	17
butanol	SW	15	6	12
	HW	15	8	27
	DS	20	12	27

[1] Pv-decayed: decayed by P. versicolor; Pm-decayed: decayed by P. monticola

[2] SW - sapwood; HW - heartwood; DS - discolored sapwood

TABLE 13.--Number of compounds detected in blocks of control and fungus-decayed tissue of white spruce.

		Treatment[1]		
Fraction	Tissue[2]	Control	Pv-decayed	Pm-decayed
ether	SW	3	5	9
	HW	4	4	7
	DS	4	1	1
butanol	SW	5	5	8
	HW	3	4	6
	DS	5	1	1

[1] Pv-decayed: decayed by P. versicolor; Pm-decayed: decayed by P. monticola

[2] SW - sapwood; HW - heartwood; DS - discolored sapwood

TABLE 14.--R_f values and color reactions of the ether-soluble compounds from woody tissue of white oak.[1]

Compound	R_f		UV		Diazotized Sulfanilic Acid	2N NaCH
	Benzene, Acetic Acid, Water (6:7:3)	6% Acetic Acid	Untreated	NH_3		
1	.00	.02	PW	YW	CBr	PG
2	.00	.09	P	Y	-	-
3	.00	.46	B	BW	-	-
4	.00	.62	P	W	-	-
5	.00	.82	L	-	-	-
6	.01	.04	P	NV	-	-
7	.01	.26	P	BW	-	-
8	.03	.64	-	BG	-	-
9	.04	.00	P	NV	-	-
10	.04	.44	B	BW	OBr	PG
11	.09	.43	-	BG	-	-
12	.09	.73	-	L	-	-
13	.10	.25	-	L	-	-
14	.10	.79	-	P	-	-
15	.13	.65	-	L	-	-
16	.17	.44	-	L	-	-
17	.23	.04	-	P	-	-
18	.24	.33	P	G	-	-
19	.34	.49	P	B	-	-
20	.43	.68	L	G	-	-
21	.49	.54	B	W	-	-
22	.61	.48	G	-	-	-
23	.68	.57	L	YG	-	-
24	.78	.60	P	-	-	-
25	.91	.00	L	-	-	-

[1]Color abbreviations: P - purple; W - white; B - blue; G - green; Y - yellow; NV - not visible; O - orange; Br - brown; L - light.

TABLE 15.--R_f values and color reactions of the butanol-soluble compounds from woody tissue of white oak.[1]

Compound	R_f		UV		Diazotized Sulfanilic Acid	2N NaOH
	Butanol, Acetic Acid, Water (4:1:5)	6% Acetic Acid	Untreated	NH_3		
1	.00	.03	P	Y	-	-
2	.00	.45	L	-	-	-
3	.00	.61	L	-	-	-
4	.10	.05	G	-	-	-
5	.14	.09	W	-	-	-
6	.19	.09	-	Y	-	-
7	.21	.04	-	P	-	-
8	.23	.65	-	-	Y	-
9	.24	.21	P	Y	YBr	-
10	.26	.21	-	Y	-	-
11	.26	.48	-	-	Y	-
12	.28	.02	P	Y	-	-
13	.28	.31	-	-	Y	-
14	.32	.08	-	Y	-	-
15	.32	.40	-	-	Y	-
16	.32	.75	-	P	-	-
17	.34	.27	L	-	-	-
18	.37	.47	L	-	-	-
19	.38	.73	-	BG	-	-
20	.39	.32	P	YBr	-	-
21	.41	.37	L	P	-	-
22	.42	.49	L	-	-	-
23	.42	.60	L	-	-	-
24	.42	.73	B	-	-	-
25	.44	.68	P	-	-	-
26	.45	.10	L	-	-	-
27	.45	.38	W	-	-	-
28	.46	.54	-	L	-	-

TABLE 15.--Continued.

Compound	R_f		UV		Diazotized Sulfanilic Acid	2N NaCH
	Butanol, Acetic Acid, Water (4:1:5)	6% Acetic Acid	Untreated	NH_3		
29	.46	.79	P	-	-	-
30	.47	.87	-	-	Y	-
31	.48	.63	-	-	YBr	-
32	.51	.71	-	L	-	-
33	.53	.46	PB	GrG	-	-
34	.57	.62	PB	GrG	-	-
35	.57	.90	-	-	BrO	Pk
36	.58	.25	P	-	-	-
37	.59	.46	-	-	YBr	GP
38	.63	.77	-	P	-	-
39	.68	.84	Y	-	-	-
40	.73	.32	P	L	-	-
41	.74	.44	P	BW	OBr	PG
42	.76	.70	-	P	-	-
43	.78	.87	-	dP	-	BrO, Pk
44	.80	.55	B	-	-	-
45	.80	.84	Y	-	-	-
46	.82	.48	P	B	-	-
47	.82	.66	L	G	-	-
48	.83	.60	L	-	-	-
49	.90	.00	B	-	-	-

[1]Color abbreviations: P - purple; L - light; G - green; W - white; B - blue; Y - yellow; Br - brown; Gr - grey; O - orange; Pk - pink; d - deep.

TABLE 16.--R_f values and color reactions of the ether-soluble compounds from woody tissue of white spruce.[1]

Compound	R_f		UV		Diazotized Sulfanilic Acid	2N NaCH
	Benzene, Acetic Acid, Water (6:7:3)	6% Acetic Acid	Untreated	NH_3		
1	.00	.02	P	Y	-	-
2	.00	.62-.81	L	-	CBr	RBr
3	.02	.15	-	-	Y	-
4	.04	.44	B	BW	-	-
5	.07	.70	-	L	-	-
6	.18	.78	-	-	YBr	RBr
7	.37	.33	-	P	-	-
8	.61	.67	-	-	Y	CBr
9	.68	.54	L	YG	-	-
10	.95	.00	L	BW	-	-

TABLE 17.--R_f values and color reactions of the butanol-soluble compounds from woody tissue of white spruce.[1]

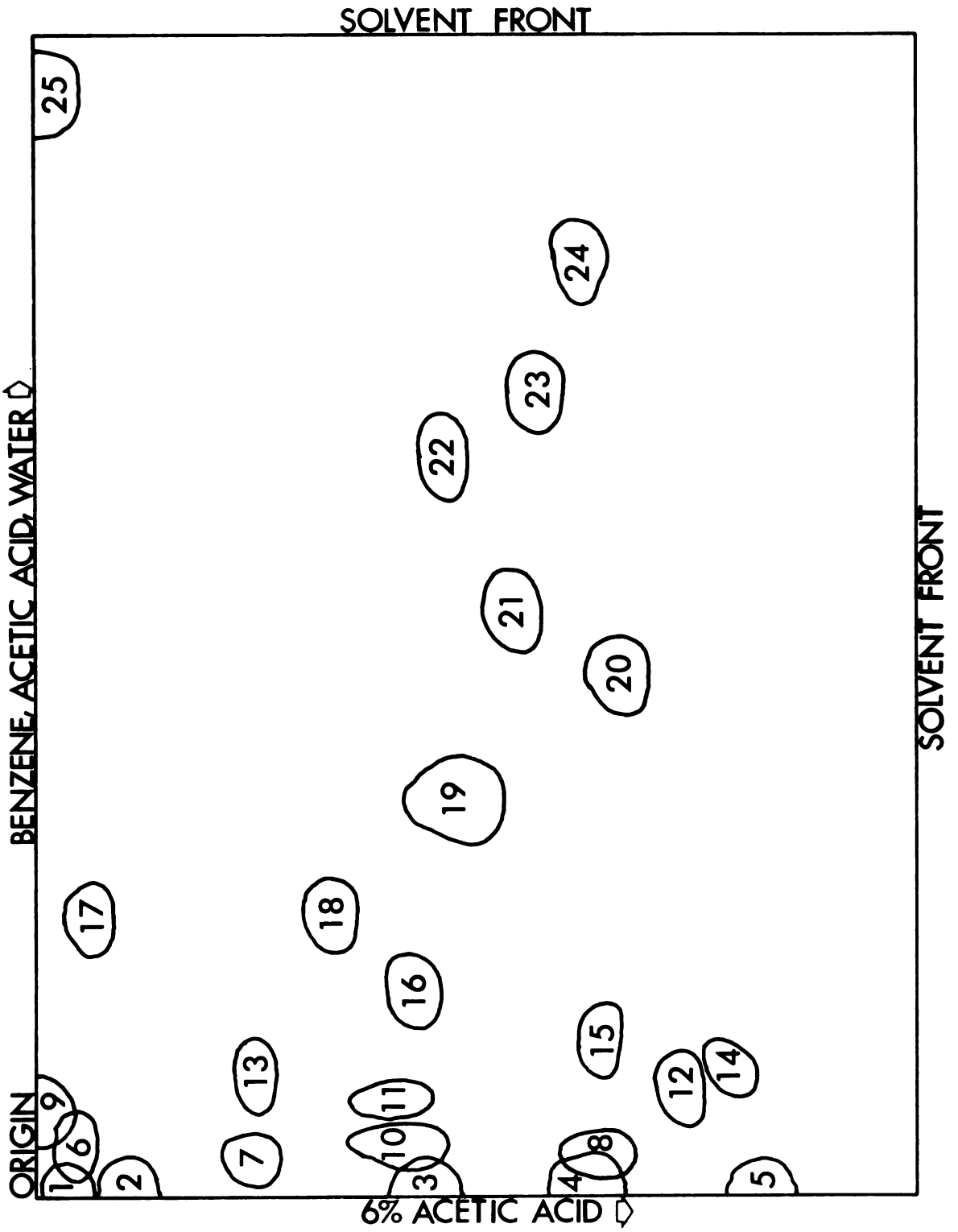
Compound Number	R_f		UV		Diazotized Sulfanilic Acid	2N NaCH
	Butanol, Acetic Acid, Water (4:1:5)	6% Acetic Acid	Untreated	NH_3		
1	.24	.03	P	-	-	-
2	.33	.03	P	-	-	-
3	.54	.21	L	-	-	-
4	.57	.28	L	-	-	-
5	.64	.82	-	-	OPk	-
6	.73	.00	BW	-	-	-
7	.74	.82	-	-	Y	RBr
8	.75	.39	P	-	-	-

TABLE 17.--Continued.

Compound	R_f		UV		Diazotized Sulfanilic Acid	2N NaOH
	Butanol, Acetic Acid, Water (4:1:5)	6% Acetic Acid	Untreated	NH_3		
9	.78	.68	P	-	-	-
10	.79	.82	-	dP	-	-
11	.82	.74	-	-	-	RBr
12	.84	.62	-	-	-	RBr
13	.86	.53	-	YG	-	-
14	.92	.00	B	-	-	-

[1]Color abbreviations: P - purple; L - light; B - blue; W - white; Y - yellow; G - green; O -orange; Br - brown; Pk - pink; R - red; d - deep.

FIGURE 15.--Composite chromatogram of the ether-soluble compounds of control and fungus-decayed tissue of white oak.


SOLVENT FRONT
BENZENE, ACETIC ACID, WATER
SOLVENT FRONT
ORIGIN
6% ACETIC ACID
1
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4
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7
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FIGURE 16.--Composite chromatogram of the butanol-soluble compounds of control and fungus-decayed tissue of white oak.

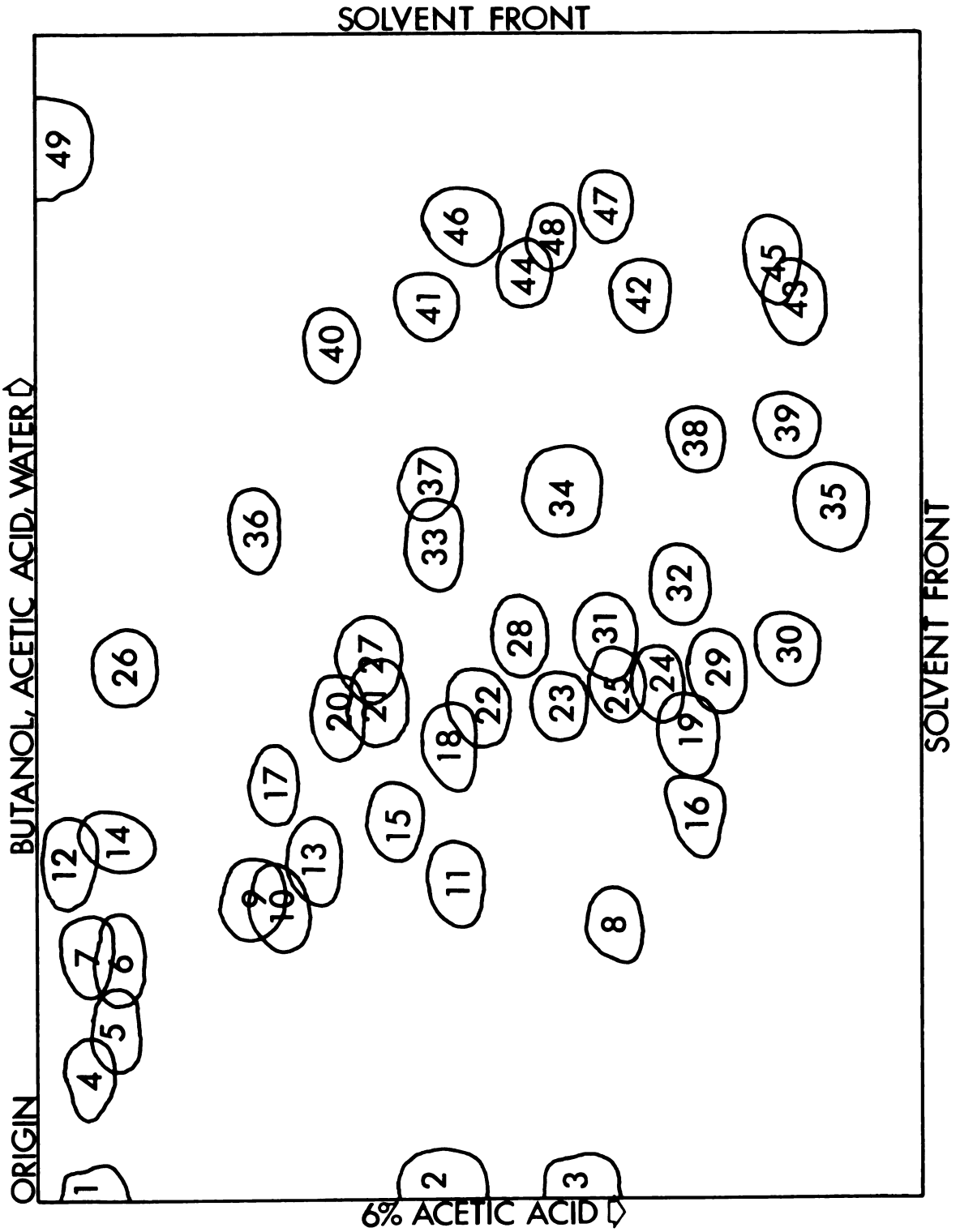
SOLVENT FRONT
BUTANOL, ACETIC ACID, WATER
ORIGIN
6% ACETIC ACID
SOLVENT FRONT
1
2
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FIGURE 17.--Composite chromatogram of the ether-soluble compounds of control and fungus-decayed tissue of white spruce.

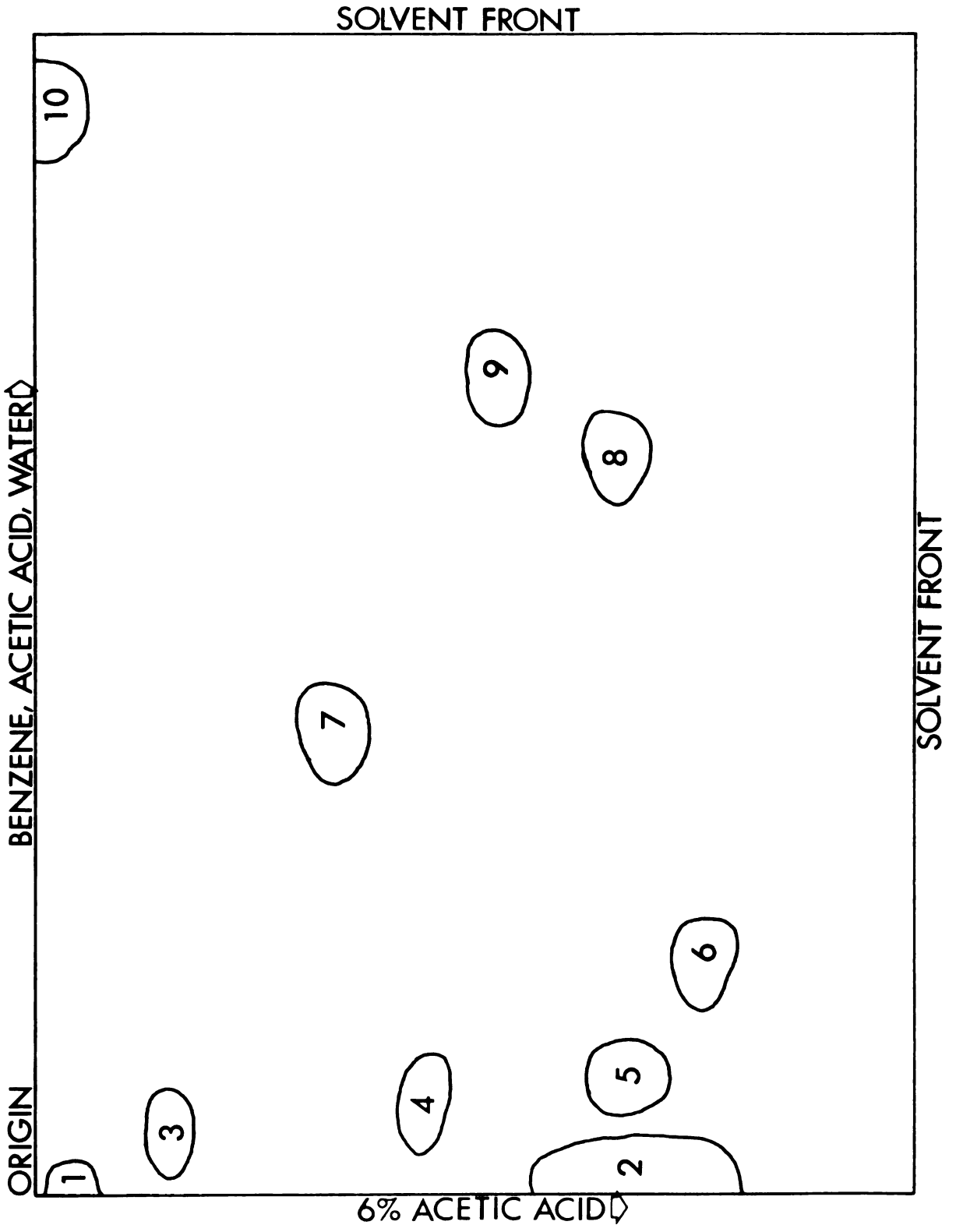
SOLVENT FRONT
BENZENE, ACETIC ACID, WATER
SOLVENT FRONT
ORIGIN
6% ACETIC ACID
1
2
3
4
5
6
7
8
9
10

FIGURE 18.--Composite chromatogram of the butanol-soluble compounds of control and fungus-decayed tissue of white spruce.

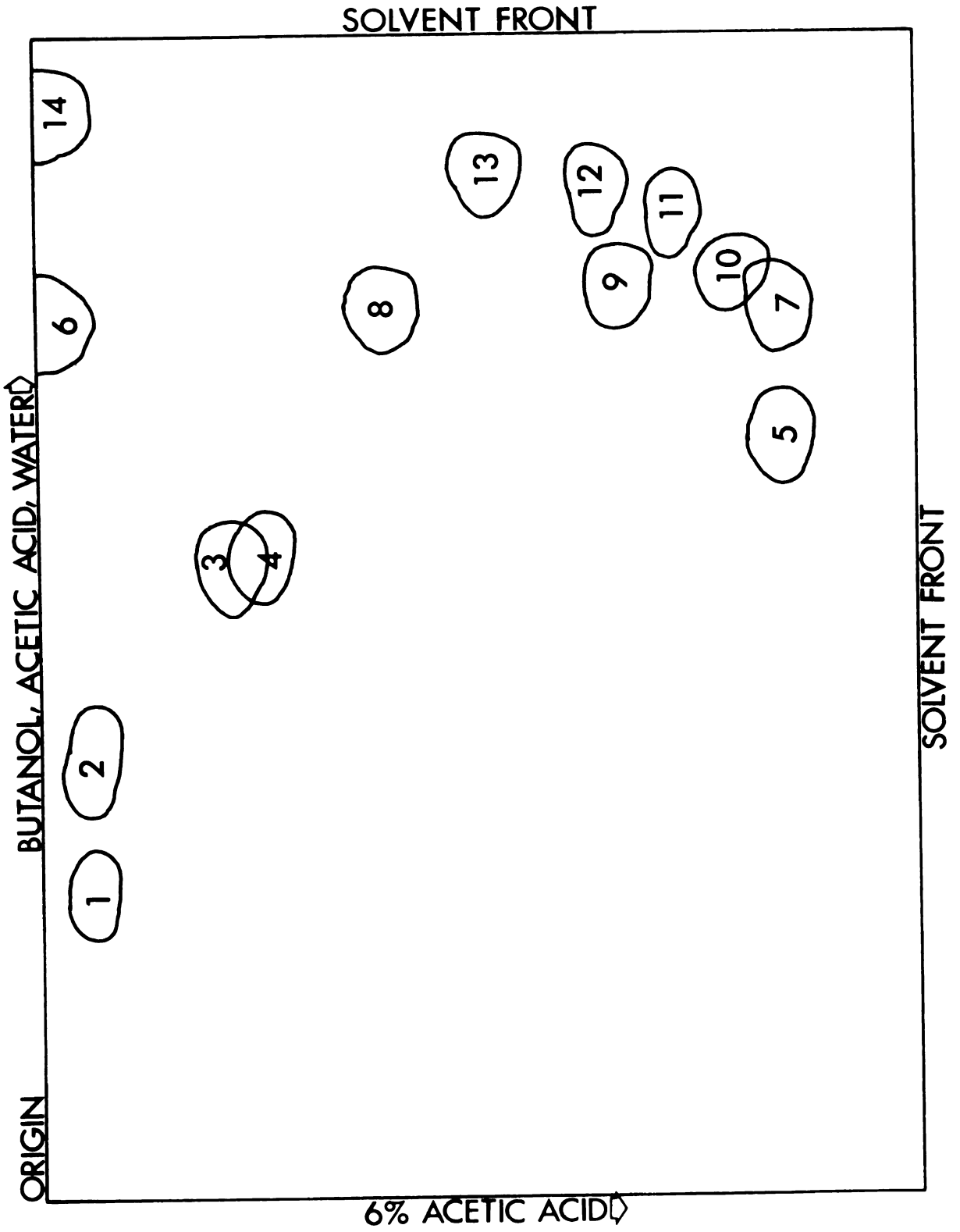
SOLVENT FRONT
14
13
12
11
10
9
8
7
6
5
4
3
2
1
BUTANOL, ACETIC ACID, WATER
SOLVENT FRONT
ORIGIN
6% ACETIC ACID

TABLE 18.--Distribution of compounds from the ether fraction in woody tissue of white oak.

Compound	Control			Decayed by Polyporous versicolor			Decayed by Poria monticola		
	DS[1]	SW	HW	DS	SW	HW	DS	SW	HW
1	P[2]	P	P	P	P	P	P	P	P
2	P	A	P	P	A	P	P	A	P
3	P	A	P	P	A	P	P	P	P
4	P	P	P	P	A	P	P	P	P
5	P	A	P	A	A	A	P	P	P
6	P	A	P	P	A	P	P	A	P
7	A	A	A	A	A	A	P	A	P
8	P	A	A	P	A	A	P	A	A
9	P	A	A	A	A	A	A	A	A
10	P	P	P	P	P	P	P	P	P
11	P	A	P	A	A	A	A	P	P
12	A	A	A	A	A	A	A	P	A
13	A	A	A	A	A	A	A	P	P
14	A	A	A	A	A	A	P	P	P
15	A	A	A	A	A	A	P	P	P
16	A	A	A	A	A	A	A	P	A
17	P	A	A	A	A	A	A	A	A
18	A	A	A	A	A	A	P	P	P
19	P	P	P	P	P	P	P	P	P
20	P	A	P	P	A	P	P	P	P
21	P	A	A	P	A	A	P	A	A
22	P	P	P	A	A	A	P	P	P
23	P	P	P	A	A	A	P	P	P
24	A	A	A	P	A	P	A	A	A
25	P	P	P	P	P	P	P	P	P

[1] DS - discolored sapwood; SW - sapwood; HW - heartwood

[2] P - detected; A - not detected

TABLE 19.--Distribution of compounds from the butanol fraction in woody tissue of white oak.

	Control			Decayed by Polyporous versicolor			Decayed by Poria monticola		
Compound	DS[1]	SW	HW	DS	SW	HW	DS	SW	HW
1	P[2]	A	A	P	A	P	P	A	P
2	P	A	A	A	A	A	P	A	A
3	P	A	A	P	A	A	P	A	P
4	A	A	A	P	A	A	A	A	A
5	A	A	A	A	A	A	P	A	A
6	A	A	A	A	A	A	P	A	A
7	A	P	A	A	A	A	A	A	A
8	A	A	A	A	A	A	P	A	P
9	A	A	P	A	A	A	P	A	P
10	A	A	A	A	A	P	A	A	A
11	A	A	A	A	A	A	A	A	P
12	P	P	P	P	P	P	P	P	P
13	A	A	A	A	A	A	A	A	P
14	A	P	P	A	A	A	A	A	A
15	A	A	A	A	A	A	A	P	P
16	P	A	A	A	A	A	A	A	A
17	A	A	A	A	A	A	A	A	P
18	P	A	A	A	A	A	P	A	A
19	P	P	A	A	A	A	A	A	A
20	P	A	P	A	A	A	P	A	P
21	A	A	A	P	A	A	P	A	A
22	A	A	A	A	A	A	A	A	P
23	A	A	A	A	A	A	P	A	A
24	P	A	A	A	P	A	A	A	A
25	P	A	A	A	A	A	A	A	A
26	A	A	A	A	A	A	A	A	P

TABLE 19.--Continued.

Compound	Control			Decayed by *Polyporous versicolor*			Decayed by *Poria monticola*		
	DS[1]	SW	HW	DS	SW	HW	DS	SW	HW
27	A[2]	A	A	A	A	A	P	A	P
28	A	A	A	A	A	A	A	A	P
29	P	P	A	A	A	A	A	A	A
30	A	P	A	A	A	A	A	A	A
31	A	P	A	A	A	A	P	P	P
32	A	A	A	A	A	A	P	P	P
33	P	P	P	P	A	P	P	A	P
34	P	P	P	P	A	P	P	P	P
35	A	P	A	A	A	A	A	A	A
36	A	A	A	A	A	A	P	A	A
37	P	P	P	A	A	A	P	P	P
38	A	A	P	A	P	P	P	P	P
39	P	P	P	A	A	A	A	A	A
40	A	A	A	A	A	A	P	P	P
41	P	A	P	P	P	A	P	P	P
42	A	A	P	A	A	A	P	P	P
43	A	A	A	A	A	A	A	A	P
44	A	A	A	A	A	A	P	A	A
45	P	P	P	A	A	A	A	A	A
46	P	P	P	P	P	P	P	P	P
47	P	A	P	P	A	A	P	A	P
48	A	A	A	P	A	A	A	A	A
49	P	P	P	P	P	P	P	P	P

[1] DS - discolored sapwood; SW - sapwood; HW - heartwood

[2] P - detected; A - not detected

TABLE 20.--Distribution of compounds from the ether fraction in woody tissue of white spruce.

Compound	Control			Decayed by *Polyporous versicolor*			Decayed by *Poria monticola*		
	DS	SW	HW[1]	DS	SW	HW	DS	SW	HW
1	P[2]	P	P	A	P	P	A	P	P
2	P	A	P	A	P	P	A	P	P
3	A	A	A	A	A	A	A	P	A
4	A	A	A	A	A	A	A	P	P
5	A	A	A	A	A	A	A	P	A
6	P	P	P	A	A	A	A	P	P
7	A	A	A	A	A	A	A	P	P
8	A	A	A	A	P	P	A	A	A
9	P	P	P	A	P	A	A	P	P
10	A	A	A	P	P	P	P	P	P

[1]HW - heartwood; SW - sapwood; DS - discolored sapwood

[2]P - detected; A - not detected

TABLE 21.--Distribution of compounds from the butanol fraction in woody tissue of white spruce.

	Control			Decayed by *Polyporous versicolor*			Decayed by *Poria monticola*		
Compound	DS[1]	SW	HW	DS	SW	HW	DS	SW	HW
1	P[2]	P	A	A	A	A	A	A	A
2	P	P	P	A	A	A	A	A	A
3	A	A	A	A	P	P	A	A	A
4	A	A	A	A	P	P	A	A	A
5	A	A	A	A	A	A	A	P	P
6	A	A	A	A	P	P	A	P	A
7	A	A	A	A	A	A	A	P	P
8	A	A	A	A	A	A	A	P	A
9	A	A	A	A	A	A	A	P	P
10	A	A	A	A	A	A	P	P	P
11	P	P	P	A	A	A	A	P	P
12	A	A	A	A	P	A	A	A	A
13	P	P	A	A	A	A	A	A	A
14	P	P	P	P	P	P	P	P	P

1 DS - discolored sapwood; SW - sapwood; HW - heartwood

2 P - detected; A - not detected

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LITERATURE CITED

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PART II

PART II

CHANGES IN MINERAL CONTENT DURING FORMATION OF DISCOLORED SAPWOOD AND HEARTWOOD IN WHITE OAK AND WHITE SPRUCE

Ash content changes during formation of heartwood and discolored sapwood. During heartwood formation, ash content decreased in Quercus alba, Maclura pomifera and Robinia pseudoacacia while it increased during formation of discolored sapwood. Ash content did not change during heartwood formation in Juglans nigra, but it increased during formation of discolored sapwood. Ash content increased during formation of heartwood and discolored sapwood in Acer saccharinum with the greatest increase in the latter tissue. An increment borer was used to induce formation of discolored sapwood (Hart, 1965; 1968).

Investigators have reported changes in inorganic elements during formation of heartwood and discolored sapwood. Formation of heartwood in Quercus rubra was accompanied by decreases in calcium, magnesium, potassium, sodium and manganese (Ellis, 1967). Phosphorous decreased

during heartwood formation in R. pseudoacacia and M. pomifera (Hart, 1968). Potassium and phosphorous decreased and manganese increased during heartwood formation in Picea abies (Bergström, 1959; Shain, 1971). In Pinus taeda, calcium, sodium, magnesium, and potassium increased from the cambium to the pith (McMillin, 1970). Levels of trace elements were not changed during heartwood formation (Hart, 1968; Ważny and Ważny, 1964).

Changes in levels of inorganic elements and nitrogen have been reported for different radial positions of the sapwood. Phosphorous and potassium decreased and calcium increased from the outer sapwood into the heartwood of Pinus radiata, Pinus nigra var. poiretiana and Pinus sylvestris except for the growth rings immediately surrounding the heartwood where phosphorous and potassium increased and calcium decreased (Wright and Will, 1958; Orman and Will, 1960). Merrill and Cowling (1966) showed that nitrogen decreased from the cambium to the heartwood boundary of several tree species. They postulated that decreases in nitrogen resulted from apposition of cellulose and lignin in cells, elution of nitrogen from vascular elements by the transpiration stream and recovery of nitrogen from dying parenchyma cells near the heartwood boundary (Cowling and Merrill, 1966).

Accumulation of calcium, magnesium, potassium, sodium, iron, manganese and numerous trace elements was observed in decayed wood of Abies grandis (Ellis, 1967). Potassium, calcium and magnesium increased in decayed wood and the reaction zone of P. abies and decayed wood of P. sylvestris (Rennerfelt and Tamm, 1962; Shain, 1971). The reaction zone of P. abies was produced in response to infection by Fomes annosus, other wood-decay fungi and mechanical injury to the sapwood (Shain, 1971). Potassium increased during formation of discolored sapwood in Q. alba, A. saccharinum, J. nigra and R. pseudoacacia. Phosphorous did not change during formation of discolored sapwood in M. pomifera and R. pseudoacacia (Hart, 1968).

Changes in mineral constituents can alter pH of woody tissue (Good, Murray and Dale, 1955). The pH of cold water extract was highest in discolored sapwood, intermediate in sapwood and lowest from heartwood of Q. alba and J. nigra (Hart, 1965). The pH was lowest from sapwood of A. saccharinum and R. pseudoacacia. The pH of the cold water extract of heartwood and discolored sapwood was similar or that from discolored sapwood was highest in these tree species (Hart, 1965; 1968). The pH of the cold water extract was highest from the reaction zone of P. abies and similar from sapwood and heartwood (Shain, 1971).

I studied changes in mineral content during formation of heartwood and discolored sapwood in white oak (Quercus alba L.) and white spruce (Picea glauca (Moench) Voss). Levels of inorganic elements, ash content and the pH of the cold water extract were measured in sapwood, heartwood and discolored sapwood of both tree species. An electron microprobe x-ray analyzer - scanning microscope was used to detect differences in levels of elements at radial positions in sapwood, heartwood and discolored sapwood. Differences in levels of elements in ray and non-ray cells were studied at these radial positions in both tree species. The effect of physiological condition of white oak at the time of injury and its effect on changes in mineral content during formation of discolored sapwood was also studied.

One objective of this research was to gain information about formation of heartwood and discolored sapwood in white spruce. The second objective was to gain more information about changes in elements during formation of discolored sapwood and heartwood in white oak, particularly as it related to differences in levels of elements at radial positions in the tree and between ray and non-ray cells at these positions.

MATERIALS AND METHODS

Location of trees, method of mechanical injury and procedures for initial preparation of the wood samples are described in Part I. Information about the trees used in this research is presented in Table 22. Blocks of tissue were dried for 72 hours on a laboratory bench, ground in a Wiley mill and passed through a 2 mm mesh screen.

Measurement of Ash Content

Ash content was measured in tissue of both tree species (Table 22). The dry weight of the crucibles was determined by heating them for 2 hours at 575 C. After cooling the crucibles in a desiccator over indicating Drierite for 15 minutes, they were weighed to the nearest 0.1 mg. A 2.2-2.4 g sample of tissue was added to each crucible and they were heated at 105 C for 24 hours. The crucibles were cooled in a desiccator for 15 minutes and weighed to the nearest 0.1 mg to determine the oven-dry weight of each sample. The samples were ashed for 3 hours at 575 C, removed and cooled in a desiccator for 15 minutes and weighed to the nearest 0.1 mg. Ash content was expressed as per cent of the oven-dry weight of the tissue for each sample.

TABLE 22.--Information about trees used in research in Part II.

Tree	Wounded	Felled	DBH[1]	SW	HW[2]	Use[3]
White Oak 1	12-21-69	3-19-70	12	10	23	A,B,D,
" 2	"	"	13	9	30	D
" 3	"	"	15	17	35	A,B,D
" 4	"	4-11-70	12	11	28	A,B,D
" 5	"	6- 4-70	13	9	35	C
" 6	"	7- 9-70	16	18	31	A,B,C
" 13	"	1-11-71	13	10	28	A,B,D
" 14	"	"	15	14	32	A,B,D
White Oak 7	4-25-70	8-17-70	12	12	39	A,B,D
" 8	"	"	14	17	27	A,B,D
" 9	"	"	14	15	26	A,B,C,D
" 10	"	"	12	18	25	A,B,C,D
" 11	"	11-12-70	13	18	24	A,B,D
" 12	"	"	16	17	28	A,B,D
White Spruce 1	4-22-70	8-19-70	11	24		A,B,D
" 2	"	"	12	24		A,B,D
" 3	"	"	13	24		A,B,D
" 4	"	"	13	24		A,B,C,D
" A	"	1- 7-71	13	26		A,B,D
" B	"	"	13	23		A,B,D

[1]diameter at 4.5 feet above ground level in cm

[2]growth rings of tissue: SW - sapwood; HW - heartwood

[3]analyses: A - ash content; B - optical emission and flame photometry; C - electron microprobe x-ray analyzer - scanning microscope; D - pH measurement

Measurement of Levels of Inorganic Elements with Optical Emission and Flame Photometry

Optical emission was used to measure levels of P, Na, Mg, Mn, Ca, Fe, Cu, B, Zn and Al in tissue from both tree species (Table 22). Samples of each tissue (equivalent to one-half g oven-dry weight) were ashed for 8 hours at 500 C, cooled to room temperature and 5 ml of an internal cobalt standard were added to each sample. Levels of each element (ppm) were measured on an Applied Research Laboratory spectrograph (Quantograph).

Levels of K were measured with flame photometry. Samples of each tissue (equivalent to one-fourth g oven-dry weight) were extracted for 2 hours with 50 ml of distilled water. The samples were shaken every 30 minutes during the extraction period. The levels of K (ppm) were determined with a Beckman flame photometer.

Measurement of pH

The pH of the cold water extract was measured for each tissue. The 3 tissues were bulked separately: white oak 1-4; 7-10; 11 and 12; 13 and 14 (discolored sapwood not bulked); white spruce 1-4; A and B (Table 22). Four g of each tissue were placed into 100 ml of distilled water and soaked for 12 hours. The pH of the water extract was measured with a Beckman zeromatic II pH meter. Distilled water without tissue (control) was treated in an identical fashion.

Electron Probe Microanalyzer Studies

Levels of inorganic and organic elements were studied with an electron microprobe x-ray analyzer - scanning microscope (Applied Research Laboratory) at different radial positions in sapwood, heartwood and discolored sapwood and in ray and non-ray cells at these positions in white oak injured in winter (5, 6) and spring (9, 10) and 1 white spruce injured in spring (4) (Table 22). The distribution of P and Ca was studied in white oak 5, 6, 9 and 10 and white spruce 4. The distribution of Mg was studied in all of the above mentioned trees except white oak 5. Manganese was studied in white oak 6, 9 and 10 and K, O, Cl and S in white oak 9 and 10 (Anderson, 1967).

Locations in ray and non-ray cells were analyzed in the outer sapwood (youngest one-third), middle sapwood (middle one-third), inner sapwood (oldest one-third), outer heartwood (2-3 growth rings next to sapwood), middle heartwood (middle one-third), inner heartwood (oldest one-third), outer discolored sapwood (youngest one-third), middle discolored sapwood (middle one-third) and inner discolored sapwood (inner one-third). A second group of positions, similar in location to those mentioned, was analyzed on the opposite side of the tree. In each section of tissue, measurements were made at 3 locations

each in ray and non-ray cells. A single 100 second count was made for each element at a location.

Sections of fresh tissue, 25-30 microns in thickness, were cut using a sliding microtome and immediately frozen on dry ice. Sections of tissue were freeze-dried at -55 F and 50-75 microns of Hg for 48 hours. Sections were mounted on polished carbon discs with Scotch double stick tape. A 100 x 80 micron area was scanned at 10 second intervals for 100 seconds. Measurements for background and tape were carried out in identical fashion.

Beam current was 15 kV and sample current 25 nano-amperes for measuring levels of P, Ca, Mg, Mn and K. For O, Cl and S, the sample current was raised to 50 nano-amperes. Lead sterate dodecinate (O), potassium acid pthalate (Mg), ammonium dihydrogen phosphate (K, Ca, P, Cl, S) and lithium fluoride (Mn) were the crystals used.

RESULTS AND DISCUSSION

Levels of Inorganic Elements, Ash Content and pH of Tissues from White Spruce

Formation of heartwood and discolored sapwood did not change levels of most inorganic elements in sapwood of white spruce. For both groups of trees, levels of K and P were reduced in discolored sapwood and heartwood while levels of Mg and Mn were reduced only in

discolored sapwood. Levels of Ca and Mn were highest in heartwood. The increase and decrease in level of elements, however, was usually not significantly different from those of the same element in sapwood (Table 23, 24).

Levels of ash were highest in heartwood, intermediate in sapwood and lowest in discolored sapwood of white spruce (Table 25). These differences, however, were significant for only one group of trees. The pH of the cold water extract was similar for sapwood and heartwood and lowest from discolored sapwood (Table 26).

Shain (1971) reported decreases of P and K and increases of Ca and Mn during heartwood formation in P. abies. In white spruce, similar changes in inorganic elements were observed during heartwood formation. The changes, however, were usually not significant. Because Shain (1971) did not statistically analyze his data, I do not know whether his differences are significant. The inorganic elements which change and the direction of change are similar for both tree species.

The pH of the cold water extract from sapwood and heartwood was similar for both tree species. That from P. abies was between 5.6-6.8 and from white spruce was 5.3 or 6.0. With respect to these criteria, formation of heartwood of white spruce was not different from P. abies.

TABLE 23.--Amounts of various inorganic elements in sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white spruce.[1]

No. of Trees	Injured Harvested	Tissue	K[2]	P	Na	Ca	Mg	Mn	Fe	Zn
						ppm of dry weight				
4	4-70 8-70	SW	417 a	37 a	6 a	658 a	79 a	34 a	1.2 a	0.5 a
		HW	217 b	0 a	5 a	1025 a	62 a	46 a	2.2 a	2.7 a
		DS	250 b	20 a	10 a	517 a	67 a	23 a	6.5 a	2.0 a
2	4-70 1-71	SW	384 a	150 a	14 a	1517 a	100 a	84 a	11 a	5.8 a
		HW	317 a	90 a	3 b	1884 a	100 a	93 a	7 a	6.0 a
		DS	350 a	75 a	4 b	983 b	84 a	56 b	9 a	3.4 a

[1]means of 3 determinations of each tissue from a tree

[2]For each set of values, averages followed by different letters differ significantly (0.05 level).

TABLE 24.--Amounts of Cu, B and Al in sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white spruce.[1]

No. of Trees	Injured Harvested	Tissue	Cu[2]	B	Al
			ppm of dry weight		
6	4-70 8-70 or 1-71	SW	1.9 a	2.9 a	8.5 a
		HW	1.8 a	3.3 a	6.5 a
		DS	2.1 a	2.9 a	7.1 a

[1]means of 3 determinations of each tissue from a tree

[2]For each set of values, averages followed by different letters differ significantly (0.05 level).

TABLE 25.--Ash content of sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white spruce.[1]

No. of Trees	Injured	Harvested	SW[2]	HW	DS
			percent of dry weight		
4	4-70	8-70	.362 a	.370 a	.327 a
2	4-70	1-71	.313 a	.356 b	.248 c

[1]means of 4 determinations of each tissue from a tree

[2]for each set of values, averages followed by different letters differ significantly (0.05 level).

TABLE 26.--pH of the cold water extract of sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white spruce.[1]

No. of Trees	Injured	Harvested	SW[2]	HW	DS
4	4-70	8-70	5.3 a	5.3 a	4.0 b
2	4-70	1-71	6.0 a	6.0 a	5.0 b

[1]means of 4 determinations of each tissue from a tree

[2]For each set of values, averaged followed by different letters differ significantly (0.05 level).

Levels of Ca, K and Mg increased greatly and the pH of the water extract was between 7.0-7.7 in the reaction zone of P. abies (Shain, 1971). Formation of discolored sapwood in white spruce was not accompanied by increases of these elements or pH of the water extract. Formation of this tissue in white spruce was very different from formation of the reaction zone in P. abies. Discolored tissue from both tree species, however, was more resistant to decay than sapwood or heartwood (Shain, 1971, Part III).

The reaction zone of P. abies was produced in response to infection by F. annosus, other decay fungi (including brown-rot fungi) and mechanical injury to the sapwood. It was not resin-soaked (acetone-solubles ca. 5% of oven-dry weight) (Shain, 1971). Discolored sapwood of white spruce was also produced in response to mechanical injury, but it was resin-soaked (heptane-solubles ca. 30%) (Part III, Appendix).

Fungus invasion and mechanical injury can expose the interior of trees to air. Low levels of resin (acetone or heptane-solubles) might not be able to completely plug the openings and evaporation of water in the transpiration stream through openings could deposit minerals in the discolored tissue. This would result in increases in levels of inorganic elements and a higher pH. High levels of resin might be able to plug the

openings and prevent evaporation of water. Increases in levels of inorganic elements and pH might be prevented. Differences observed in levels of inorganic elements and pH between discolored tissue in P. abies and white spruce may result from different levels of resin in each tissue.

Levels of Inorganic Elements, Ash Content and pH of Tissues from White Oak

Formation of heartwood and discolored sapwood changed levels of inorganic elements present in sapwood of white oak. Levels of K increased during formation of discolored sapwood and decreased during heartwood formation. Levels of P, Mg and Mn decreased during heartwood formation. When all trees were statistically analyzed together, levels of Cu, B and Al increased during formation of discolored sapwood.

Calcium levels were sometimes higher in discolored sapwood and lower in heartwood compared with levels in sapwood. Levels of P decreased and Mg and Mn increased or decreased during formation of discolored sapwood. These levels, however, were usually not significantly different from those observed for the same element in sapwood (Table 27, 28).

TABLE 27.--Amounts of various inorganic elements in sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white oak.[1]

No. of Trees	Injured Harvested	Tissue	K[2]	P	Na	Ca	Mg	Mn	Fe	Zn
			ppm of dry weight							
3	12-69 4-70	SW	1192 a	290 a	55 a	1136 a	200 a	75 a	11.0 a	0.3 a
		HW	833 b	65 b	48 a	1075 a	133 b	26 b	6.8 a	0.0 a
		DS	1067 a	240 a	36 a	1383 a	183 a	81 a	17.0 a	0.6 a
1	12-69 7-70	SW	1350 a	147 a	12 a	1057 a	114 a	71 a	15.0 a	2.9 a
		HW	1350 a	0 b	2 a	1050 a	33 b	18 b	69 b	2.5 a
		DS	1900 b	118 a	22 a	1433 b	117 a	74 a	16.3 a	4.7 a
1	12-69 1-71	SW	633 a	270 a	10 a	1067 a	133 a	93 a	10.3 a	1.0 a
		HW	433 b	90 b	12 a	900 a	100 a	26 b	6.3 a	1.0 a
		DS	600 a	210 a	17 a	1600 a	100 a	72 c	12.4 a	3.7 a
1	12-69 1-71	SW	667 a	330 a	24 a	2700 a	233 a	88 a	14.2 a	7.8 a
		HW	333 b	120 b	5 a	900 b	100 b	36 b	8.1 a	1.0 a
		DS	2400 c	210 b	58 a	1633 b	133 b	67 c	8.3 a	3.7 a

TABLE 27.--Continued.

No. of Trees	Injured Harvested	Tissue	K[2]	P	Na	Ca	Mg	Mn	Fe	Zn
			ppm of dry weight							
4	4-70	SW	1175 a	98 a	23 a	1017 a	71 a	82 a	0.0 a	0.0 a
	8-70	HW	538 b	0 b	15 a	400 b	0 b	24 b	0.0 a	0.0 a
		DS	3383 c	95 a	171 b	1368 a	94 a	80 a	0.0 a	0.0 a
2	4-70	SW	1484 a	117 a	41 a	1050 a	91 a	90 a	0.0 a	0.5 a
	11-70	HW	867 a	0 a	19 a	1133 a	0 b	32 a	0.5 a	0.0 a
		DS	2600 b	57 a	69 a	1300 a	83 b	76 a	1.5 a	0.0 a

[1]means of 3 determinations of each tissue from a tree

[2]For each set of values, averages followed by different letters differ significantly (0.05 level).

TABLE 28.--Amounts of Cu, B and Al in sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white oak.[1]

No. of Trees	Injured	Tissue	Cu[2]	B	Al
			ppm of dry weight		
12	12-69 or 4-70	SW	4.9 a	4.0 a	7.7 a
		HW	2.9 b	4.3 a	5.3 a
		DS	8.0 c	4.9 b	21.6 b

[1]means of 3 determinations of each tissue from a tree

[2]For each set of values, averages followed by different letters differ significantly (0.05 level).

Levels of ash were usually higher in discolored sapwood, intermediate in sapwood and lowest from heatwood. In 1 of 2 white oak wounded in winter and harvested 1 year later, high ash levels were detected in discolored sapwood. Discolored sapwood from the other tree had ash levels between those of sapwood and heartwood (Table 29).

The pH of the cold water extract was lowest in heartwood, intermediate in sapwood and usually highest in discolored sapwood. In 1 of 2 white oak wounded in winter and harvested 1 year later, high pH values were detected in discolored sapwood. Discolored sapwood from the other tree had a pH value between those of sapwood and heartwood (Table 30).

Physiological condition of white oak at the time of mechanical injury affected development of discolored sapwood. Increases in K levels and ash content were not observed until 7 months after mechanical injury when trees were wounded in December, but increases in K levels and ash content were observed within 4 months after mechanical injury when trees were wounded in late April (Table 27, 29).

Ellis (1967) reported decreases in Ca, Mg, K, Na and Mn during heartwood formation in Q. rubra. Heartwood of Q. alba also had lesser amounts of K than sapwood (Hart, 1968). During heartwood formation, P levels

TABLE 29.--Ash content of sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white oak.[1]

No. of Trees	Injured	Harvested	SW[2]	HW	DS
			percent of dry weight		
3	12-69	4-70	.464 a	.374 a	.482 a
1	12-69	7-70	.405 a	.269 b	.623 c
1	12-69	1-71	.441 a	.167 b	.328 c
1	12-69	1-71	.498 a	.257 b	1.154 c
4	4-70	8-70	.406 a	.225 a	1.419 b
2	4-70	11-70	.456 a	.439 a	1.131 b
12			.439 a	.299 b[3]	

[1]means of 4 determinations of each tissue from a tree

[2]For each set of values, averages followed by different letters differ significantly (0.05 level).

[3]For each set of values, averages followed by different letters differ significantly (0.05 level).

TABLE 30.--pH of the cold water extract of sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white oak.[1]

No. of Trees	Injured	Harvested	SW[2]	HW	DS
4	12-69	4-70	5.6 a	4.2 b	5.6 a
1	12-69	1-71	6.0 a	4.1 b	4.5 b
1	12-69	1-71	6.0 a	4.1 b	6.8 c
4	4-70	8-70	5.3 a	4.2 b	6.5 c
2	4-70	11-70	5.7 a	4.4 b	6.2 c

[1]means of 4 determinations of each tissue from trees bulked as indicated in Materials and Methods section

[2]For each set of values, averages followed by different letters differ significantly (0.01 level).

decreased in R. pseudoacacia and M. pomifera while Mg only decreased in the latter species (Hart, 1968).

Heartwood formation in white oak is accompanied by similar changes in inorganic elements as that observed with other species of hardwoods. Levels of P, K, Mg, Mn and sometimes Ca were lower in heartwood than in sapwood. Cowling and Merrill (1966) postulated that trees recovered N from cells in sapwood before their transformation into heartwood for use elsewhere in the tree. A similar mechanism may operate for inorganic elements, especially for those important in cellular metabolism. Additional evidence to support this suggestion is presented later in Part II.

Formation of discolored sapwood is also accompanied by changes in levels of inorganic elements present in the sapwood. In several species of hardwoods, K levels increased during formation of discolored sapwood. Higher levels of Ca and Mg were detected in discolored sapwood than in sapwood of M. pomifera and R. pseudoacacia (Hart, 1968).

Differences and similarities were observed between formation of discolored sapwood in white oak and that observed with other hardwood species. During formation of discolored sapwood, large increases in K and smaller increases in Cu, B and Al were observed. Levels of Ca were sometimes higher in discolored sapwood

than in sapwood. Small decreases of P and increases or decreases of Mg and Mn were also detected in discolored sapwood. Changes in levels of Mg, P and Mn were usually not significantly different from levels detected in sapwood.

Ellis (1967) reported that microorganisms such as decay and stain fungi change the mineral composition in discolored tissue of trees. The role microorganisms played in the accumulation of inorganic elements in discolored sapwood of white oak was not investigated. Because they are very effective at accumulating trace elements in discolored tissue, increases in Cu, B and Al may be primarily due to the presence of microorganisms in discolored sapwood of white oak. A wide variety of microorganisms are associated with processes of discoloration and decay in hardwoods (Shigo and Sharon, 1970).

There is another possible mechanism for accumulation of inorganic elements in discolored sapwood of white oak. Borer holes remained unplugged during the investigation period. Evaporation of water from the transpiration stream of the tree at the surface of the borer hole could deposit minerals in discolored tissue and large increases in levels of inorganic elements could result.

Hart (1965) reported that ash content and pH of the cold water extract was lowest in heartwood, intermediate in sapwood and highest in discolored sapwood of white oak. Similar results were obtained with my white oak.

Increases in levels of inorganic elements were accompanied by increases in ash content and pH of the cold water extract while decrease in levels of inorganic elements were accompanied by decreases in ash content and pH. Similar relationships between mineral content and pH have been reported (Good, Murray and Dale, 1955). In R. pseudoacacia, however, heartwood had lower levels of ash than sapwood, but a higher pH (Hart, 1968). High K levels appear to be most responsible for increases in ash content and pH of discolored sapwood in white oak. Only when high levels of K were detected in discolored sapwood, were increases in ash content and pH observed (Table 31).

Physiological condition of the host at the time of mechanical injury affected development of discolored sapwood in white oak. One question was whether mechanical injury in winter actually delayed formation of discolored sapwood or whether formation of discolored sapwood did not occur because the tree was dormant and proceeded at the same rate as formation of discolored tissue did in trees wounded in spring after tree growth had begun.

TABLE 31.--Relationship between K, Ca, ash content and pH of the cold water extract in sapwood (SW) and discolored sapwood (DS) of white oak.[1]

			SW				DS			
No. of Trees	Injured	Harvested	K	Ca	Ash	pH	K	Ca	Ash	pH
3	12-69	4-70	1192	1136	.464	5.5	1067	1383	.482	5.6
1	12-69	7-70	1350	1057	.405	ND[2]	1900	1433	.623	ND[2]
1	12-69	1-71	667	2700	.498	6.0	2400	1633	1.154	6.8
1	12-69	1-71	633	1067	.441	6.0	600	1600	.328	4.5
4	4-70	8-70	1175	1017	.406	5.3	3383	1368	1.419	6.5
2	4-70	11-70	1484	1050	.456	5.7	2600	1300	1.131	6.2

[1]K, Ca - means of 3 determinations of each tissue from a tree (ppm of dry weight); ash content - means of 4 determinations of each tissue from a tree (per cent of dry weight); pH - means of 4 determinations of each tissue bulked as indicated in Materials and Methods section.

[2]ND - not determined.

Potassium accumulation was a characteristic of discolored sapwood and appeared to accumulate in discolored tissue when the tree was growing. Significant increases in K in discolored sapwood had occurred in trees wounded in winter within 2 months after growth of the trees had started. No accumulation was observed during the 5 months after mechanical injury in winter when the trees were dormant. This observation suggests that mechanical injury in winter to white oak does not actually delay formation of discolored sapwood, per se. Accumulation of K with upward movement of water in the xylem also suggests that evaporation of water from the transpiration stream through the borer holes might at least be partially responsible for the high mineral content of this tissue.

Electron Probe Microanalyzer Studies
White Spruce

In white spruce wounded in spring, P levels decreased (0.01 level) from outer to inner sapwood. Levels of P in inner sapwood were not different from those in heartwood. No difference in P levels was detected at different positions in heartwood. Levels of P in inner discolored sapwood were less (0.01 level) than those in outer and middle discolored sapwood.

Levels of P were higher (0.01 level) in ray than non-ray cells in sapwood, but not in heartwood and discolored sapwood. The difference between levels of P in ray and non-ray cells decreased (0.01 level) from outer to inner sapwood (Table 32).

Levels of Ca were not different from each other in outer and inner sapwood, but were greater in middle (0.05 level) than in outer sapwood. Calcium levels in outer heartwood were higher (0.01 level) than in middle and inner heartwood which were not different from each other. Levels of calcium in inner discolored sapwood were greater (0.05 level) than in outer and middle discolored sapwood. At every position sampled, Ca levels were higher in ray than non-ray cells (0.01 level) of sapwood and heartwood, but not discolored sapwood (Table 33).

Levels of Mg were not different from each other in sapwood and heartwood and Mg levels were not different from each other in discolored sapwood. Higher Mg levels (0.01 level) were detected in heartwood than in sapwood. Ray cells in discolored sapwood had higher (0.05 level) levels of Mg than non-ray cells (Table 34).

Electron Probe Microanalyzer Studies
White Oak

In white oak injured in winter, P levels decreased (0.01 level) from outer to inner sapwood. No change in levels of P was observed at different positions in heartwood. Levels of P decreased (0.05 level) from outer to

ABBREVIATIONS

The following abbreviations are used in Tables 32-46.

R	ray cells
NR	non-ray cells
OSW	outer sapwood
MSW	middle sapwood
ISW	inner sapwood
OHW	outer heartwood
MHW	middle heartwood
IHW	inner heartwood
ODS	outer discolored sapwood
MDS	middle discolored sapwood
IDS	inner discolored sapwood
BKGD	background
5%	significant (0.05 level) above background
1%	significant (0.01 level) above background
ND	not determined

TABLE 32.--Phosphorous levels at different radial positions in sapwood, heartwood and discolored sapwood of white spruce injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	390	2.592	251	2.400
MSW	257	2.410	219	2.342
ISW	215	2.333	201	2.305
OHW	212	2.328	219	2.342
MHW	223	2.350	216	2.336
IHW	205	2.313	196	2.293
ODS	540	2.733	325	2.513
MDS	497	2.697	377	2.577
IDS	223	2.350	223	2.350
BKGD	163	2.213		
5 % (SW, HW)	185	2.267		
1 % (SW, HW)	195	2.290		
5 % (DS)	215	2.334		
1 % (DS)	242	2.385		

TABLE 33.--Calcium levels at different radial positions in sapwood, heartwood and discolored sapwood of white spruce injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	2416	3.383	2080	3.318
MSW	2711	3.433	2495	3.397
ISW	2541	3.405	2224	3.347
OHW	3199	3.505	2904	3.463
MHW	2680	3.428	2478	3.394
IHW	2755	3.440	2692	3.430
ODS	2056	3.313	1893	3.277
MDS	2104	3.323	1862	3.270
IDS	2438	3.387	2285	3.357
BKGD	1446	3.160		
5 % (SW, HW)	1596	3.203		
1 % (SW, HW)	1664	3.221		
5 % (DS)	1630	3.212		
1 % (DS)	1714	3.234		

TABLE 34.--Magnesium levels at different radial positions in sapwood, heartwood and discolored sapwood of white spruce injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	604	2.781	566	2.753
MSW	620	2.793	633	2.802
ISW	605	2.782	571	2.757
OHW	708	2.850	641	2.807
MHW	639	2.806	619	2.792
IHW	724	2.860	663	2.822
ODS	724	2.860	598	2.777
MDS	712	2.853	620	2.793
IDS	645	2.810	558	2.747
BKGD	575	2.760		
5 % (SW, HW)	629	2.799		
1 % (SW, HW)	654	2.816		
5 % (DS)	654	2.816		
1 % (DS)	690	2.839		

inner discolored sapwood. At each position sampled in sapwood and discolored sapwood, P levels were greater in ray than non-ray cells (0.01 level). No differences were detected between P levels in ray and non-ray cells of heartwood (Table 35).

Levels of Ca were lower (0.05 level) in outer than in middle and inner sapwood. Calcium levels decreased between inner sapwood and outer heartwood and were highest in inner heartwood (0.01 level). At each position sampled in sapwood, heartwood (0.01 level) and discolored sapwood (0.05 level), Ca levels were higher in ray than non-ray cells (Table 36).

Levels of Mg did not change from outer to inner sapwood. Magnesium levels decreased from inner sapwood to outer heartwood and increased from outer to inner heartwood (0.01 level). Higher Mg levels were detected (0.01 level) in ray than non-ray cells of sapwood and discolored sapwood, but not heartwood. Levels of Mg in ray cells were greater (0.01 level) in outer than in middle and inner discolored sapwood (Table 37).

Levels of Mn decreased from outer to inner sapwood and inner sapwood to outer heartwood (0.01 level). No differences in Mn levels were observed at different positions in heartwood and in discolored sapwood. At

TABLE 35.--Phosphorous levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in winter.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	529	2.724	369	2.568
MSW	359	2.555	270	2.432
ISW	312	2.495	234	2.370
OHW	206	2.314	206	2.314
MHW	ND	ND	ND	ND
IHW	218	2.339	207	2.316
ODS	440	2.640	285	2.456
MDS	345	2.538	232	2.366
IDS	281	2.450	220	2.343
BKGD	169	2.229		
5 % (SW, HW)	181	2.258		
1 % (SW, HW)	186	2.270		
5 % (DS)	181	2.258		
1 % (DS)	194	2.288		

TABLE 36.--Calcium levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in winter.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	2850	3.456	2560	3.409
MSW	3060	3.487	2710	3.434
ISW	3070	3.488	2650	3.424
OHW	2880	3.460	2380	3.377
MHW	ND	ND	ND	ND
IHW	3460	3.540	3000	3.478
ODS	3410	3.533	2870	3.459
MDS	3040	3.484	2700	3.432
IDS	3110	3.493	2790	3.446
BKGD	1707	3.232		
5 % (SW, HW)	1799	3.255		
1 % (SW, HW)	1837	3.264		
5 % (DS)	1906	3.280		
1 % (DS)	1996	3.300		

TABLE 37.--Magnesium levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in winter.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	584	2.767	459	2.662
MSW	530	2.725	492	2.692
ISW	543	2.735	448	2.652
OHW	389	2.590	374	2.573
MHW	ND	ND	ND	ND
IHW	543	2.735	518	2.715
ODS	612	2.787	460	2.663
MDS	501	2.700	451	2.655
IDS	489	2.690	431	2.635
BKGD	375	2.575		
5 % (SW, HW)	396	2.598		
1 % (SW, HW)	405	2.608		
5 % (DS)	400	2.602		
1 % (DS)	411	2.614		

every position sampled in sapwood and heartwood, Mn levels were higher (0.01 level) in ray than non-ray cells. Levels of Mn in ray and non-ray cells of discolored sapwood were not different from each other (Table 38).

In white oak injured in spring, P levels decreased from outer to inner sapwood (0.01 level). The difference between levels of P in ray and non-ray cells also decreased (0.01 level). No change in levels of P occurred in heartwood. In discolored sapwood, P levels in ray cells were greater (0.01 level) in middle than in outer and inner discolored sapwood. Ray cells in outer discolored sapwood had higher P levels (0.05 level) than those in inner discolored sapwood. No differences in P levels were detected at different positions in non-ray cells of discolored sapwood.

Every position sampled in sapwood and discolored sapwood had higher levels of P in ray than non-ray cells (0.01 level). No differences were observed in P levels between ray and non-ray cells in heartwood. More P was present in sapwood than in heartwood (0.01 level) and a greater proportion of P was detected in ray cells of discolored sapwood than in sapwood (Table 39).

Levels of Ca were not different from each other at positions sampled in sapwood and discolored sapwood. Levels of Ca increased from outer to inner heartwood

TABLE 38.--Manganese levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in winter.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	626	2.797	524	2.722
MSW	572	2.758	459	2.662
ISW	555	2.745	476	2.678
OHW	436	2.640	382	2.582
MHW	ND	ND	ND	ND
IHW	407	2.610	352	2.547
ODS	656	2.817	467	2.670
MDS	482	2.683	391	2.593
IDS	524	2.720	521	2.717
BKGD	311	2.493		
5 % (SW, HW)	336	2.527		
1 % (SW, HW)	348	2.542		
5 % (DS)	387	2.588		
1 % (DS)	424	2.628		

TABLE 39.--Phosphorous levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	1090	3.038	447	2.651
MSW	662	2.821	375	2.574
ISW	567	2.754	362	2.559
OHW	272	2.435	260	2.415
MHW	258	2.413	258	2.413
IHW	274	2.438	280	2.448
ODS	881	2.945	370	2.569
MDS	1930	3.287	356	2.552
IDS	584	2.767	303	2.482
BKGD	187	2.273		
5 % (SW, HW)	203	2.309		
1 % (SW, HW)	211	2.325		
5 % (DS)	226	2.355		
1 % (DS)	245	2.390		

(0.01 level). Except for discolored sapwood and outer and middle sapwood, higher Ca levels were observed in ray than non-ray cells (0.01 level) (Table 40).

No differences in levels of Mg and Mn were observed at positions sampled in sapwood and discolored sapwood. Levels of both elements decreased from inner sapwood to outer heartwood and increased from outer to inner heartwood (0.01 level) (Tables 41, 42).

Higher Mg levels were detected (0.01 level) in ray than non-ray cells of sapwood and discolored sapwood. The difference between levels of Mg in ray and non-ray cells was greater (0.01 level) in outer than in middle and inner sapwood. No difference in levels of Mg were observed between ray and non-ray cells of heartwood (Table 41).

Higher levels of Mn were detected (0.01 level) in ray than non-ray cells of sapwood and heartwood. No differences between levels of Mn in ray and non-ray cells were detected in discolored sapwood (Table 42).

Levels of K increased from outer to inner sapwood, decreased from inner sapwood to outer heartwood and increased from outer to inner heartwood (0.01 level). Changes in K levels at different locations in discolored sapwood were not significant. Higher K levels were detected in ray than non-ray cells in sapwood (0.01 level) and heartwood (0.05 level), but not in discolored

TABLE 40.--Calcium levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	2230	3.350	2220	3.348
MSW	2300	3.363	2300	3.363
ISW	2330	3.368	2180	3.340
OHW	2230	3.349	2050	3.313
MHW	2300	3.363	2110	3.325
IHW	2610	3.418	2320	3.366
ODS	2020	3.307	1910	3.283
MDS	1960	3.293	1960	3.293
IDS	1970	3.296	1920	3.285
BKGD	1514	3.180		
5 % (SW, HW)	1593	3.202		
1 % (SW, HW)	1626	3.211		
5 % (DS)	1633	3.213		
1 % (DS)	1683	3.226		

TABLE 41.--Magnesium levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	929	2.968	770	2.887
MSW	839	2.924	807	2.907
ISW	851	2.930	774	2.889
OHW	620	2.793	629	2.799
MHW	690	2.840	688	2.838
IHW	837	2.923	811	2.909
ODS	1050	3.023	765	2.884
MDS	1020	3.012	772	2.888
IDS	1170	3.071	699	2.845
BKGD	575	2.760		
5 % (SW, HW)	606	2.783		
1 % (SW, HW)	620	2.793		
5 % (DS)	656	2.817		
1 % (DS)	693	2.841		

TABLE 42.--Manganese levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	439	2.643	400	2.602
MSW	431	2.635	417	2.621
ISW	420	2.624	390	2.592
OHW	390	2.592	357	2.553
MHW	394	2.596	375	2.575
IHW	434	2.638	379	2.579
ODS	360	2.557	378	2.578
MDS	358	2.554	342	2.535
IDS	369	2.568	353	2.548
BKGD	309	2.490		
5 % (SW, HW)	328	2.517		
1 % (SW, HW)	338	2.529		
5 % (DS)	337	2.528		
1 % (DS)	350	2.544		

sapwood. More K was present in sapwood (0.01 level) than in heartwood (Table 43).

Levels of Cl, S and O were not different from each other at positions sampled in sapwood, heartwood and discolored sapwood. Levels of Cl were greater (0.01 level) in ray than non-ray cells in sapwood and heartwood, but not in discolored sapwood. Levels of S were higher (0.01 level) in ray than non-ray cells of all tissues while levels of O were not different from each other in ray and non-ray cells of any tissue (Tables 44, 45, 46).

I observed different patterns of distribution for elements at different radial positions in sapwood of white oak and white spruce. Phosphorous levels decreased from outer to inner sapwood in both tree species. In 1 group of oaks, Mn followed a similar pattern. The distribution of P and, in 1 instance, Mn was the same as that reported previously for N (Merrill and Cowling, 1966). In white oak, Mg and Mn followed a different distribution than that of P. Decreases in these elements occurred at the heartwood boundary. Even in the 1 group of oaks where Mn had a similar distribution as P, Mn also decreased significantly at the heartwood boundary. Potassium levels in oak increased from outer to inner sapwood. At the heartwood boundary, levels of this

TABLE 43.--Potassium levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	5210	3.717	2692	3.430
MSW	5795	3.763	2945	3.469
ISW	6715	3.827	3184	3.503
OHW	2559	3.408	2089	3.320
MHW	2742	3.438	2618	3.418
IHW	3027	3.481	2891	3.461
ODS	9886	3.995	13102	4.118
MDS	13105	4.119	7980	3.902
IDS	11803	4.073	10698	4.029
BKGD	984	2.993		
5 % (SW, HW)	1079	3.033		
1 % (SW, HW)	1122	3.050		
5 % (DS)	1331	3.124		
1 % (DS)	1511	3.179		

TABLE 44.--Chlorine levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	2648	3.423	2297	3.361
MSW	2884	3.460	2512	3.400
ISW	3090	3.490	2377	3.376
OHW	2793	3.446	2444	3.388
MHW	2898	3.462	2637	3.421
IHW	2979	3.474	2577	3.411
ODS	3891	3.590	3945	3.596
MDS	3420	3.534	4678	3.670
IDS	3112	3.493	3468	3.540
BKGD	1280	3.107		
5 % (SW, HW)	1406	3.148		
1 % (SW, HW)	1463	3.165		
5 % (DS)	1556	3.192		
1 % (DS)	1691	3.228		

TABLE 45.--Sulfur levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	1055	3.023	800	2.903
MSW	950	2.978	781	2.893
ISW	1017	3.007	765	2.884
OHW	950	2.978	794	2.900
MHW	939	2.973	824	2.916
IHW	944	2.975	822	2.915
ODS	1084	3.035	918	2.963
MDS	1130	3.053	948	2.977
IDS	1117	3.048	897	2.953
BKGD	606	2.783		
5 % (SW, HW)	654	2.816		
1 % (SW, HW)	676	2.830		
5 % (DS)	659	2.819		
1 % (DS)	682	2.834		

TABLE 46.--Oxygen levels at different radial positions in sapwood, heartwood and discolored sapwood of white oak injured in spring.

	R		NR	
Tissue	CP100S	Log_{10} Count	CP100S	Log_{10} Count
OSW	12701	4.104	11608	4.071
MSW	12602	4.101	11007	4.044
ISW	13205	4.122	12405	4.095
OHW	14409	4.161	12306	4.092
MHW	13109	4.120	12309	4.093
IHW	14309	4.158	14509	4.164
ODS	16303	4.213	18504	4.268
MDS	16600	4.220	19105	4.282
IDS	17901	4.253	18705	4.273
BKGD	2224	3.347		
5 % (SW, HW)	2427	3.385		
1 % (SW, HW)	2518	3.401		
5 % (DS)	2383	3.377		
1 % (DS)	2455	3.390		

element greatly decreased. The distribution of K in sapwood of white oak is opposite to that reported for N (Merrill and Cowling, 1966).

Levels of some elements remained unchanged at different radial positions in sapwood and heartwood. These included Cl, S, O (oak) and Mg (spruce). The distribution of Mg in white spruce was different from that of P. taeda where levels of Mg increased from cambium to pith (McMillin, 1970). More Mg, however, was detected in heartwood than in sapwood of white spruce. Calcium levels were lowest in outer sapwood of white spruce and 1 group of white oaks. The distribution of Ca resembled that reported for several Pinus spp where Ca levels increased from outer sapwood to the heartwood boundary (Wright and Will, 1958; Orman and Will, 1960).

My data show that elements are recovered from cells in sapwood before or during their transformation into heartwood. Phosphorous, K, Mg and Mn levels decreased across the sapwood or at the heartwood boundary. The mechanism for recovery must act very quickly. Potassium levels were highest in inner sapwood, but greatly decreased in outer heartwood. Different tree species are not always able to recover the same elements. White spruce was unable to recover Mg, but recovery occurred in white oak during heartwood formation. Recovery occurred from ray and non-ray cells.

Chlorine, S, O and Ca were not recovered before heartwood formation in white oak. Oxygen forms part of the woody matrix of the tree and most of this element is probably not available for recovery. Any recovery of O would probably not be detected because of the small percentage of the total O present in the cell actually involved.

Studies with Hordeum vulgare, barley, (Greenway and Thomas, 1965) and Phaseolus vulgaris, red kidney bean, (Biddulph et al. 1958) showed Ca, Cl and S were not readily retranslocated from the original place of deposit in these plants. If a similar situation occurred with white oak this might explain why Ca, Cl and S were not recovered. Ziegler (1966) showed, however, that ^{35}S was recovered from ray cells of 1 year old Fagus sylvatica near the pith of the tree. Whether recovery would occur in trees at the age of my oaks is not known. Juvenile and adult stages in a tree are different in several characteristics from each other and both oak and beech, according to Robbins (1957) have juvenile and adult forms.

Merrill and Cowling (1966) postulated 2 mechanisms for recovery of N from dying parenchyma cells. Nitrogen was removed along ray parenchyma back towards the cambium or recovered by elution with the transpiration

stream. Some evidence exists to support the first idea. Ziegler (1966) showed that significantly higher amounts of $^{35}SO_4$ were present in ray cells than in vascular elements in 1 year old F. sylvatica until the pith was reached. Activity which remained in ray cells after removal of the soluble ^{35}S did not account for the different levels observed between ray cells and vascular elements. He concluded that translocation of S occurred in ray cells and that removal of S from ray cells at the pith occurred through rays in the tree. A mechanism for transport of elements along rays was not proposed. Retranslocation of P in H. vulgare was from mature to immature leaves, but controlled by the older tissue (Greenway and Gunn, 1966). Whether similar methods for regulation of retranslocation of elements exist in both tree species, is completely speculative.

Recovery of elements from dying cells is not restricted to cells in sapwood. Recovery of nitrogenous and phosphorous compounds has been observed from senescencing leaves of forest trees (Zimmerman, 1964). In annual plants, N, K, P, S, Cl and sometimes Mg were recovered from senescencing tissue (Biddulph, 1959). Calcium and Mn were not recovered. Similarities and differences exist between elements which are recovered and not recovered by the 2 phenomena.

Elements which are required for cellular metabolism were detected in greater amounts in ray cells of sapwood. More P, Mg, Mn, Ca, K, Cl and S were detected in ray cells than in vascular elements. Oxygen was the only element studied which did not have higher levels in ray cells. This element contributes to the woody matrix of the tree and any accumulation in ray cells would probably not be detected.

More Mn, Ca, K, S and Cl were observed in ray cells than in vascular elements in heartwood. Sulfur, Cl and Ca were not recovered from cells before their transformation into heartwood and high levels of these elements in ray cells of heartwood was expected. High Mn and K levels in ray cells of heartwood suggest that recovery of these elements might not have been completed before heartwood formation occurred.

Levels of Ca, Mg and Mn increased from outer to inner heartwood. Merrill and Cowling (1966) observed a similar phenomenon with N. They suggested that diffusion of N from the pith was 1 possible explanation. Cells in annual increments nearest the pith are usually shorter in length and have thinner walls. In these cells, the higher ratio of cytoplásm to cell wall substance would result in increases in levels of N. If a similar situation occurred with Ca, Mg or Mn, either of their suggestions might also explain my results.

Relationships between distribution of elements at radial positions in sapwood were changed after mechanical injury. Phosphorous levels decreased between middle and inner discolored sapwood of white spruce instead of across the entire tissue as in sapwood. In ray cells of white oak injured in spring, P levels were highest in middle discolored sapwood and higher in outer than in inner discolored sapwood. Levels of P in non-ray cells were not changed at different radial positions in discolored sapwood. Levels of P decreased from outer to inner sapwood. Differences in K and Mn levels were not observed at radial positions in discolored sapwood while levels of K increased from outer to inner sapwood and levels of Mn decreased from outer to inner sapwood.

Differences between levels of elements in ray and non-ray cells of sapwood were changed after mechanical injury. In white oak, differences were not observed between levels of K, Mn and Cl in ray and non-ray cells. Differences were detected in sapwood and heartwood.

Selective accumulation of elements might occur in ray cells of discolored sapwood. Greater proportions of P (oaks injured in spring) and Mg (oaks injured in spring, spruces) in discolored sapwood were detected in ray cells than in sapwood. The central role of both

elements in cellular metabolism is well documented (Devlin, 1966). Though highly speculative, this might suggest that increased synthesis of chemical constituents occurred in these cells.

Physiological condition of white oak at the time of mechanical injury might have influenced changes in distribution of P and Mg in ray and non-ray cells of discolored sapwood. Greater proportions of both elements in discolored sapwood were detected in ray cells than in sapwood only when trees were injured in spring.

SUMMARY

I measured levels of inorganic elements, ash content and pH of the cold water extract of discolored sapwood, heartwood and sapwood of white oak and white spruce. Levels of P, Na, Ca, Mg, Mn, Fe, Cu, B, Zn and Al were measured with optical emission and K with flame photometry.

Changes in levels of inorganic elements were detected in both tree species during formation of heartwood and discolored sapwood. Potassium and P decreased in heartwood and discolored sapwood of white spruce, but Mg and Mn decreased only in discolored sapwood. Calcium and Mn were highest in heartwood. Few changes,

however, were significantly different from levels of the same element in sapwood. Potassium increased in discolored sapwood of white oak, but decreased in heartwood. Phosphorous, Mg and Mn decreased in heartwood while Cu, B and Al increased in discolored sapwood. Calcium sometimes increased and decreased in discolored sapwood or decreased in heartwood.

Ash content was highest in heartwood, intermediate in sapwood and lowest from discolored sapwood of white spruce. Differences between tissues were not always significant. The pH of the cold water extracts were similar from sapwood and heartwood and lowest from discolored sapwood. Ash content was highest in discolored sapwood, intermediate in sapwood and lowest in heartwood of white oak. The pH of the cold water extract was highest in discolored sapwood, intermediate from sapwood and lowest in heartwood.

An electron microprobe x-ray analyzer - scanning microscope was used to detect differences in levels of elements at radial positions in sapwood, heartwood and discolored sapwood. Differences in levels of elements in ray and non-ray cells were studied at these radial positions in both tree species. Phosphorous, Ca and Mg were studied in both tree species while Mn, K, Cl, S and O were also studied in white oak.

Chlorine, S, O (white oak) and Mg (white spruce) were not affected by changes in radial positions in sapwood and heartwood. Phosphorous decreased from outer to inner sapwood and Ca was lowest in outer sapwood of both tree species. Magnesium and Mn decreased between inner sapwood and outer heartwood. Potassium increased from outer to inner sapwood, but decreased from inner sapwood to outer heartwood. Calcium, Mg, Mn and K increased from outer to inner heartwood in white oak. In white oak, more P, Mg (DS, SW), Ca, S (DS, SW, HW), Mn, K and Cl (SW, HW) were detected in ray cells than in vascular elements. In white spruce, more P (SW), Ca (SW, HW) and Mg (DS) were detected in ray cells than in vascular elements.

The distribution of elements in ray and non-ray cells at different radial positions in discolored sapwood of white spruce injured in spring and white oak injured in winter was not greatly different from that observed in sapwood. Phosphorous decreased between middle and inner discolored sapwood while Ca was highest in inner discolored sapwood of white spruce. Calcium and Mn were not changed at different radial positions in discolored sapwood of white oak. More drastic changes were detected in discolored sapwood of white oak injured

in spring. In ray cells, more P was detected in middle than in outer discolored sapwood which had more P than inner discolored sapwood. No difference in P was detected at different positions in non-ray cells. Differences in K were not observed at radial positions in discolored sapwood.

Physiological condition of white oak at the time of mechanical injury affected development of discolored sapwood. Increases in K levels and ash content were not observed until 7 months after mechanical injury when trees were wounded in December, but increases in K levels and ash content were observed within 4 months after mechanical injury when trees were wounded in late April.

With respect to the criteria investigated in this study, formation of discolored sapwood and heartwood are different from each other in white oak. Formation of discolored sapwood was sometimes different from heartwood formation in white spruce. Formation of discolored sapwood in white oak is very different from that observed for white spruce.

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PART III

PART III

THE DURABILITY OF SAPWOOD, HEARTWOOD AND DISCOLORED SAPWOOD OF WHITE OAK AND WHITE SPRUCE TO *POLYPOROUS VERSICOLOR* AND *PORIA MONTICOLA*

In most trees, the trunk is composed of sapwood and heartwood. In living trees, sapwood is usually more resistant than heartwood to decay fungi. After harvest, sapwood of many tree species is more susceptible to decay fungi than heartwood. Mechanical injury or attack by microorganisms may cause discoloration to sapwood (discolored sapwood). Discolored sapwood may be more resistant to wood-decay organisms than sapwood.

Considerable variation in decay resistance has been reported for heartwood of *Quercus alba*. The variability (6-36% of oven-dry weight) was as great whether trees were from similar or widely scattered locations (Scheffer, Englerth and Duncan, 1949). The sapwood of *Q. alba* is susceptible to decay (Hart, 1964; Zabel, 1948) while heartwood of *Picea* spp is susceptible to decay (Scheffer and Cowling, 1966).

Discolored sapwood may be more resistant to decay fungi than sapwood. Discolored sapwood of Q. alba, Robinia pseudoacacia and Maclura pomifera was more resistant to decay by Polyporous versicolor than sapwood (Hart and Johnson, 1970). The reaction zone of Fomes annosus-infected Pinus taeda and Picea abies, incipiently decayed wood (both species) and heartwood (P. taeda) were more resistant than sapwood (both species) and heartwood (P. abies) to decay by F. annosus (Shain, 1967; 1971). Discolored sapwood of Juglans nigra and Acer saccharinum, however, was as susceptible as sapwood to decay by P. versicolor (Hart, 1964).

Chemical constituents which are deposited during formation of heartwood and discolored sapwood are thought to be responsible for the increase in decay resistance of both tissues. Removal of methanol-soluble compounds from heartwood of several eucalypts increased the susceptibility of this tissue to decay fungi (Rudman and Da Costa, 1961). Compounds which were soluble in ether and methanol appeared to be responsible for the durability of Tectona grandis heartwood to decay organisms (Rudman and Da Costa, 1959). The hot water extract from heartwood of Q. alba tested in vitro was toxic to linear growth of Lenzites trabea (Zabel, 1948). Extraction of resistant tissues of F. annosus-infected P.

taeda with acetone increased their susceptibility to decay by F. annosus (Shain, 1967). Shain and Hillis (1971) suggested that hydroxymatairesinol and the alkaline conditions of the reaction zone of F. annosus-infected P. abies contributed to the durability of this tissue to F. annosus.

I studied the development of decay-resistant discolored sapwood in white oak (Quercus alba L.) and white spruce (Picea glauca (Moench) Voss). One objective of this study was to determine whether mechanical injury to sapwood of white spruce produced discolored sapwood that was more resistant to decay than sapwood and heartwood. Another objective was to determine the effect of physiological condition of white oak at the time of mechanical injury on the development of decay-resistant discolored sapwood. Recently, Hart and Hillis (unpublished data) suggested that the durability of heartwood of Q. alba to P. monticola appeared to result from the presence of several ellagitannins in this tissue. The role of ellagitannins in the durability of discolored sapwood to P. monticola was also investigated.

MATERIALS AND METHODS

Location of trees, method of mechanical injury and initial preparation of wood samples are described in Part I. White oaks and white spruces which were used in Part II were also utilized in this investigation. Information about these trees is presented in Table 22 in Part II.

Measurement of Durability of Discolored Sapwood, Sapwood and Heartwood

The resistance of discolored sapwood, sapwood and heartwood to decay was measured with the agar block method (McNabb, 1958). Tissues of white oaks and white spruces were exposed to Polyporous versicolor L. ex Fr. (USDA isolate Madison 697), a white-rot organism, and Poria monticola Murr. (USDA isolate Madison 698), a brown-rot organism.

Blocks were dried for 14 days at 40 C and weighed to determine the initial dry weight of the blocks. After incubation with the fungus, blocks were removed from the decay chambers and the fungus was carefully scraped from the blocks. Blocks were immediately weighed to determine their moist weight. The final dry weight was determined after drying the blocks for 48 hours at 105 C. Weight loss was expressed as percent

of the initial oven-dry weight of the block. Blocks which were not exposed to the decay fungi served as controls.

Blocks of each tissue, approximately 15 x 8 x 8 mm in size, were cut with the longest dimension parallel to the radial axis of the tree. The blocks were autoclaved for 5 minutes at 121 C and placed aseptically into the decay chambers 14 days after the chambers had been inoculated with the test organism. The decay chambers were incubated at 26 C for 6 weeks.

Eight oz. French square bottles capped with unlined aluminum lids were used for decay chambers. Each bottle contained 32 ml of 3% (w/v) malt extract and 1.5% (w/v) agar in distilled water. Bottles were autoclaved at 121 C for 40 minutes, cooled to room temperature and inoculated with a small piece of mycelium from the outer margin of a 2 week old culture of the test organism. A 4 mm glass rod was placed on the agar to support the block above the medium.

Extraction of Tissue of White Oak for Ellagitannins

White oak 11, 12, 13 and 14 (Part II, Table 22) were used to investigate the distribution of ellagitannins. Sapwood, heartwood and discolored sapwood of white oak injured in spring (11, 12) were bulked separately while sapwood and heartwood of white oak injured in

winter (13, 14) were bulked separately. Discolored sapwood of these trees was extracted individually because previous work (Part II) had showed that these tissues were different from each other. Oven-dried material prepared as described in Part I was used.

Four g of each tissue were extracted for 48 hours on a "wrist-action" reciprocal shaker with 250 ml of acetone, water (9:1 v/v). The extraction was repeated 2 more times and the extracts were combined. They were reduced to 200 ml in a rotary evaporator at 40 C. Extracts were washed with six 50 ml portions each of hexane, chloroform and ethyl acetate and the washings were discarded. Extracts were reduced to near-dryness in a rotary evaporator at 40 C and brought to 1 ml with acetone, water (9:1 v/v).

Two-dimensional paper chromatography was used to study the ellagitannins. Fifty microliters of each extract were applied with a micropipette onto Whatman 3 MM chromatography paper. Papers were irrigated in the first direction with 6% acetic acid and in the second direction with butanol, 27% acetic acid (1:1). Chromatograms were viewed under shortwave ultraviolet light and sprayed with 0.05% p - nitrobenzediazonium tetrafluoborate (pNA) and oversprayed with 20% sodium

carbonate. Chromatograms were also sprayed with NSSC (15 g Na_2SO_3: 3.5 Na_2CO_3: 350 ml H_2O) and examined at 3 minutes and 12 hours after spraying. The R_f value and color reaction of each ellagitannin was determined.

RESULTS AND DISCUSSION

Durability of Discolored Sapwood, Sapwood and Heartwood to Polyporous versicolor and Poria monticola

Discolored sapwood from both tree species was more resistant than sapwood (oak) or sapwood and heartwood (spruce) to decay by P. versicolor and P. monticola (Tables 47, 48). My results with white oak are the same as those reported previously with this species (Hart and Johnson, 1970). Shain (1971) reported that the reaction zone of P. abies produced in response to fungus invasion and mechanical injury to sapwood was more resistant to decay by F. annosus than sapwood and heartwood. My results show that mechanical injury to sapwood of white spruce also produced discolored sapwood more resistant than sapwood and heartwood to decay by at least 2 decay fungi. The mechanism for resistance in each tissue is very different (Appendix).

Physiological condition of white oak at the time of mechanical injury influenced the development of decay-resistant discolored sapwood. When trees were

TABLE 47.--Weight loss (% of oven-dry weight) of sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white spruce after 6 weeks exposure to Poria monticola and Polyporous versicolor.[1]

		Poria monticola			Polyporous versicolor		
No. of Trees	Injured Harvested	SW[2]	HW	DS	SW	HW	DS
4	4-70 8-70	44.3 a	48.1 a	29.4 b	17.1 a	18.3 a	3.4 b
2	4-70 1-71	38.2 a	41.2 a	17.5 b	7.5 a	7.4 a	1.9 b

[1]Means of 3 determinations of DS and 4 determinations of SW and HW from a tree.

[2]For each set of values, averages followed by different letters differ significantly (0.05 level).

TABLE 48.--Weight loss (% of oven-dry weight) of sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white oak after 6 weeks exposure to Poria monticola and Polyporous versicolor.[1]

		Poria monticola			Polyporous versicolor		
No. of Trees	Injured Harvested	SW [2]	HW	DS	SW	HW	DS
4	12-69 4-70	21.8 a	9.9 b	29.1 a	15.8 a	3.0 a	20.6 a
1	12-69 6-70	25.7 a	12.1 b	19.5 a	30.2 a	11.8 b	34.4 a
1	12-69 7-70	19.8 a	12.9 b	3.9 c	28.7 a	10.7 b	21.0 c
1	12-69 1-71	24.8 a	7.5 b	21.8 a	32.7 a	7.8 b	20.9 c
1	12-69 1-71	28.7 a	10.5 b	0.3 c	36.0 a	11.9 b	15.5 b
4	4-70 8-70	20.0 a	12.1 b	1.0 c	39.2 a	6.8 b	18.8 c
2	4-70 11-70	12.6 a	4.7 b	3.0 b	42.6 a	6.8 b	29.6 c

[1]Means of 3 determinations of DS and 5 determinations of SW and HW from a tree.

[2]For each set of values, averages followed by different letters differ significantly (0.05 level).

wounded in December, discolored sapwood durable to both decay fungi was not observed until 7 months after mechanical injury while durable discolored sapwood was observed within 4 months after mechanical injury when trees were wounded in late April (Table 48). Other aspects about this subject are presented in Part II.

The effect of season on formation of discolored sapwood has been investigated with Pinus sylvestris (Lyr, 1967) and Pinus resinosa (Jorgensen, 1961). In P. sylvestris, formation of discolored sapwood (resin flow and accumulation of pinosylvin) occurred between late April and early December. In P. resinosa, formation of discolored sapwood (accumulation of pinosylvin) occurred in November and January, but not in June and July. In both cases, the durability of discolored sapwood was not evaluated. My results appear to be similar to those observed with P. sylvestris. Formation of discolored sapwood in white oak, however, was much different from that observed in P. sylvestris (Lyr, 1967).

Discolored sapwood from white oak was more resistant to P. monticola than to P. versicolor while the reverse was true for white spruce. Rudman (1963) showed that most extractives toxic to wood-decay fungi

had a limited spectrum of activity. If extractives were responsible for durability of discolored sapwood in either or both tree speices, they might be less effective against one of the wood-decay organisms. Mechanisms other than extractives may contribute to the durability of discolored sapwood. As with extractives, they could be less effective against one of the wood-decay fungi.

Distribution of Ellagitannins in Tissues of White Oak

Four ellagitannins were detected in tissues of white oak. The R_f value and color reaction of each compound are given in Table 49 and the distribution of these compounds in the 3 tissues of the tree is presented in Table 50. Three of the 4 ellagitannins were detected in sapwood and discolored sapwood (one exception) and all 4 ellagitannins were detected in heartwood. The color intensity of ellagitannins in heartwood extracts was greater than observed for the other tissues. These compounds are the same as those detected in heartwood of Q. alba (Hart, personal communication) and reported to contribute to the durability of this tissue to P. monticola (Hart and Hillis, unpublished data).

TABLE 49.--Characteristics of the ellagitannins from woody tissue of white oak.[4]

Compound Number	Butanol, 27% Acetic Acid, Water (1:1)	6% Acetic Acid	UVS[1]	NSSC[2]		pNA[3]	
				3 mins	12 hrs	before overspray	after overspray
TZ	.05	.35-.55	P	P to PkR	pT	Br	T
1	.20	.47	P	Pk to RO and OBr	GrBr	Br	T
2	.17	.54	P	PBr to P	pRBr	Br	T
3	.14	.58	P	P to PR	GrBr	Br	T

[1]ultraviolet light - 2537 A

[2]NSSC - 15 g NA_2SO_3; 3.5 g Na_2CO_3; 350 ml water

[3]pNA - 0.05% p-nitrobenzendiazonium tetrafluoborate in water

[4]Color abbreviations: P - purple; Pk - pink; R - red; O - orange; Br - brown; T - tan; Gr - grey; p - pale.

TABLE 50.--Distribution of ellagitannins in sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white oak.

Compound	SW(S)[1]	HW(S)	DS(S)	SW(W)	HW(W)	DS(W1)	DS(W2)
1	P[2]	P	P	P	P	P	A
2	P	P	P	P	P	P	A
3	P	P	P	P	P	P	A
TZ	A	P	A	A	P	A	A

[1](S) - injured in spring; (W) - injured in winter: SW, HW and DS of 2 trees wounded in spring bulked while SW and HW of 2 trees wounded in winter bulked, but DS of these trees extracted separately. Previous decay studies showed that SW from white oaks was susceptible, HW was resistant and DS resistant (S, W2) or susceptible (W1) to decay by Poria monticola and Polyporous versicolor.

[2]P - detected; A - not detected

My results show that the presence of ellagitannins is not necessary for discolored sapwood to be durable to P. monticola. Discolored sapwood of 1 white oak (W 2) was very resistant to decay by P. monticola (weight loss of 0.3%), but ellagitannins were not detected in this tissue. This observation suggests that the 4 ellagitannins are not responsible for formation of discolored sapwood that is resistant to decay by P. monticola.

Discolored sapwood of white oak was more resistant to decay by P. monticola when the pH of the cold water extract from this tissue was above 6.0 (Table 51). A liquid medium was used to measure the effect of pH upon in vitro growth of this fungus. The buffered (0.05 M) medium contained (mg/l): glucose, 20,000; asparagine, 2000; KH_2PO_4, 1000; $MgSO_4 \cdot 7H_2O$, 500; biotin, 0.005; thiamine, 0.1. Two ml of a micronutrient solution which contained (mg/l): $Fe(NO_3)_3 \cdot 9H_2O$, 723.5; $ZnSO_4 \cdot 7H_2O$, 439.8; $MnSO_4 \cdot 4H_2O$, 203.0 were added and the medium was brought to 1 liter with distilled water. Portions of the medium were adjusted to the proper pHs with NaOH or 6 N HCl and sterilized by millipore filtration. Twenty-five ml of medium were added to 300 ml erlenmyer

TABLE 51.--Relationship between pH of the cold water extract and decay resistance of discolored sapwood of white oak.[1]

Characteristic	Tree	SW	DS	HW[2]
pH	11, 12	5.7 a	6.2 b	4.4 c
	13	5.9 a	4.5 b	4.1 c
	14	6.0 a	6.8 b	4.1 c
Weight loss (% of oven-dry weight)	11, 12	12.6 a	3.0 b	4.7 b
	13	24.8 a	21.8 a	7.5 b
P. monticola	14	28.7 a	0.3 b	10.5 c
Weight loss (% of oven-dry weight)	11, 12	42.6 a	29.6 b	6.8 c
	13	32.7 a	20.9 b	7.8 c
P. versicolor	14	36.0 a	15.5 b	11.9 b

[1]white oaks 11, 12 - injured in spring; white oaks 13, 14 - injured in winter

[2]SW - sapwood; HW - heartwood; DS - discolored sapwood; For each set of values, averages followed by different letters differ significantly (0.05 level).

flasks and the flasks were inoculated with 1 ml of mycelial suspension (aerial mycelium ground in 100 ml of sterile distilled water). Flasks were incubated on laboratory shelves up to 3 weeks at 26 C.

At 7 day intervals, growth of P. monticola was measured by filtering cultures through tared Whatman No. 3 filter paper which was previously dried for 12 hours at 40 C, oven-dried for 1.5 hours at 105 C, cooled in a desiccator over indicating Drierite for 15 minutes and weighed. The filtered medium was saved and the pH measured with a Beckman zeromatic pH meter.

Poria monticola grew between pH 3.5-6.0. Best growth was achieved when the fungus was able to reduce the pH of the medium during the incubation period (Table 52). These results suggest that high pH might play some role in the development of decay-resistant discolored sapwood of white oak to P. monticola. The effect of high pH on fungus growth in vitro, however, may be very different from that observed in vivo. Additional data is needed before firm conclusions are possible. High K levels appear to be primarily responsible for the high pH of the cold water extract from discolored sapwood (Part II).

TABLE 52.--Growth of Poria monticola in a buffered, defined liquid medium.[1]

Buffer	Initial[2] pH	7 Days[3] Growth	7 Days[3] pH	14 Days Growth	14 Days pH	21 Days Growth	21 Days pH
Tartaric Acid 0.05 M	3.5	34	3.3	41	3.2	46	3.2
	4.0	36	3.9	45	3.8	58	3.8
	4.5	36	4.2	45	4.2	79	4.3
	5.0	35	4.6	49	4.4	91	4.4
MES[4] 0.05 M	5.5	31	4.9	99	3.6	107	4.0
	6.0	0	5.8	55	5.5	95	3.7
Phosphate[5] 0.05 M	6.0	0	6.0	0	5.8	44	5.7
	6.5	0	6.5	0	6.5	0	6.5
	7.0	0	7.0	0	7.0	0	7.0

[1]growth: mg of oven-dry weight of mycelium.

[2]pH adjusted with 6 N HCl (tartaric acid) or NaOH (MES, phosphate)

[3]means of 3 determinations at each pH

[4]MES - 2-(N-morpholino) ethanesulfonic acid

[5]phosphate buffer - stock solution of sodium phosphate, dibasic and potassium phosphate, monobasic

Discolored sapwood was resistant to decay (0.05 level) by P. versicolor when the pH of the cold water extract from this tissue was 4.5 (Table 51). This observation suggests that the agent(s) responsible for durability of discolored sapwood to P. monticola and P. versicolor may be different. Different factor(s) appear to be responsible for the durability of heartwood of Q. alba to P. monticola and P. versicolor (Hart and Hillis, unpublished data).

SUMMARY

The durability of discolored sapwood of white oak and white spruce to Poria monticola and Polyporous versicolor was measured. The agar-block method was used. Discolored sapwood from both tree species was more resistant than sapwood (oak) or sapwood and heartwood (spruce) to decay by both decay fungi.

Physiological condition of white oak at the time of mechanical injury influenced the development of decay-resistant discolored sapwood. Durability of discolored sapwood to both decay fungi was not observed until 7 months after mechanical injury when trees were wounded in December, but was observed within 4 months after mechanical injury when trees were wounded in late April.

The distribution of ellagitannins in tissues of white oak was studied with two-dimensional paper chromatography. Four compounds were detected in heartwood and 3 were detected in sapwood and discolored sapwood (one exception). Ellagitannins were not necessary for discolored sapwood to be durable to P. monticola.

High pH levels of discolored sapwood of white oak might contribute to the durability of this tissue to P. monticola. Growth of the fungus in vitro did not occur above pH 6.0. Resistance of discolored sapwood to decay by P. monticola was observed when the pH of the cold water extract from this tissue was above 6.0.

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APPENDIX

APPENDIX

ANALYSIS OF LIGNANS IN WHITE SPRUCE WITH GAS CHROMATOGRAPHY

Samples of sapwood, heartwood and discolored sapwood were cut from white spruces A and B (Part II, Table 22). Each wood sample was dried on a laboratory bench for 72 hours and ground in a Wiley mill to pass through a 2 mm mesh screen. Preliminary experiments indicated that the high resin content of discolored sapwood interfered with the chromatographic analysis of lignans. Hence each sample was first extracted for 6 hours in a Soxhlet apparatus with n-heptane before being extracted with methanol. The percentage of each sample which was soluble in the 2 solvents was determined (Table 53).

Quantitative estimates of lignans present in each tissue were made by GLC using a Varian 2100 gas chromatograph with flame ionization detectors. A glass column 2 meters long of 3 mm inside diameter packed with 3.6% Apiezon L on 80-100 mesh DMCS Chromosorb W was used. The carrier gas (nitrogen) flow rate was

TABLE 53.--Heptane and methanol solubilities of white spruce wood (% oven-dry basis).

Tissue[1]	Heptane	Methanol
A-SW	2.0	3.6
A-HW	1.2	1.15
A-DS	29.3	6.2
B-SW	2.4	3.4
B-HW	1.7	1.42
B-DS	34.0	10.0

[1]SW - sapwood; HW - heartwood; DS - discolored sapwood

50 ml/min, while hydrogen and air flow rates were 35 ml/min and 350 ml/min. Detector and injection temperatures were 250 C and initial oven temperature (200 C) was increased 1 C per min to a final temperature of 240 C.

Seventy microliters of TMS mixture-hexamethyldisilazane:trimethylchlorosilane:pyridine (2:1:10 v/v/v) - and 30 microliters of BSA (N,O-bis (tri-methylsilyl) acetamide) were successively added to a known amount (approximately 1 mg) of the vacuum dried methanol extract. This mixture was briefly heated with a match and after 15 min 5 microliters were injected into the chromatograph. Known amounts of matairesinol, liovil and conidendrin were silylated in a similar fashion for calibration purposes. Previous work (Shain and Hillis, 1971; Krahmer, Hemingway and Hillis, 1970) had shown that calibration curves for these lignans showed a linear response over the range of concentration that lignans were detected in wood. Authentic hydroxymatairesinol and pinoresinol were also used to establish relative retention times (RRT). The 5 authentic silylated lignans were used as markers both alone and in combination with the various silylated methanol extracts. RRT for the silylated lignans were: hydroxymatairesinol 1.00; liovil 0.80; matairesinol 1.13; conidendrin 1.28; pinoresinol 1.68. Authentic lignans and extracts were also run under previously reported

chromatographic conditions (Shain and Hillis, 1971; Krahmer, Hemingway and Hillis, 1970) for comparative purposes. Two or more analyses were made of each sample.

Compounds with the same retention time as authentic silylated liovil, hydroxymatairesinol, conidendrin and pinoresinol (trace amounts) were present. Matairesinol was not present in detectable amounts. Relative values for peak areas for liovil, hydroxymatairesinol and conidendrin are given in Table 54.

There is no indication from this work that mechanical injury to the sapwood of white spruce resulted in an increase in hydroxymatairesinol or in any of the other lignans studied. At a concentration of approximately 0.10%, hydroxymatairesinol appears to have little effect on the decay resistance of the wood against Poria monticola and Polyporous versicolor. Shain and Hillis (1971) did report that 0.1% of hydroxymatairesinol in an agar medium reduced growth of Fomes annosus by 25%. The increased decay resistance shown for discolored sapwood of white spruce must be due to some other factor than lignan concentration as the decay susceptible sapwood and heartwood contained the same lignans in approximately the same concentration as did the injured tissue. The response of white spruce sapwood to mechanicl injury

TABLE 54.--Relative concentration of hydroxymatairesinol, liovil and conidendrin in the sapwood (SW), heartwood (HW) and discolored sapwood (DS) of white spruce.

	A			B		
	SW	HW	DS[2]	SW	HW	DS[2]
Hydroxy-matairesinol	172[1]	72	67	215	142	207
Liovil	39	34	22	54	47	45
Conidendrin	20	29	25	4	32	50

[1]A value of 170 is equal to 0.10% of the oven-dry wood.

[2]Amount of liovil in DS was difficult to measure accurately because of other compounds with similar retention times.

(no significant changes in lignans present and acid pH) is in marked contrast to the response of Picea abies sapwood to invasion by F. annosus (large increases in hydroxymatairesinol and alkaline pH) as reported by Shain and Hillis (1971). They also reported that hydroxymatairesinol and conidendrin were not present in detectable quantities in the sapwood of P. abies, but liovil was present in similar amounts to those reported here for white spruce.

The values reported in this study for the heartwood of white spruce agree very closely with the values reported for the heartwood of P. abies by Freudenberg and Knof (1957). They are also quite similar to the data reported by Goldschmid and Hergert (1961) for the sapwood of Tsuga heterophylla. Hydroxymatairesinol (0.255%) was found to be present in a ratio of about 5:1 to that of conidendrin (0.05%). Only a trace of pinoresinol (0.009%) and no matairesinol were present. Freudenberg and Niedercorn (1958 as reported by Goldschmid and Hergert) stated that the cambial sap of P. abies contained hydroxymatairesinol and pinoresinol.

Weinges (1960) reported both quantitative and qualitative changes in wound resin lignan composition of P. abies after injury while Shain and Hillis (1971) reported only an accumulation of normal heartwood lignans

after attack by F. annosus. In this study, neither quantitative or qualitative differences were observed in response to injury. Hence this work is additional evidence to support the hypothesis that the accumulation of lignans in plant tissue is quite variable as previously reported by Krahmer, Hemingway and Hillis (1970).

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