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ABSTRACT

CONVERSION OF SAPWOOD TO NORMAL HEARTWOOD AND DISCOLORED SAPWOOD IN SWAMP WHITE OAK, QUERCUS BICOLOR WILLD.

By

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The object of this investigation was to compare the sequence of events in the conversion of sapwood to heartwood and to discolored sapwood in swamp white oak, Quercus bicolor Willd., using cytological and histochemical techniques. An increment borer was used to initiate the formation of discolored sapwood.

In the sapwood-heartwood transition zone, starch grains were absent. Starch grains disappeared from cells in the discolored sapwood and from cells of the sapwood immediately adjacent to the discolored area eight days after wounding. Lipids occurred in moderate amounts in the sapwood and transition zone, and were found in trace amounts in the heartwood. Lipids decreased to trace amounts in the discolored sapwood.

In heartwood formation, the nuclei became oblong to egg-shaped in the transition zone. Nuclei appeared larger and nucleoli more distinct than in the annual rings of the middle sapwood. At the heartwood boundary, the nucleolus disappeared and the nucleus began to degenerate. With the

formation of discolored sapwood, nuclei appeared to round-up or assumed an oblong shape after the disappearance of starch and after the cells had lost their ability to reduce triphenyl tetrazolium chloride. The nucleoli were quite evident in these nuclei. In other instances, degeneration of the nucleus was observed similar to that observed in the formation of heartwood. No increase in the size of nuclei was noted in the formation of discolored sapwood.

Tyloses were present in the springwood vessels in all annual rings except the outermost annual rings of sapwood. Tannins occurred in moderate amounts at the heartwood boundary, but decreased slightly moving towards the pith. Tannins were absent in the sapwood and increased to moderate amounts in the discolored sapwood. Catechol and catechol derivatives were absent from the sapwood, occurred in moderate to abundant amounts at the heartwood boundary and occurred in trace amounts in the heartwood. They developed in the discolored sapwood between 12 and 16 days after wounding and disappeared immediately prior to the accumulation of amorphous deposits in large amounts.

Amorphous deposits occasionally occurred in trace amounts in the heartwood, but accumulated in copious amounts in the discolored sapwood. The accumulation of amorphous deposits preceded the disintegration of the nucleus in the formation of discolored sapwood. In heartwood formation,

extractives are deposited after the disappearance of the nucleus.

Parenchymatous cells within the heartwood and in the annual ring of sapwood adjacent to the heartwood boundary were unable to reduce the triphenyl tetrazolium chloride to formazan. In the discolored sapwood, cells were not capable of reducing triphenyl tetrazolium chloride eight days after wounding.

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AND DISCOLORED SAPWOOD IN SWAMP WHITE
OAK, QUERCUS BICOLOR WILLD.

By

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Dedicated

to

Sandra Jean

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INTRODUCTION

The study of the formation of heartwood is difficult because of its central location in the tree. Mechanical injury to the sapwood may result in discoloration. The damaged cells darken prematurely and resemble heartwood in color. If the sequence of events in the conversion of sapwood to discolored sapwood were similar to that of sapwood to heartwood, information about the transformation to heartwood could be gathered using discolored sapwood. A review of literature indicates that studies of the transformation processes for both tissues have (1) been largely ignored and (2) if studied, conducted independently from each other.

The purpose of this investigation was to compare changes in cells of the sapwood during their conversion to heartwood and to discolored sapwood in a single species of tree using cytological, anatomical and histochemical studies.

LITERATURE REVIEW

Heartwood is composed of dead cells that originate from internal physiological processes. The presence or absence of color does not indicate the presence or absence of heartwood. Discolorations initiated through external stimuli are termed wound-initiated discolorations (Shigo, 1965). These may be centrally located within the tree, false heartwood, or originate in the sapwood, discolored sapwood. False heartwood can only occur in trees lacking normal heartwood. Discolored sapwood can form in trees containing normal heartwood, and results from an injurious disturbance which kills the parenchymatous cells in the sapwood. The injured tissue darkens prematurely and resembles normal heartwood in appearance. Whether false heartwood formation is similar to the formation of discolored sapwood is not known.

The factor responsible for initiating the series of events which leads to the formation of heartwood is unknown. A variety of factors has been suggested which initiate the transformation process, but none has conclusive support.

Stewart (1966) states that the heartwood is a depository for excretions from living cells in the sapwood. These extraneous materials, toxic or inhibitory with respect

to the sapwood, are translocated to the center of the tree, accumulate to lethal concentrations, and cause the death of living cells. The continued translocation of excretions results in outward movement of the sapwood-heartwood boundary.

Erdtman (1955) believes that heartwood formation is directed from the cambium since no heartwood is formed when the cambium is damaged. Compounds are produced and transported from the cambium via the vascular rays to the dead portion of the tissue.

A second hypothesis concerning heartwood formation is that the polyphenols are not translocated from the cambial region but are formed in situ and that their formation is intimately connected to the cytological breakdown of the ray cells. Investigations with European timbers indicate that mitochondria retain their reductive capacity only in the outermost sapwood rings, and that starch grains and nuclei disappear in the transition zone. Aerobic respiration ceases and dehydrogenases favor the production of colorless phenols. After the starch disappears, the enzyme system of the ray cells is altered, allowing phenols which diffuse radially inward from the inner sapwood to be oxidized. The colorless phenols are oxidized with the passage of time. (Frey-Wyssling and Bosshard, 1959)

Assembling single observations from separate investigations indicates the possible sequence of the cytological

breakdown of the ray and xylem parenchyma during heartwood formation. Starch grains are considered to be the raw material and energy source for extractive formation. They are the first structure to disappear from the ray and xylem cells and the disintegration of the cell nucleus appears to be the last step prior to heartwood formation (Fahn and Arnon, 1963). Much current evidence indicates that the nucleus disintegrates prior to the formation of extractives in large amounts (Hillis and Inoue, 1966). Extractives originate in the parenchyma tissue; during or after cell death they are capable of migrating to adjacent tissue (Sachs et al., 1966). The differences in the polyphenols of the sapwood, heartwood, and wound sapwood support in situ formation of the polyphenols in response to certain stimuli (Hillis, 1967).

The isolation of fungi from the heartwood of myrtle beech, Nothofagus cunninghamii Oerst, and yellow carbeen, Sloanea woollsii F. Muel, led Chattaway (1952) to believe that the primary stimulus in the sapwood-heartwood transformation 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.

Respiration studies utilizing pedunculate oak indicated a zone of increased respirational activity in the inner sapwood. The increase in respirational activity was followed with a sharp decrease in respirational activity at the sapwood-heartwood boundary. This zone of intensified respiration was considered to be "the site of the sapwood-heartwood transformation." (Zelawski, 1960) However studies with European timbers indicated that the transition zone was not a zone of intensified metabolism, but rather a region of altered metabolism (Frey-Wyssling and Bosshard, 1959).

In trees lacking a clear demarcation between the sapwood and heartwood, a gradual darkening of the sapwood causes the central core of the tree to have a pronounced deeper color than the outer surrounding wood. The dark central core may contain starch and living cells but shows other features associated with normal heartwood.

Yazawa and Ishida (1965) noticed a zone of intermediate wood between the sapwood and heartwood in some Japanese timbers. In comparison to the sapwood, nuclei were smaller, the concentration of DNA in them increased and RNA was almost undetectable in the intermediate zone (Higuchi et al., 1964; Fukazawa and Higuchi, 1966). Investigations of the transition zone of "tawa," Beilschmiedia tawa (A. Cunn.) Benth et Hook. f. ex Kirk, illustrated a tendency for the cell nucleus to increase in size in

the transition zone and therefore indicate an increase in cell activity (Bosshard, 1968).

In the literature, red heart, pathological heartwood, brown-heart, and frost heart appear as synonyms for false heartwood. Most studies of false heartwood have been with European beech. False heartwood formation has been attributed to external oxidizing agents (Zycha, 1958; Buchholz, 1958; Bosshard, 1965), wounding stimuli (Paclt, 1953; van der Meiden, 1959; Schopfer, 1961), severe cold (Larsen, 1943), and micro-organisms (Champbell and Davidson, 1941; Siegle, 1967; Ohman, 1968). Jorgensen (1962) and Dietrichs (1964) consider false heartwood formation identical with normal heartwood formation.

Necasany (1956) summarized the terminology and reconciled the varied explanations for the cause of false heartwood. He concluded that formation of false heartwood is different from the formation of normal heartwood. In the formation of false heartwood, cell death is rapid, extractives coagulate in the lumen of cells, and the vitality of the cells is high at the time of their conversion to heartwood. In the formation of normal heartwood, cell death results from natural aging, extractives impregnate the cell wall, and the vitality of the cells is very low at the time of their conversion to heartwood.

Air moisture relationships have been considered responsible for the formation of red heart in European beech.

Abnormal withdrawals of moisture reserves result in the inflow of atmospheric oxygen into the tissue. The death of the parenchyma cells and the formation of tyloses results from the continued withdrawal of moisture and accumulation of atmospheric oxygen in the vessel elements. Characteristic discolorations are not dependent on fungal activity, rather they are caused by oxidative processes. Age of the tree, site factors, and climate are important factors in the formation of red heart of European beech. (Zycha, 1948)

The concern for wounds as entrance courts for decay organisms has encouraged the study of induced formation of discolored sapwood, primarily with increment borers (Meyer and Haywood, 1936; Campbell, 1939; Lorenz, 1944; Hepting et al., 1949; Toole and Gammage, 1959). In diffuse-porous species, extensive vertical discoloration of one to several feet was observed; in ring-porous species, vertical discolorations were limited to a few inches. In both instances, little or no horizontal extension of the discolored area occurred beyond the diameter of the borer hole (Hepting et al., 1949).

Some investigators considered discolored sapwood to be identical with normal heartwood (Meyer and Haywood, 1936; Campbell, 1939; Chattaway, 1952; Esau, 1953; McNabb et al., 1959; Jorgensen, 1962). Hepting and Blaisdell (1936) considered the protective zone which develops in red gum to be similar to heartwood. Shain (1967) reached a similar

conclusion in studying the attack by Fomes annosus on loblolly pine. Other investigators were noncommittal on this point (Lorenz, 1944; Hepting et al., 1949; Roth, 1950; Toole and Gammage, 1959). Discolored sapwood is similar in morphological characteristics to normal heartwood, but differs significantly in chemical characteristics from normal heartwood (Hart, 1965, 1968). He concluded that "discolored sapwood should be considered a distinct, unique tissue, and not a precocious development of heartwood."

Erdtman (1955) believed that polyphenols deposited in the wound heartwood "were similar to those accumulating in the heartwood." Hillis (1967) reported that polyphenols formed in situ in the wound heartwood were different from those forming in situ in heartwood.

Some authors consider micro-organisms responsible for some discolorations of the sapwood. More than one-fourth of the isolations from the discolored sapwood of yellow-poplar yielded bacteria, but very few yielded fungi (Roth, 1950). Micro-organisms 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 nature of the wounding stimulus appears immaterial (McNabb et al., 1959; Hart, 1965).

Sucoff et al. (1967) studied the sequence of events in Populus tremuloides Michx. for the first 10 days after wounding. Nuclei of some cells were reduced in size 2 days after wounding, tissue browning was evident 7 days after wounding, and 8 days after wounding many of the ray cells were dead and phenols had begun to accumulate in these dead cells. Lipids disappeared within the period of investigation. In spite of these alterations, most cells remained unchanged during the 10-day period after wounding. Discoloration advanced fastest near the heartwood boundary, but the nucleus had begun to disintegrate in these cells through natural aging. Pigments which result in discoloration of the sapwood appeared to originate from parenchyma cells with coincident disintegration of the nucleus and cytoplasm. Superficially, at least, the discoloration processes in the sapwood resemble heartwood formation as described by Frey-Wyssling and Bosshard (1959).

MATERIALS AND METHODS

A ring-porous species, swamp white oak (Quercus bicolor Willd.), was used to compare the sequence of events in the formation of heartwood and discolored sapwood. The wood lacks characteristic odor or taste, is usually straight-grained, heavy to very heavy in weight, and hard to very hard (Panshin and DeZeeuw, 1964). Trees were located on poorly-drained soil approximately 20 miles north of Lansing, Michigan. All trees were dominant or co-dominant within the forest stand.

Three series of 8 trees each were studied. Series A included trees felled 0, 2, 4, 6, 8, 10, 12, and 14 days after wounding and series B and C included trees felled 0, 4, 8, 12, 16, 20, 24, and 28 days after wounding. The number of rings of sapwood, of heartwood, date bored, and date felled were recorded for series A, B, and C; in addition, diameter of the tree was recorded for series B and C (Table 1). Four holes were bored with an increment borer between 4.5 and 5.5 feet above the ground and all wounds were left unplugged. Sterile technique was not employed. In some cases, the increment borer penetrated the heartwood.

Samples of sapwood, heartwood, and discolored sapwood were collected from the same transverse section removed

TABLE 1.--Characteristics of trees used for cytological and histochemical studies and measurements of oxygen consumption.

Series	Tree No ^a			Rings SW ^b	Rings HW ^c	DBH ^d
A	0	7/23/68	7/23/68	6	11	-
	2	7/9/68	7/11/68	6	14	-
	4	7/9/68	7/13/68	6	11	-
	6	7/9/68	7/15/68	6	9	-
	8	7/9/68	7/17/68	7	9	-
	10	7/9/68	7/19/68	6	11	-
	12	7/9/68	7/21/68	7	9	-
	14	7/9/68	7/23/68	6	7	-
B	0	8/4/68	8/4/68	10	10	4.7
	4	8/4/68	8/8/68	7	9	6.3
	8	8/4/68	8/12/68	7	9	3.9
	12	8/4/68	8/16/68	7	14	4.0
	16	8/4/68	8/20/68	7	11	3.7
	20	8/4/68	8/24/68	8	14	4.0
	24	8/4/68	8/28/68	7	15	6.0
	28	8/4/68	9/1/68	7	10	4.5
C	0	10/6/68	10/6/68	-	-	-
	4	10/10/68	10/14/68	10	20	4.5
	8	10/10/68	10/18/68	7	16	4.0
	12	10/10/68	10/22/68	7	19	3.7
	16	10/10/68	10/26/68	8	16	4.0
	20	10/10/68	10/30/68	7	11	4.0
	24	10/10/68	11/3/68	7	14	4.0
	28	10/10/68	11/7/68	7	11	3.8

^aDays after wounding the tree was felled.

^bSapwood.

^cHeartwood.

^dDiameter of tree at breast height expressed to nearest one-tenth inch.

from the tree. The fourth annual ring of sapwood, the transition zone between the sapwood and heartwood, and fourth annual ring in from the heartwood boundary were used to study the formation of heartwood and discolored sapwood. Discolored sapwood tissue was removed from immediately below the borer hole. Fresh sections of tissue were cut on a sliding microtome 10 to 20 microns in thickness, mounted in 50% glycerine-gelatin, observed under a light microscope, and photographed.

Presence of Starch

Fresh radial samples were placed in a 70% ethanol solution containing 2% iodine-potassium iodide for 3 to 5 minutes. The appearance of a deep purple color indicates the presence of starch.

Presence of Tannins (unspecific)

Small blocks of fresh material were placed in a 0.1N HCL solution containing 0.5 to 1.0% ferric sulfate. After 3 to 5 minutes the blocks were removed and sliced into sections with a razor blade. A blue precipitate indicates the presence of tannins. (Reeve, 1959)

Presence of Catechol or Catechol Derivatives

The nitroso reaction was used to detect the presence of catechol and catechol derivatives. To fresh radial sections, equal volumes of (1) 10% sodium nitrate, (2) 20%

urea, and (3) 10% acetic acid were added in sequence. After 3 to 4 minutes, 2 volumes of 2N sodium hydroxide were applied to the sections. A nitroso derivative forms with the addition of the sodium nitrate. The addition of a base forms the salt of the nitroso derivative, a colored compound.

Catechol and chlorogenic acid give a dark cherry-red color. Hydroquinone, orcinol, pyrogalllic acid, quercitrin, and rutin yield a reddish-brown color. Mixtures of catechol and chlorogenic acid with lesser amounts of quercitrin and rutin result in the formation of a cherry-red color. Greater amounts of these flavones in mixtures with either catechol or chlorogenic acid give a cherry-red color which turns red-brown or dark orange with the passage of time (Reeve, 1951).

Presence of Lipids

Sudan black B was used to detect total lipids. The dye will give a blue to black color in the presence of fats, oils, waxes, free fatty acids, and phospholipids. Fresh radial sections of tissue were placed in (1) 50% ethyl alcohol for 3 to 5 minutes, (2) placed in a 70% ethyl alcohol solution containing Sudan black B for 15 to 25 minutes, and (3) differentiated in 50% ethyl alcohol for 1 minute. Oils accumulate the dye much more readily than fats; as a result, solid lipids may stain poorly. (Jensen, 1962)

Ability of Cells to Reduce TTC to Formazan

Triphenyl tetrazolium chloride (TTC) measures dehydrogenase activity within the cell. It is reduced in actively metabolizing cells to formazan, a pink to reddish compound. Blocks were submerged in fresh aqueous 1% TTC and placed in the dark for 24 hours. After 24 hours, the blocks were removed and sliced into sections with a razor blade. The presence or absence of a red color was noted in parenchymatous cells (Hart, 1965).

Presence and Condition of the Nucleus

Fresh radial sections were placed in Mayer's hemalum for 20 to 30 minutes. The stain is highly selective for metabolic (resting) nuclei. The nucleus stains purple and the nucleolus appears deep blue to black in color. (Sass, 1951).

Distribution and Detection of Tyloses

Prior to observation, fresh radial and transverse sections were placed in 1% NaOH for 3 to 5 minutes to remove any gum deposits present in the vessels. (Chattaway, 1949)

Observations for Amorphous Deposits

Fresh, unstained, radial sections were used. The removal of gum deposits allowed amorphous deposits to be distinguished from tyloses.

The Reductive Capacity of Mitochondria

Fresh radial sections of tissue were placed in a 1:20,000 aqueous solution of Jannus green B for 15 to 30 minutes and observed under oil immersion. The appearance of a green color indicates the ability of mitochondria to perform reductive reactions.

Measurement of Oxygen Consumption

Oxygen consumption was measured for the sapwood, heartwood, and discolored sapwood. Blocks were chiseled from 0.6 cm thick transverse sections. The discolored sapwood was removed from immediately below the borer hole. Block dimensions were 1.0 cm in length (radial direction), 0.2 cm in width (tangential direction), and 0.6 cm in thickness. Two blocks were removed from each tissue end to end in the radial direction. Each tissue was replicated 3 times for series A and B and sapwood and discolored sapwood were replicated 5 times and heartwood 3 times for series C. The end-to-end pairing of sapwood and discolored sapwood blocks in the radial direction allowed measurement of total oxygen consumption for all tissues. Measurements were made at 30-minute intervals for 3 hours.

Whatman filter paper (No. 1) was placed in the bottom of each flask and 0.3 ml of distilled water was added. Two-tenths ml of 5N KOH was pipetted into the center well and a wick was inserted to absorb the CO₂ produced. All blocks

were air-dried 48 hours at 100 C and the oxygen consumption was expressed as μ l of oxygen consumption per mg of dry weight of tissue. In series C, blocks were placed in TTC for 24 hours to measure the ability of cells to reduce TTC prior to air-drying 48 hours at 100 C.

The mean oxygen consumption was calculated for the sapwood, heartwood, and discolored sapwood for each tree. Oxygen consumption was expressed as per cent of sapwood oxygen consumption (100%) for series A, B, and C. A standard "t" test was used to determine the significance of deviations of mean values of discolored sapwood and heartwood from the mean value of the sapwood for individual trees.

RESULTS

Appearance of Discoloration in Sapwood Tissue

Some discoloration developed in the sapwood of trees felled the same day as wounded. A pale brownish color was evident in the tissue immediately surrounding the borer hole. At 16 days after wounding, the discoloration was similar to the heartwood. Thereafter, the discoloration in the sapwood was much darker than the heartwood when wet and somewhat darker when dry. The heartwood contained bands of alternating light and dark colors and the discolored sapwood was uniform in color (Figure 1).

Vertical discoloration between 1.5 to 3.0 cm above and below the borer hole was noted for trees wounded during the summer. Vertical discoloration was 0.75 cm or less above and below the borer holes for trees wounded in the autumn. In all trees, horizontal spread of the discoloration was restricted to approximately the diameter of the borer hole.

Histochemical Changes in the Transformation of Sapwood to Discolored Sapwood

Iodine-potassium iodide indicated that starch grains disappeared from the discolored sapwood 8 days after wounding in trees wounded in the summer (Figures 2a and 2b). Single

starch grains or a single cell containing very few starch grains were present in discolored sapwood at later dates. Starch grains disappeared from discolored sapwood 12 to 16 days after wounding in trees felled in autumn. Starch grains were present in trace amounts at later dates.

The $\text{Fe}_2(\text{SO}_4)_3$ test indicated a moderate increase in tannins 10 to 12 days after wounding when compared with the sapwood. The increase occurred primarily along the interface between the sapwood and discolored sapwood.

Catechol or derivatives appeared between 12 and 16 days after wounding (Figures 3a and 3b). During this time, the cherry-red color was present in moderate amounts. Cathechol or its derivatives disappeared from the discolored sapwood 16 days after wounding (Figure 3c).

In general, lipids decreased in the discolored sapwood 12 to 16 days after wounding. In 80% of the trees examined after this decrease, lipids were present in the discolored sapwood in at least trace amounts. In the remaining 20% of the trees examined, no lipids were observed 2 weeks after injury. In certain instances, 24 and 28 days after wounding in series B and 20 days after wounding in series C, lipids appeared to be present in amounts equal to those in uninjured sapwood (Figure 4).

Cells capable of reducing TTC to formazan decreased to scattered individual cells 8 days after wounding and completely disappeared 14 to 16 days after wounding.

Cytological Changes in the Transformation
of Sapwood to Discolored Sapwood

Nuclei, stained with hemalum, appeared to round-up 12 to 14 days after wounding (Figure 5). A slight increase in size was noted. Condition of nuclei within individual cells varied considerably from 16 days after wounding until termination of the investigation. In some cells, the nucleus had lost the ability to stain and had begun to disintegrate (Figure 6). In other cells, nuclei appeared capable of active metabolism and nucleoli were visible (Figure 7).

No differences in number of tyloses were noted in the sapwood and discolored sapwood. Ray and xylem parenchyma in the discolored sapwood turned light to cherry-red with application of 1% NaOH between 12 and 16 days after wounding (Figure 8). Except for single trace instances, the red color was not present in the discolored sapwood prior to or after these dates. The color was uniform in distribution and occurred in moderate quantity. The color was not noted in uninjured sapwood.

Amorphous deposits began to appear 10 to 12 days after wounding. A very decided increase in amorphous deposits occurred at 16 days after wounding, evident even to the unaided eye (Figures 9a, 9b, and 9c).

Results using Jannus green B were not conclusive. Positive results using any tissue were exceedingly difficult to evaluate.

Figure 10 summarizes the cytological changes in sapwood following mechanical injury.

Figure 1.--Comparisons between the color of the discolored sapwood and heartwood 28 days after wounding when dry (top) and wet (bottom).

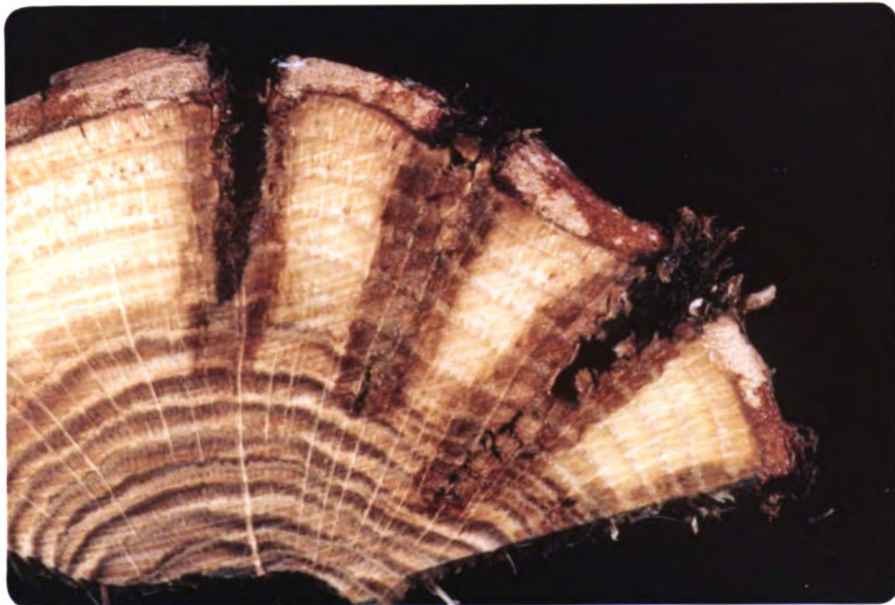
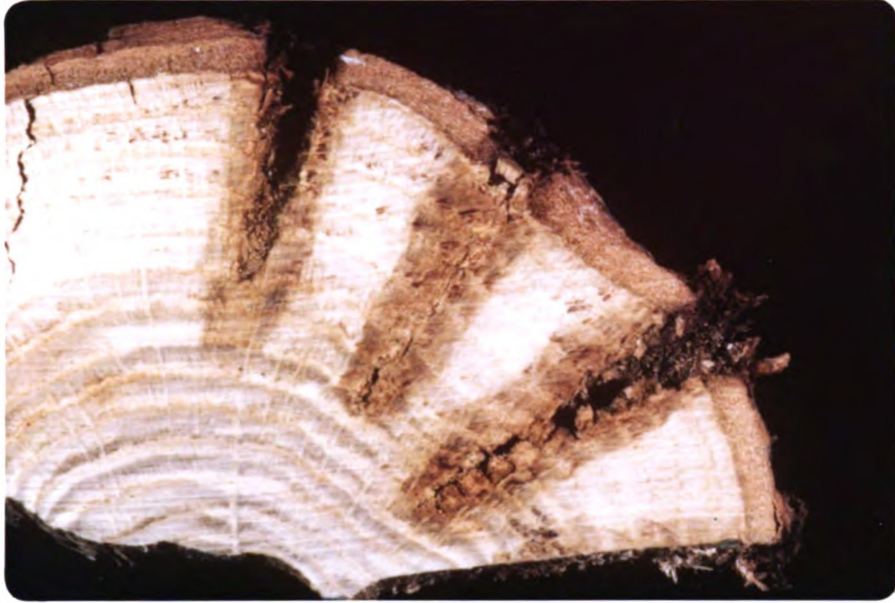


Figure 2a.--The disappearance of starch grains from the ray parenchyma at 0 (top) and 4 (bottom) days after wounding. (Radial section--10 to 20 microns in thickness--430X-IKI)

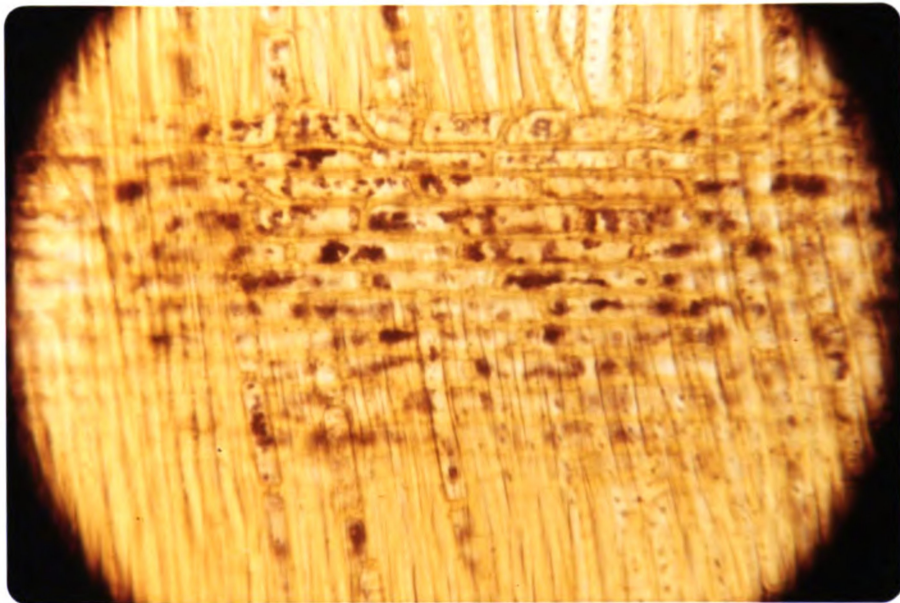
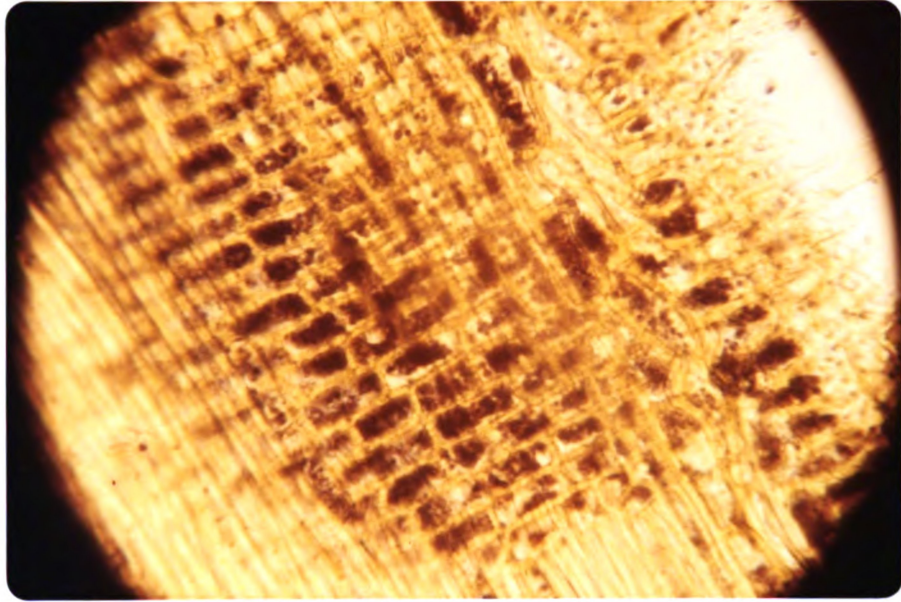


Figure 2b.--The absence of starch grains from the ray parenchyma 8 days after wounding (radial section--10 to 20 microns in thickness--430X-IKI).

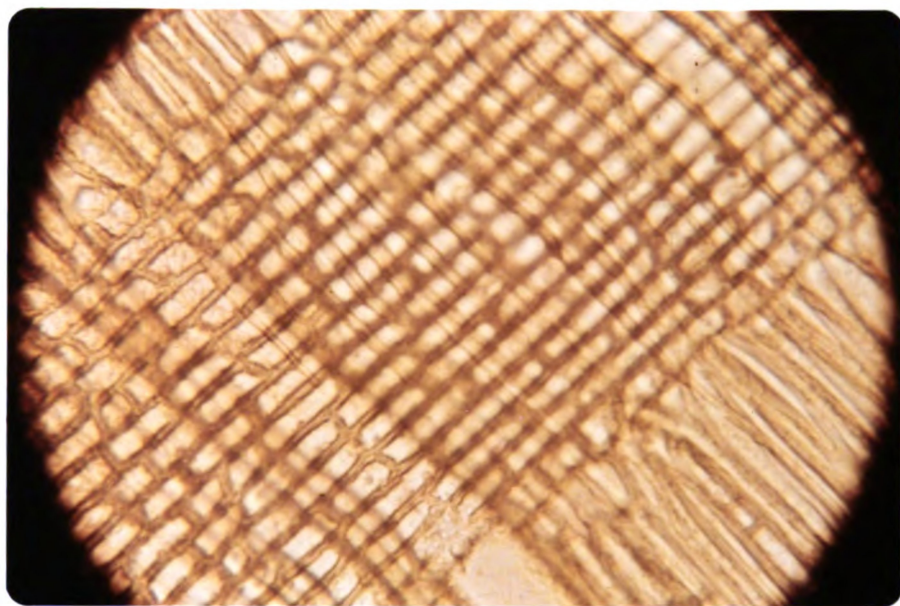


Figure 3a.--The occurrence of catechol or its derivatives in the discolored sapwood between 12 and 16 days after wounding. (Radial section--10 to 20 microns in thickness--970X-Nitroso)

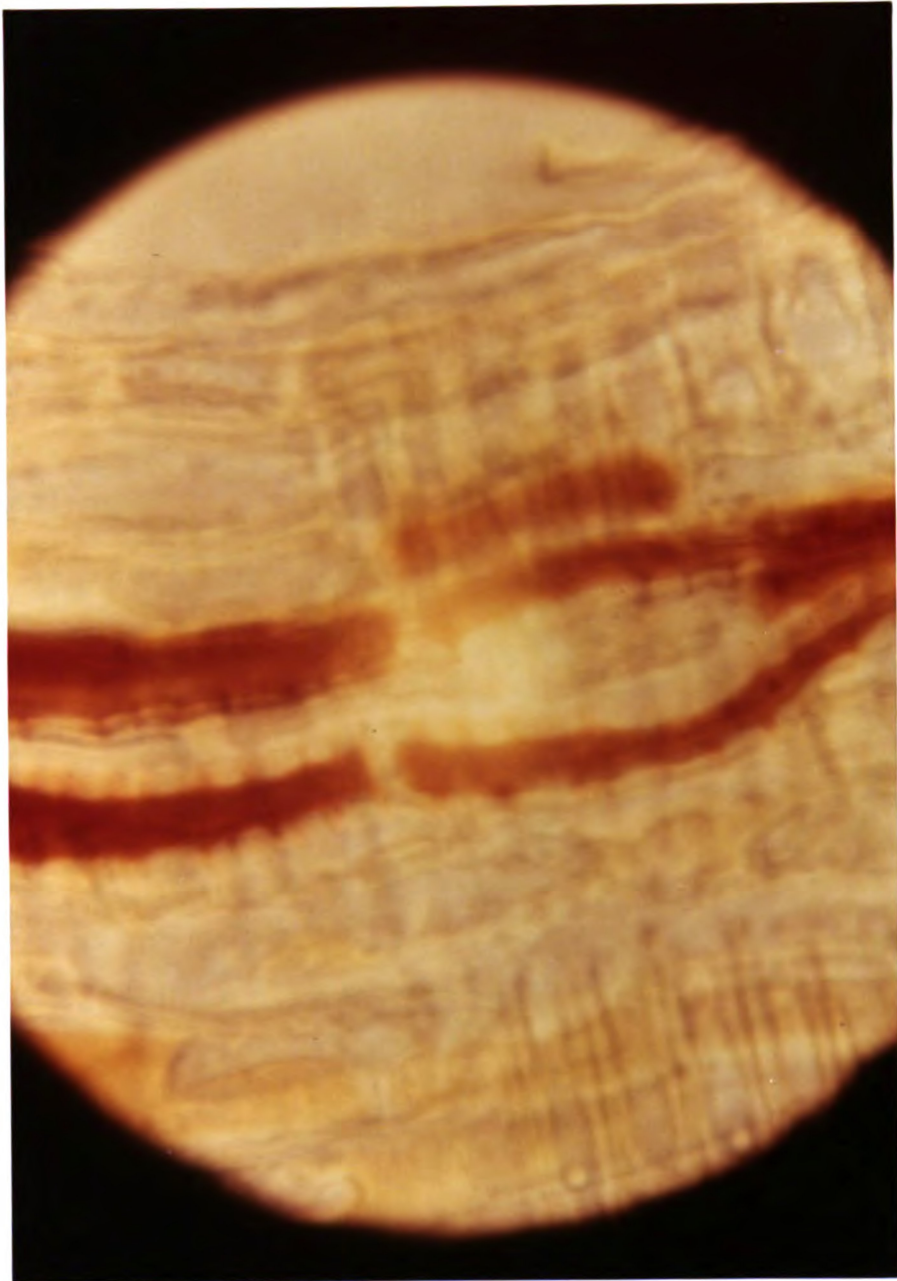


Figure 3b.--The occurrence of catechol or its derivatives in the discolored sapwood between 12 and 16 days after wounding. (Radial section--10 to 20 microns in thickness--430X-Nitroso)

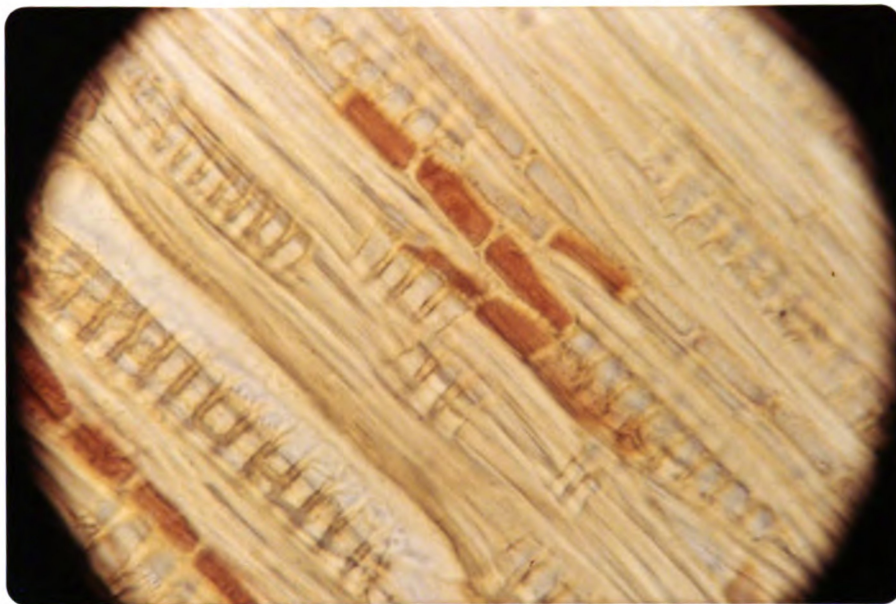


Figure 3c.--The absence of catechol or its derivatives in the discolored sapwood 24 days after wounding. (Radial section--10 to 20 microns in thickness--430X-Nitroso)



Figure 4.--The presence of lipids in the discolored sapwood at 10 days after wounding (top) and 24 days after wounding (bottom). (Radial section--10 to 20 microns in thickness--430X-Sudan black b)

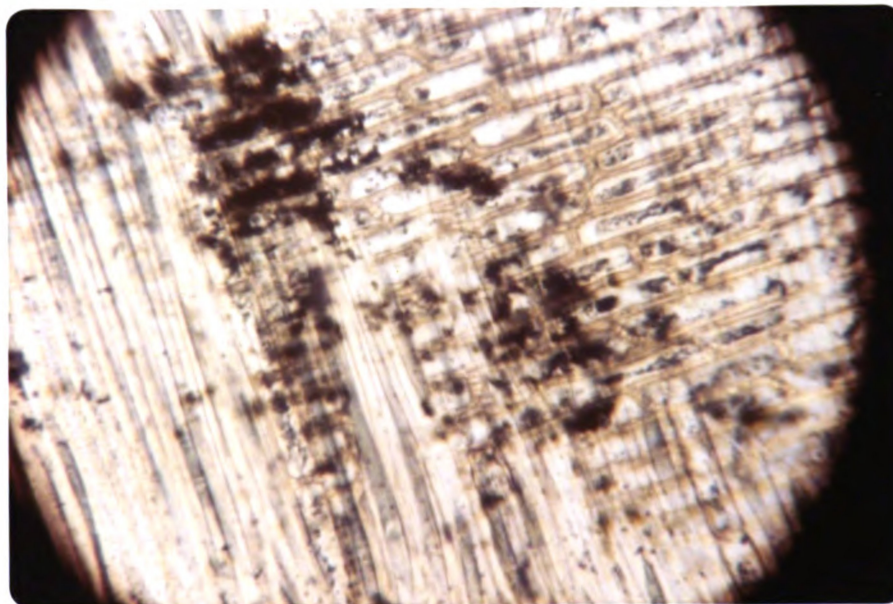
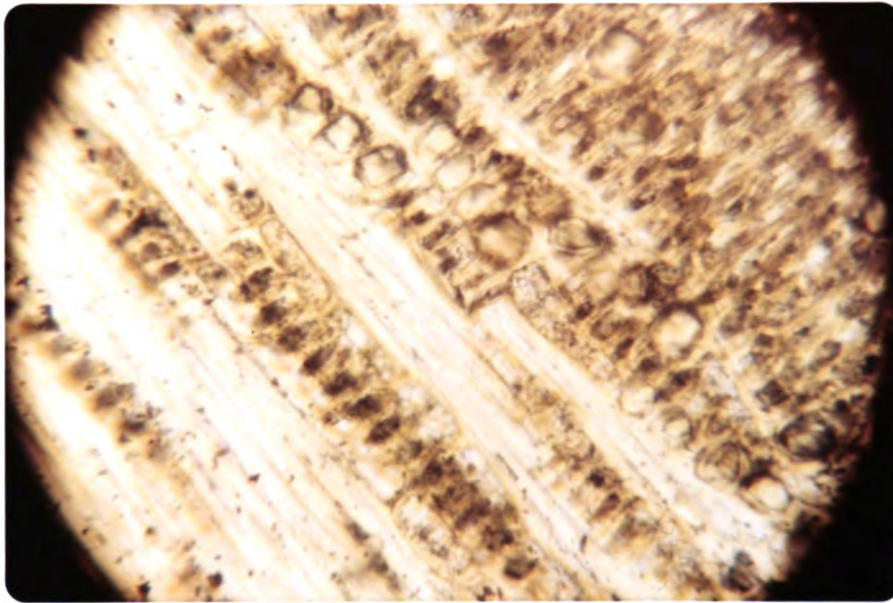


Figure 5.--The possible rounding-up of the nucleus in the xylem parenchyma moving from the sapwood (top) to discolored sapwood (bottom) is illustrated. (Radial section--10 to 20 microns in thickness--970X-Hemalum)

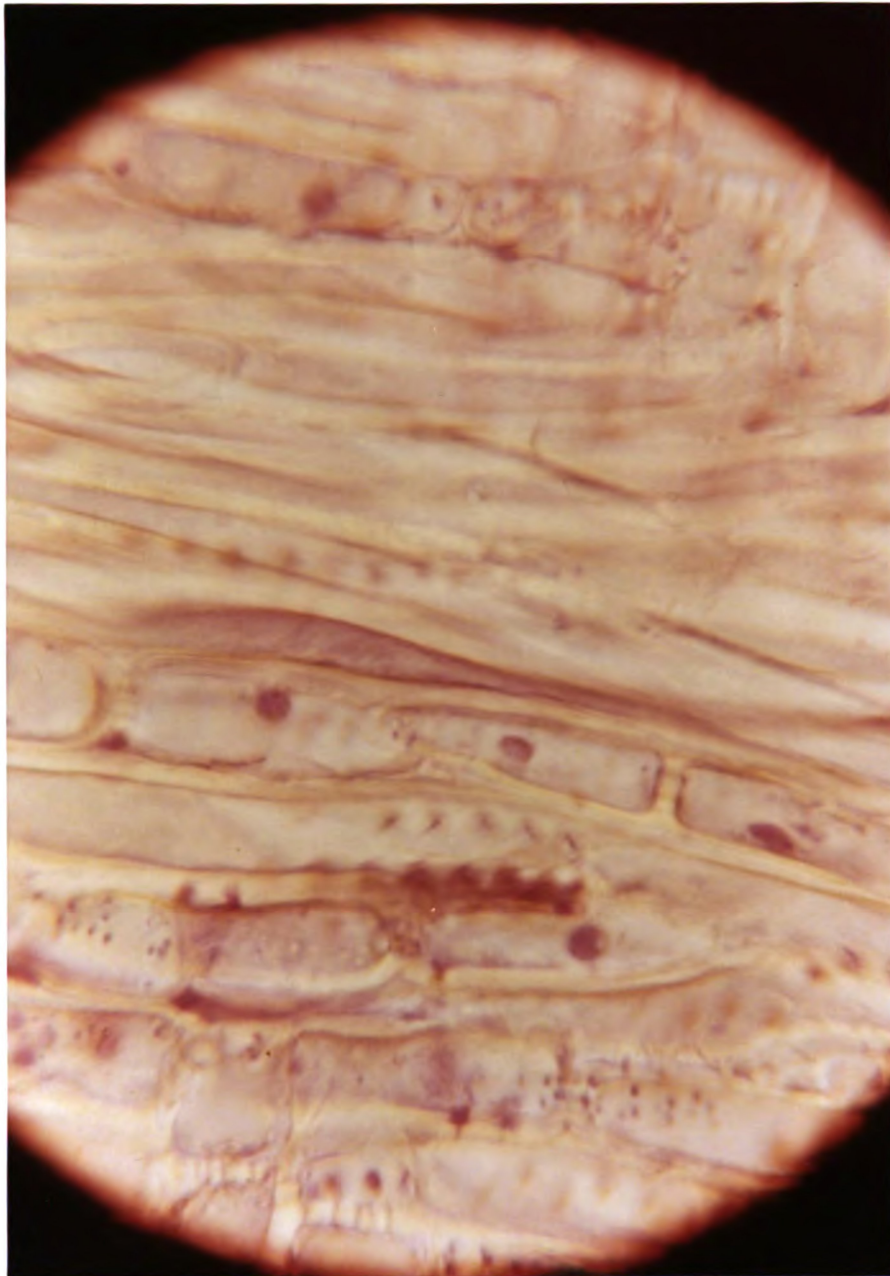


Figure 6.--Nuclei within the discolored sapwood
20 days after wounding, note disintegration of nuclei.
(Radial section--10 to 20 microns in thickness--
970X-Hemalum)

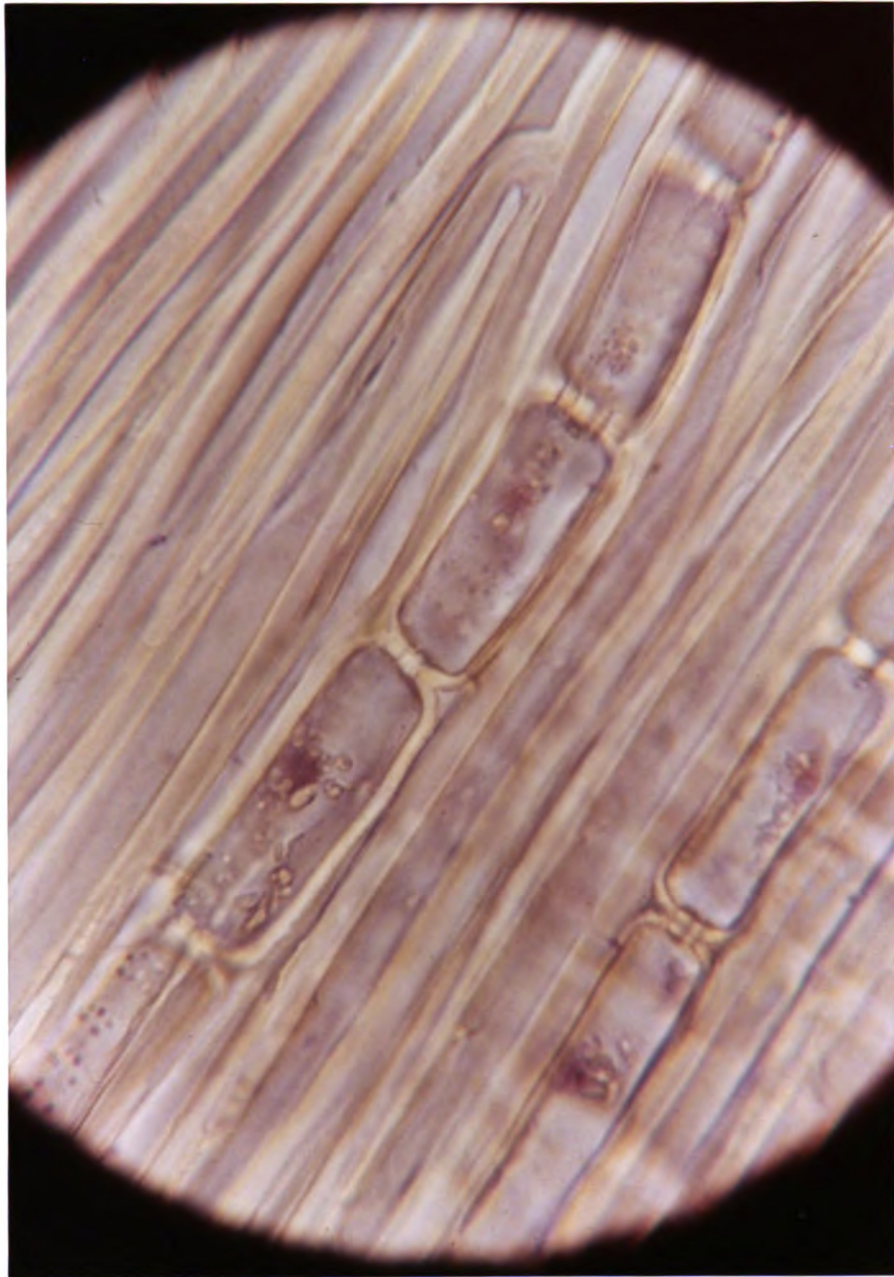


Figure 7.--Nuclei within the discolored sapwood 24 days after wounding, note the presence of nucleoli in the nuclei. (Radial section--10 to 20 microns in thickness--970X-Hemalum)

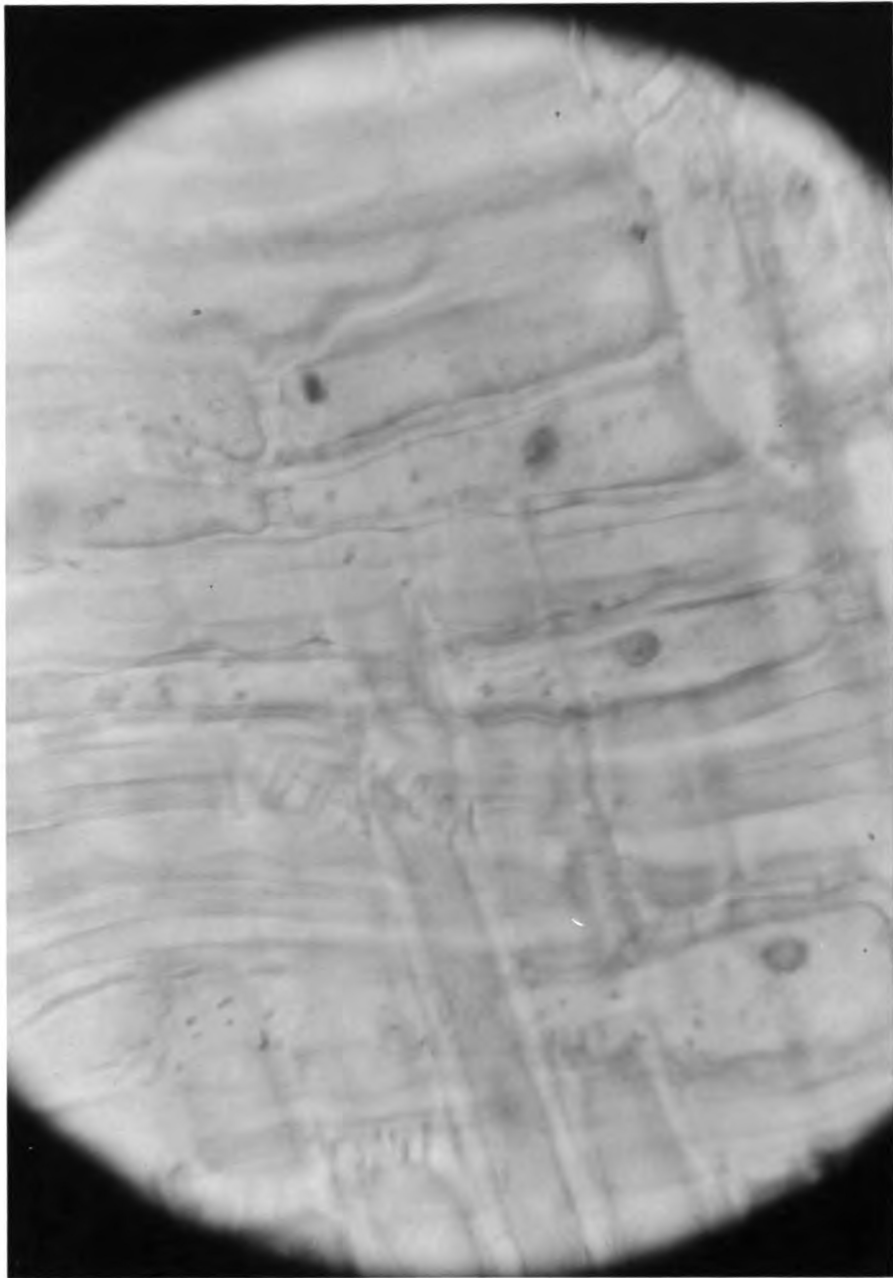


Figure 8.--The appearance and distribution of the light to cherry-red color in cells of the discolored sapwood after application of 1% NaOH 12 days after wounding. (Radial section--10 to 20 microns in thickness--430X-1% NaOH)

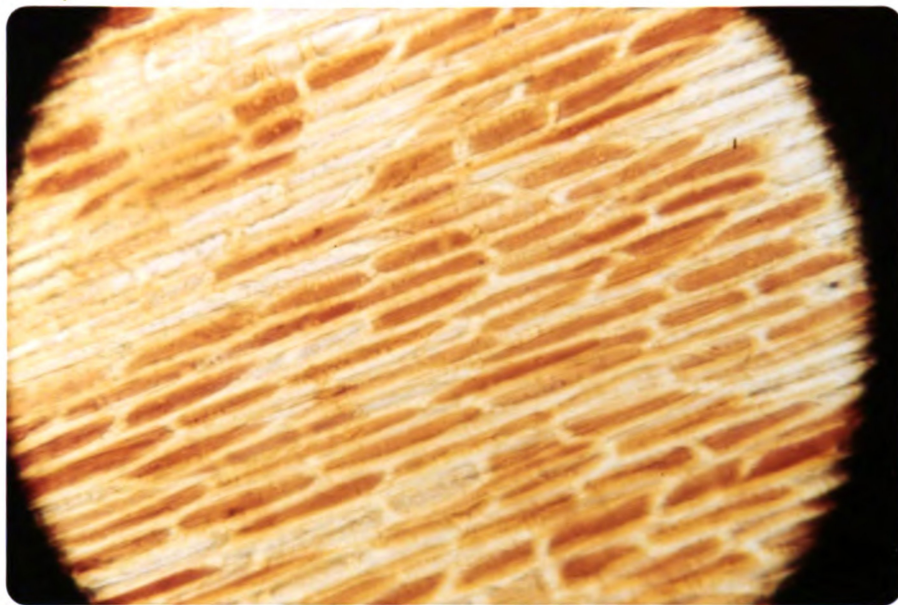


Figure 9a.--The occurrence of amorphous deposits in the discolored sapwood 28 days after wounding, similar conditions were present 16 days after wounding. (Radial section--10 to 20 microns in thickness--430X-Unstained)

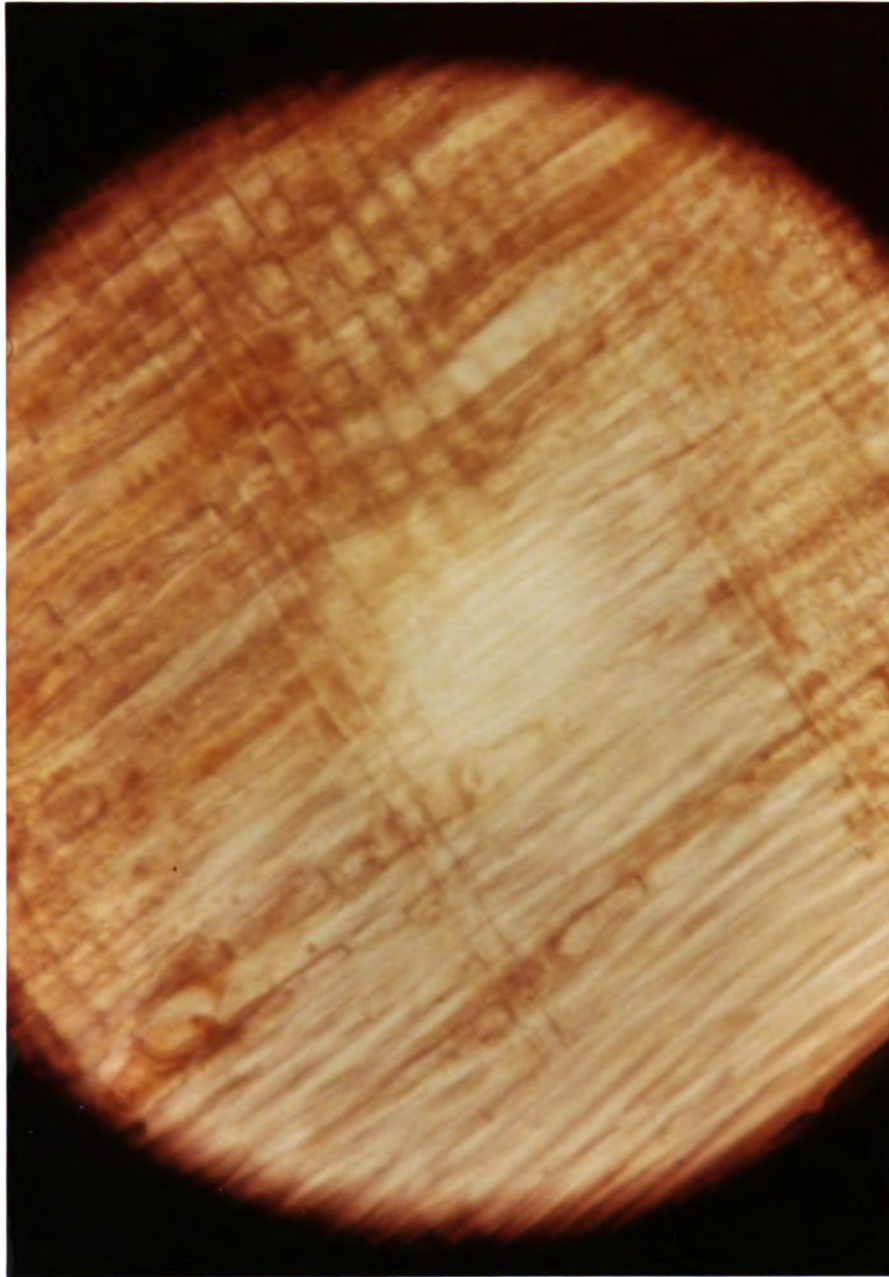


Figure 9b.--The occurrence of amorphous deposits in the discolored sapwood 28 days after wounding, similar conditions were present 16 days after wounding. (Radial section--10 to 20 microns in thickness--970X-Jannus green B)

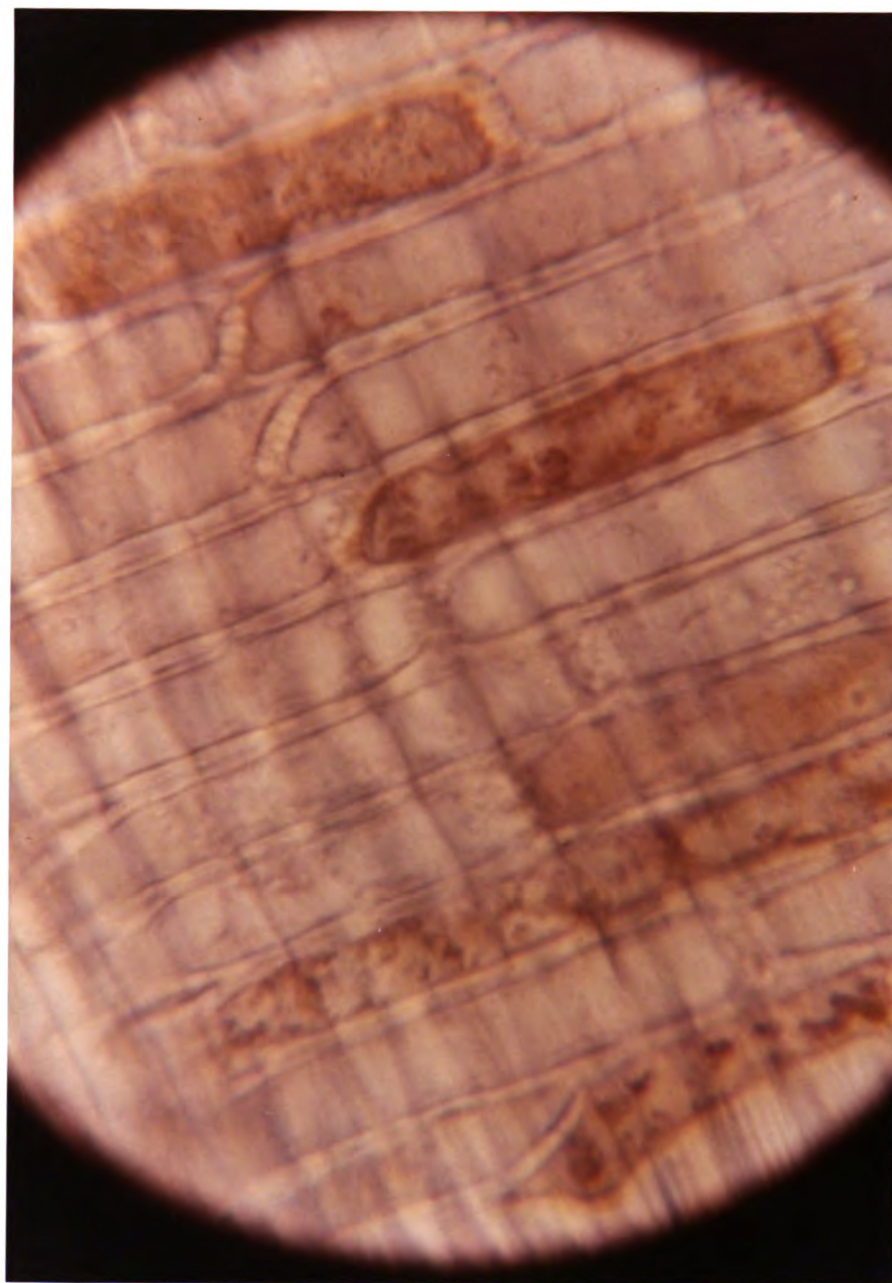


Figure 9c.--The occurrence of amorphous deposits in the discolored sapwood 28 days after wounding, similar conditions were present 16 days after wounding. (Radial section--10 to 20 microns in thickness--430X-Unstained)

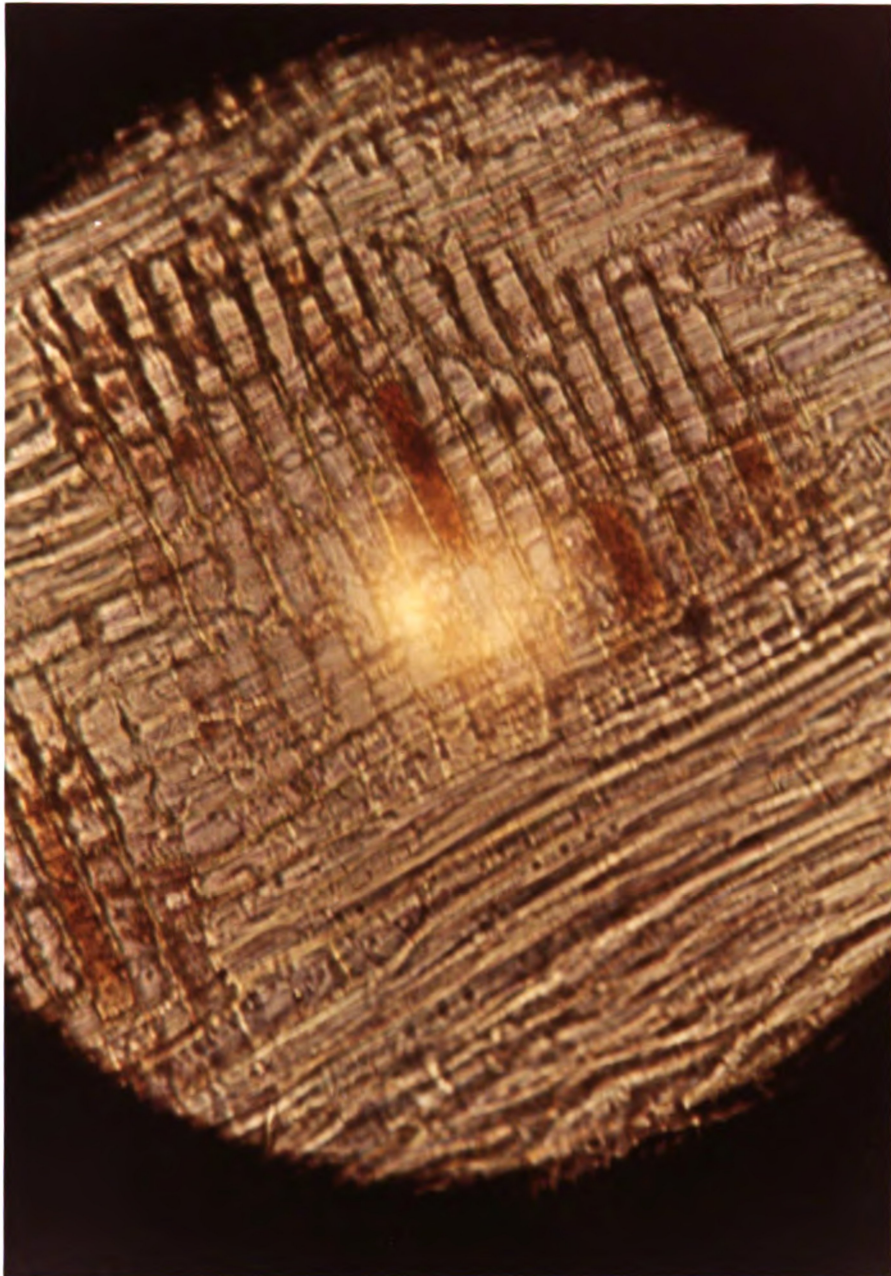
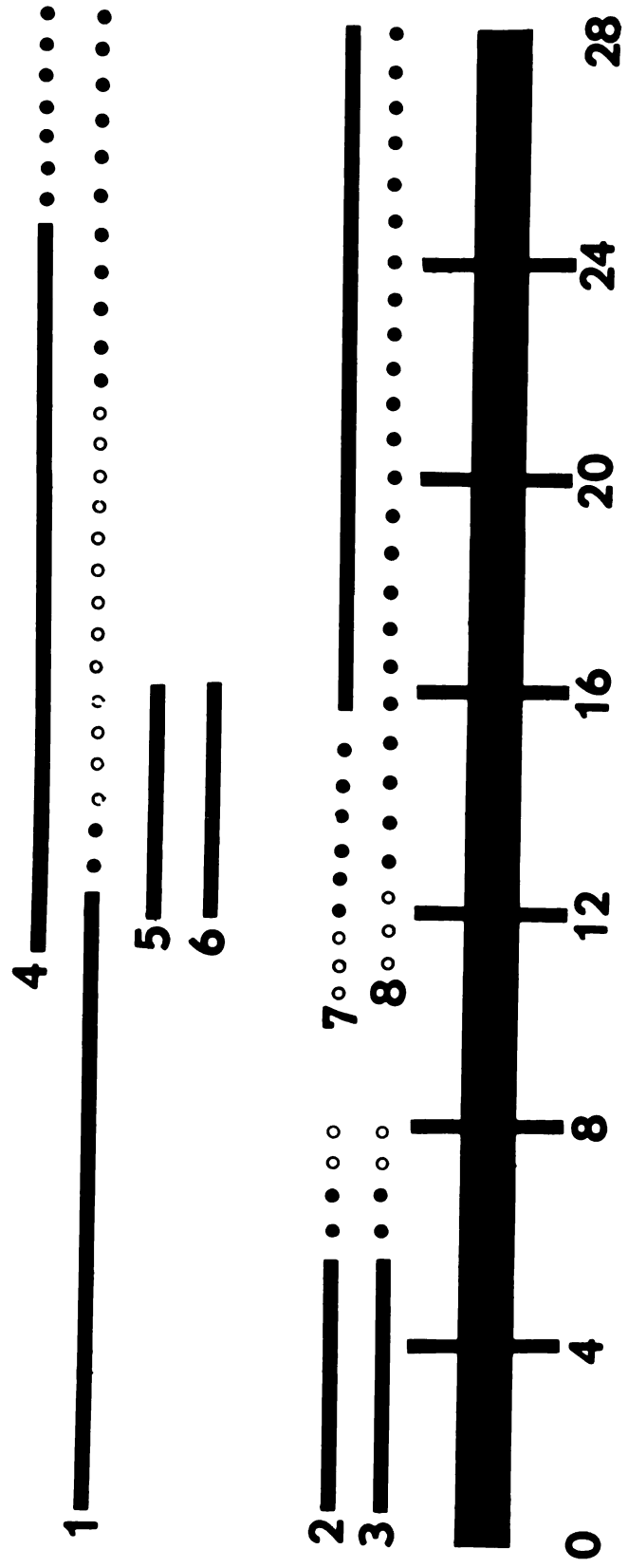


Figure 10.--Cytological changes in sapwood following mechanical injury.

Solid lines and dots indicate positive results.

- (1) Lipids
- (2) Starch grains
- (3) Parenchyma reduce TTC
- (4) Nuclei
- (5) Catechol or its derivatives
- (6) 1% NaOH gives a cherry-red color to parenchyma
- (7) Amorphous deposits
- (8) Tannins

CYTOLOGICAL CHANGES IN SAPWOOD FOLLOWING MECHANICAL INJURY



DAYS AFTER WOUNDING

ABUNDANT — MODERATE ... TRACE ...

Histochemical Changes in the Transformation of Sapwood to Heartwood

Iodine-potassium iodide indicated that starch grains were absent from the zone of the sapwood-heartwood transformation in 21 of 24 trees examined. In the remaining 3 trees, single, scattered starch grains or cells which contained few to many starch grains were present. The heartwood lacked starch grains (Figures 11a and 11b).

The application of $\text{Fe}_2(\text{SO}_4)_3$ showed tannins were present in moderate to abundant amounts within the zone of the sapwood-heartwood transformation, primarily adjacent to the heartwood boundary. Tannins were present in moderate quantity in the heartwood.

The nitroso reaction indicated that catechol or its derivatives occurred in the zone of the sapwood-heartwood transformation in moderate quantity in 14 of 24 trees examined, in abundant amounts in 3 of 24 trees examined, and in trace amounts in 7 of 24 trees examined (Figure 12). They were present in the heartwood in trace amounts in 12 of 24 trees examined, moderate amounts in 6 of 24 trees examined, and absent from 6 of 24 trees examined. The sapwood lacked catechol or its derivatives (Figure 13).

Lipids were absent from the zone of the sapwood-heartwood transformation in 8 of 24 trees examined, but occurred in moderate amounts the remainder of the time. Lipids were present in the heartwood in trace amounts in

20 of 24 trees examined, otherwise lipids were absent from the heartwood (Figure 14).

Cells in the annual ring of sapwood adjacent to the heartwood and in the heartwood itself were not capable of reducing TTC to formazan. This would indicate inactivation of dehydrogenases in these cells.

Cytological Changes in the Transformation of Sapwood to Heartwood

Nuclei appeared oblong to egg-shaped and were present in moderate numbers in the zone of the sapwood-heartwood transformation (Figure 15). Nucleoli were frequently present. The surface area of nuclei appeared to be larger than those of the middle sapwood (Figure 16). Nuclei were lacking in the heartwood or were not capable of holding the hemalum stain.

Tyloses were abundant in the zone of the sapwood-heartwood transformation and in the heartwood (Figure 17). As with the discolored sapwood, sapwood and heartwood, tyloses were largely restricted to early wood vessels. One per cent NaOH gave a light to cherry-red color in 21 of 24 trees examined in the zone of the sapwood-heartwood transformation. Cells yielding the color were moderate to abundant in occurrence. A cherry-red color, having an irregular pattern of occurrence resulted from application of 1% NaOH to the heartwood (Figure 18).

Amorphous deposits were rarely present in the heartwood in more than trace amounts (Figure 19). The zone of the sapwood-heartwood transformation contained amorphous deposits in trace amounts in 2 of 24 trees examined.

Comparisons between cytological changes in cells in the transformation of sapwood to heartwood are presented in Figure 20 and comparisons between the sequence of events in the transformation of sapwood to heartwood and discolored sapwood are presented in Table 2.

Figure 11a.--The disappearance of starch grains from the ray parenchyma of the sapwood in the formation of heartwood, sapwood (top) and zone of the sapwood-heartwood transformation (bottom). (Radial section--10 to 20 microns in thickness--430X-IKI)

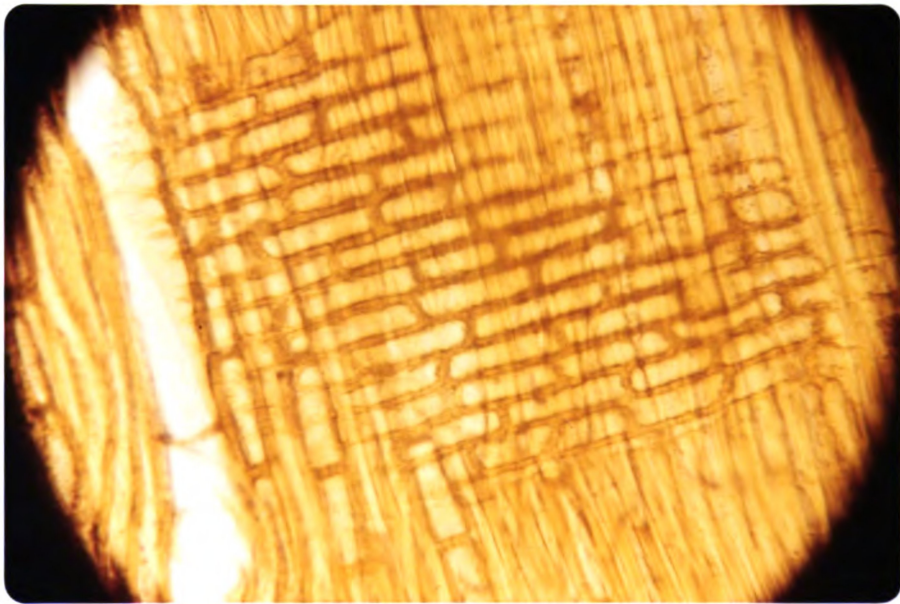
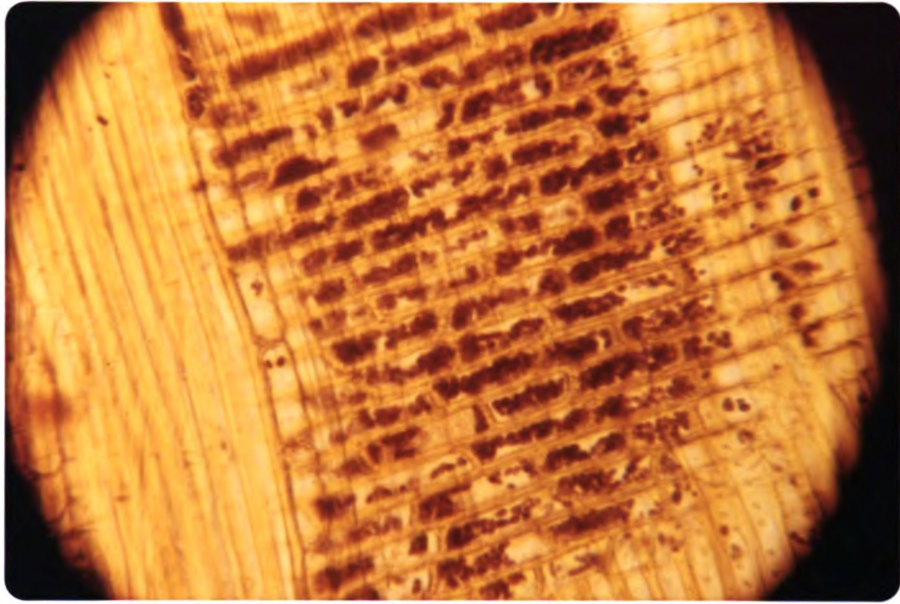


Figure 11b.--The disappearance of starch grains from the ray parenchyma of the sapwood in the formation of heartwood, heartwood. (Radial section--10 to 20 microns in thickness--430X-IKI)

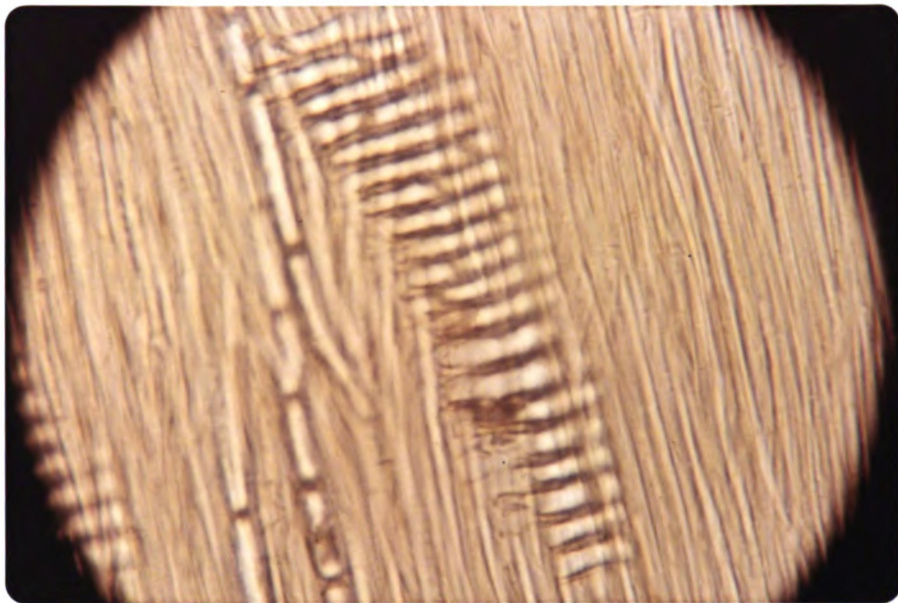


Figure 12.--The occurrence of catechol or its derivatives in the zone of the sapwood-heartwood transformation (top) and heartwood (bottom). (Radial section--10 to 20 microns in thickness--430X-Nitroso)

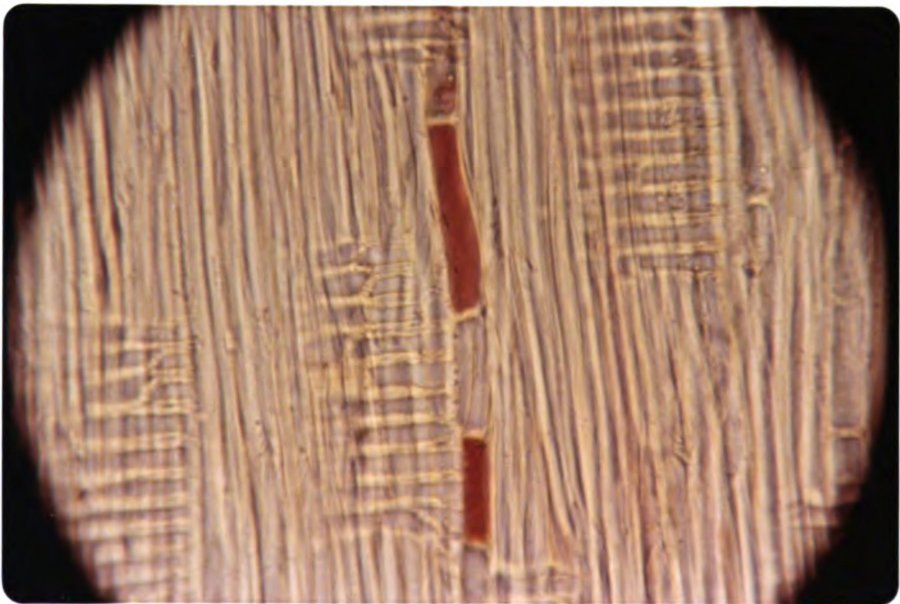
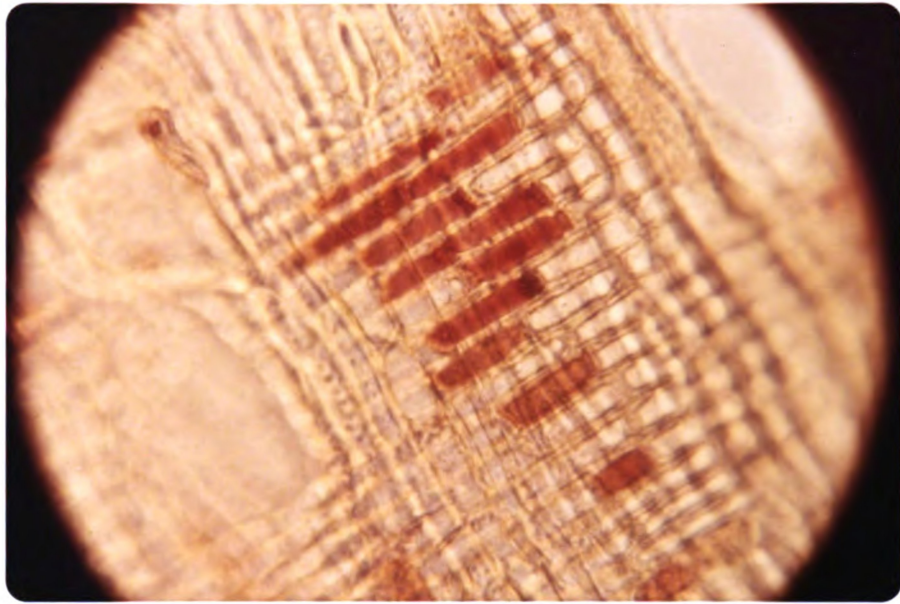


Figure 13.--The absence of catechol and catechol derivatives from the sapwood. (Radial section--10 to 20 microns in thickness--100X-Nitroso)



Figure 14.--The appearance of lipids in the heartwood. (Radial section--10 to 20 microns in thickness--430X-Sudan black B)

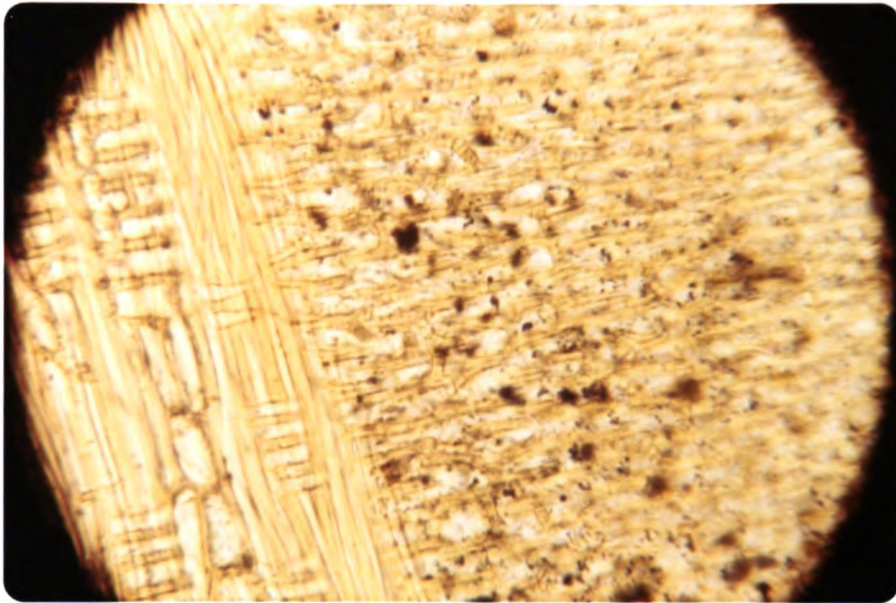


Figure 15.--The appearance of the nucleus in the zone of the sapwood-heartwood transformation. (Radial section--10 to 20 microns in thickness--970X-Hemalum)

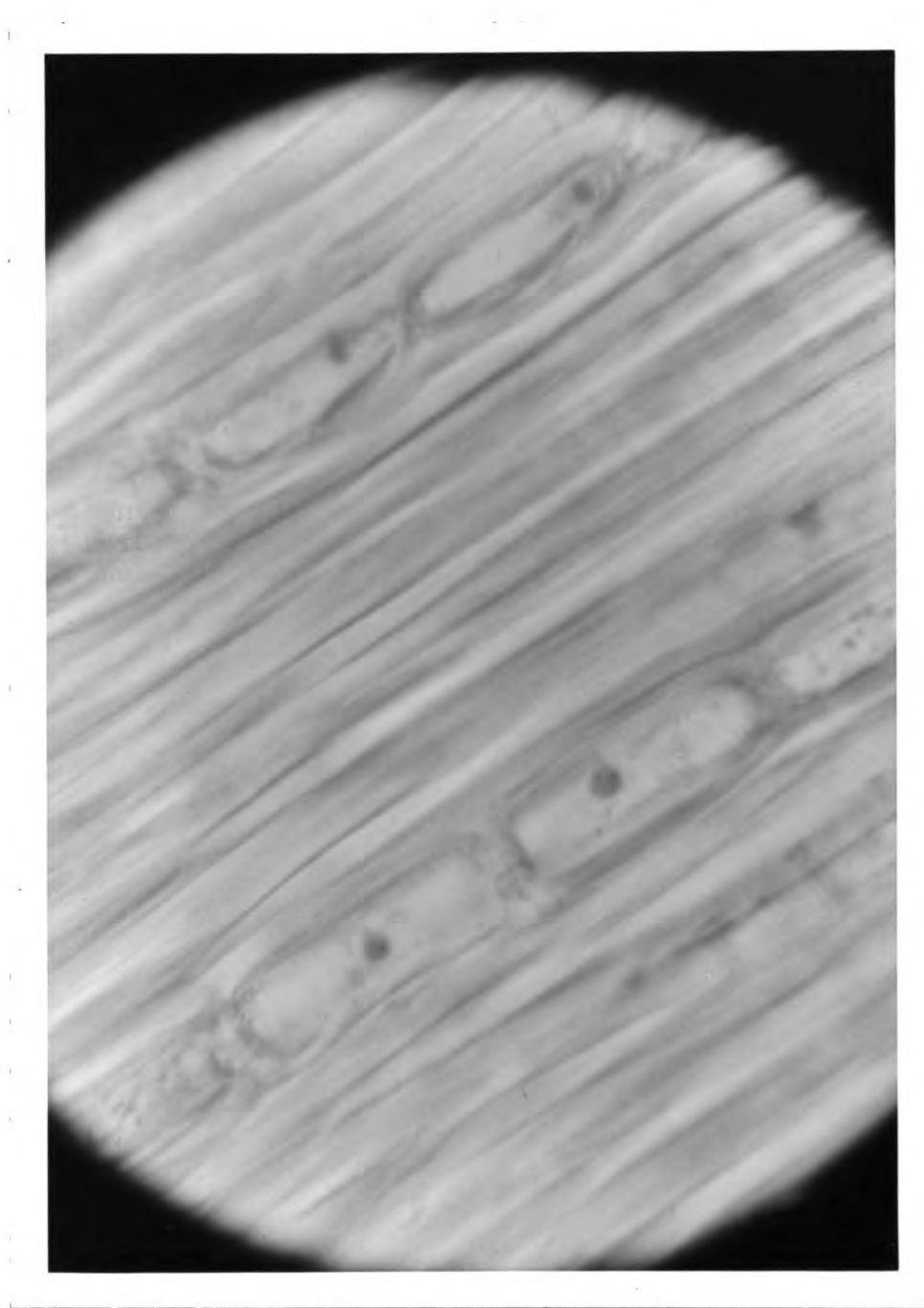


Figure 16.--The appearance of nuclei in the middle sapwood. (Radial section--10 to 20 microns in thickness--970X-Hemalum)

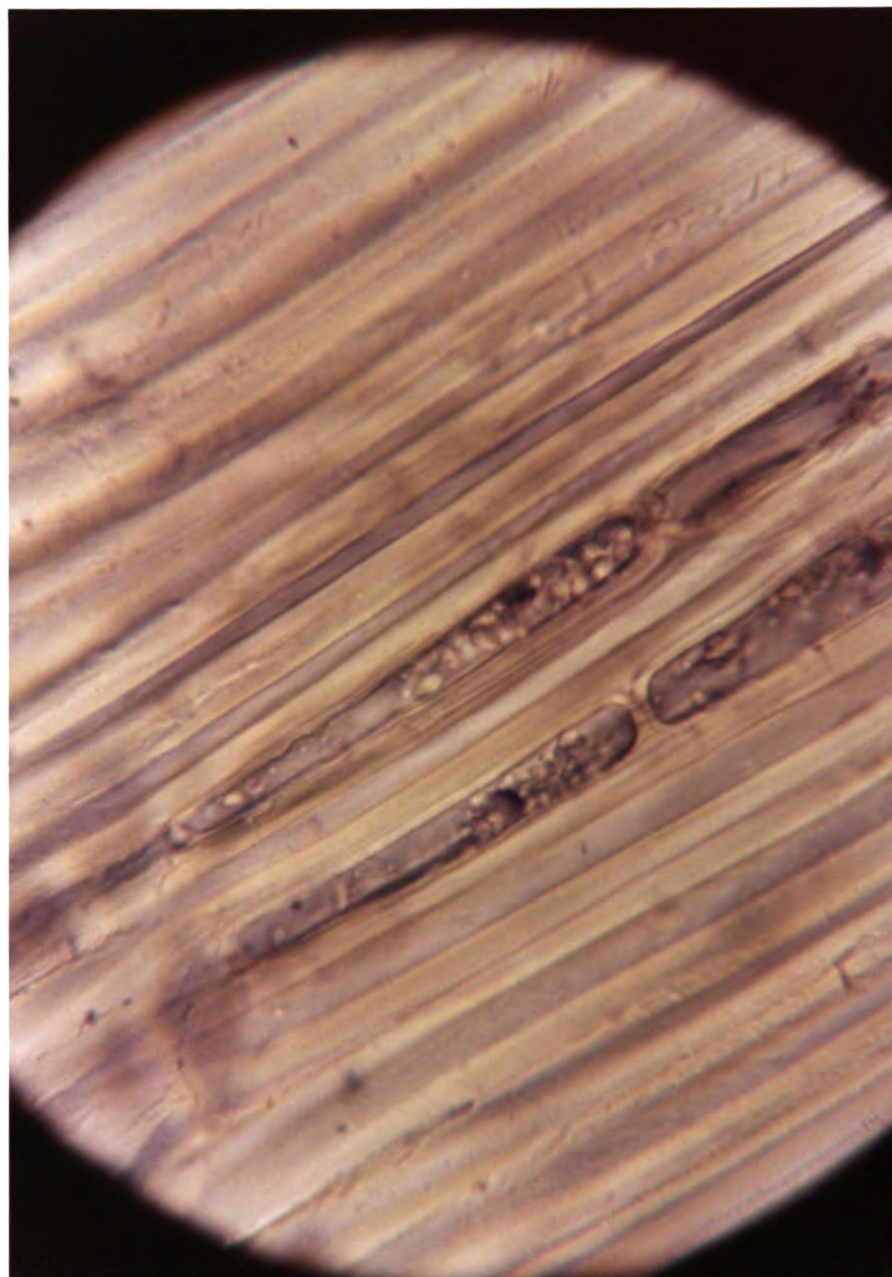


Figure 17.--Tyloses in the heartwood, note the extremely thin walls (less than 2 microns in thickness). (Transverse [top] and radial [bottom] sections--10 to 20 microns in thickness--430X-1% NaOH)

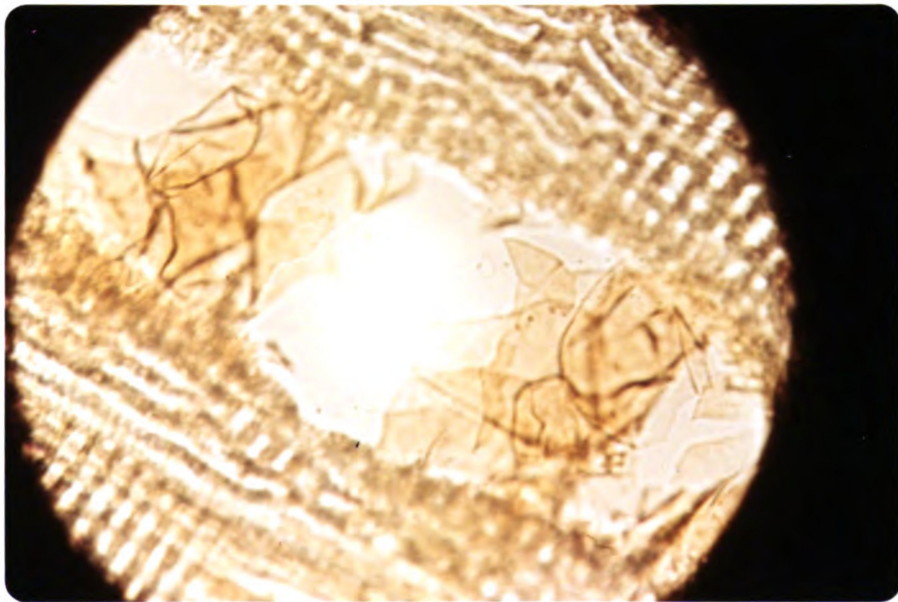
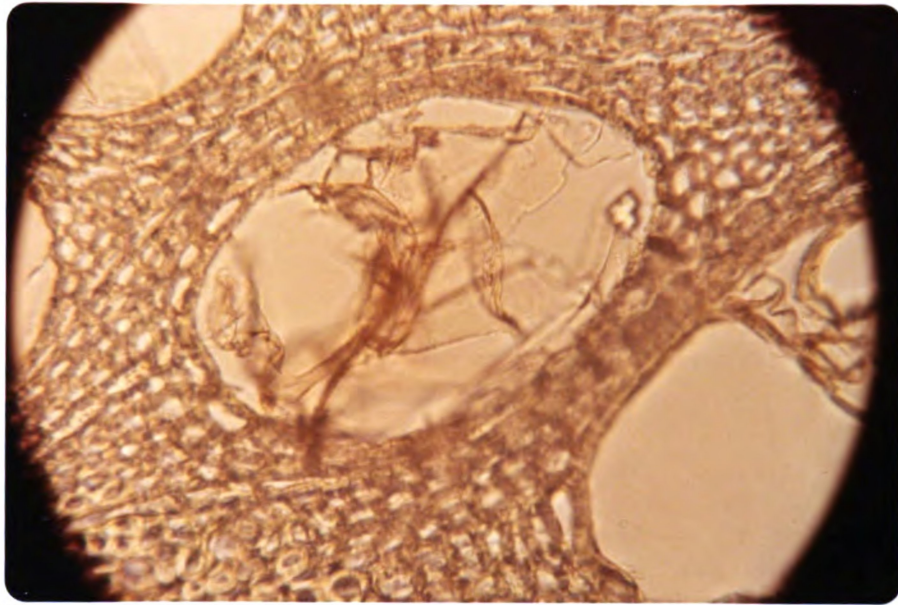


Figure 18.--The appearance of the light to cherry-red color in the zone of the sapwood-heartwood transformation (top) and heartwood (bottom). (Radial section--10 to 20 microns in thickness--430X-1% NaOH)

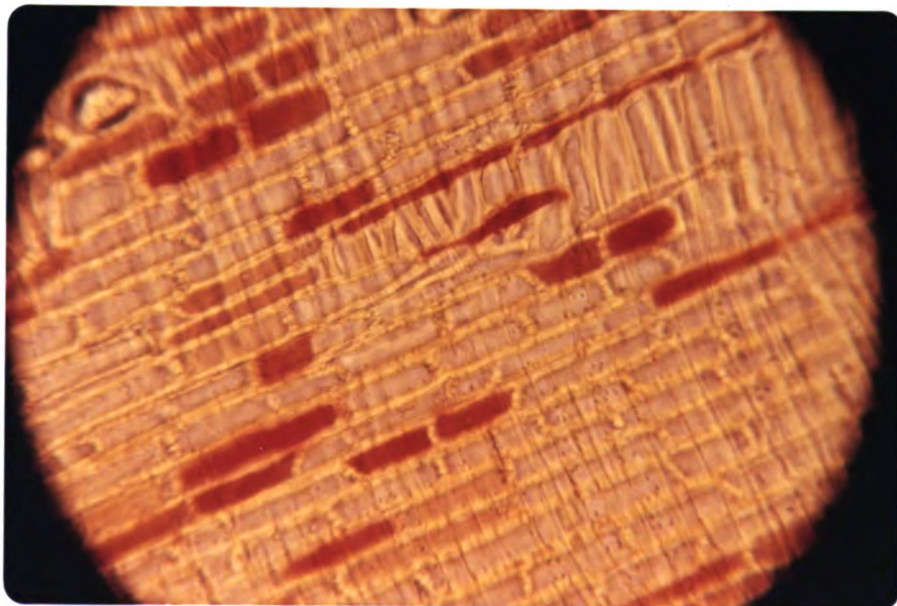
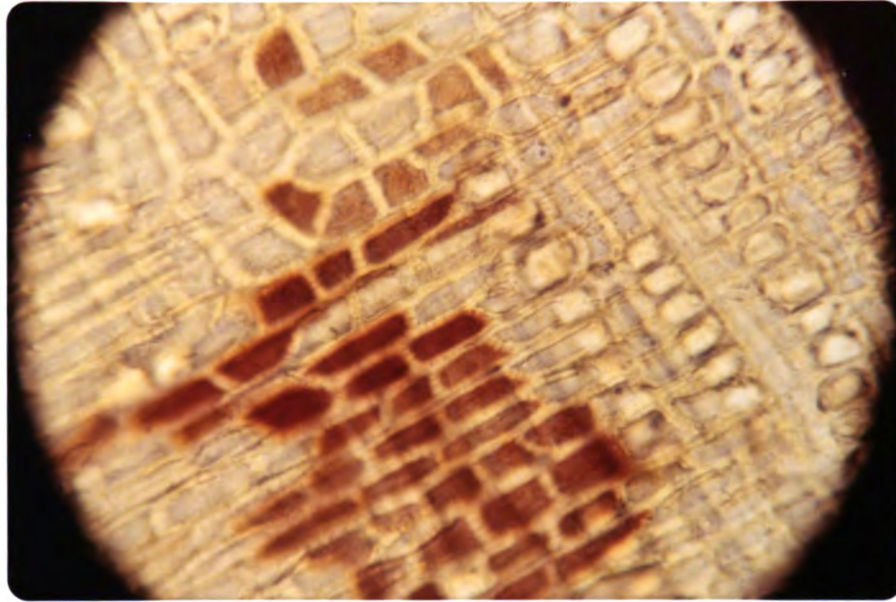


Figure 19.--The appearance of amorphous deposits
in the heartwood. (Radial section--10 to 20 microns
in thickness--430X-Unstained)

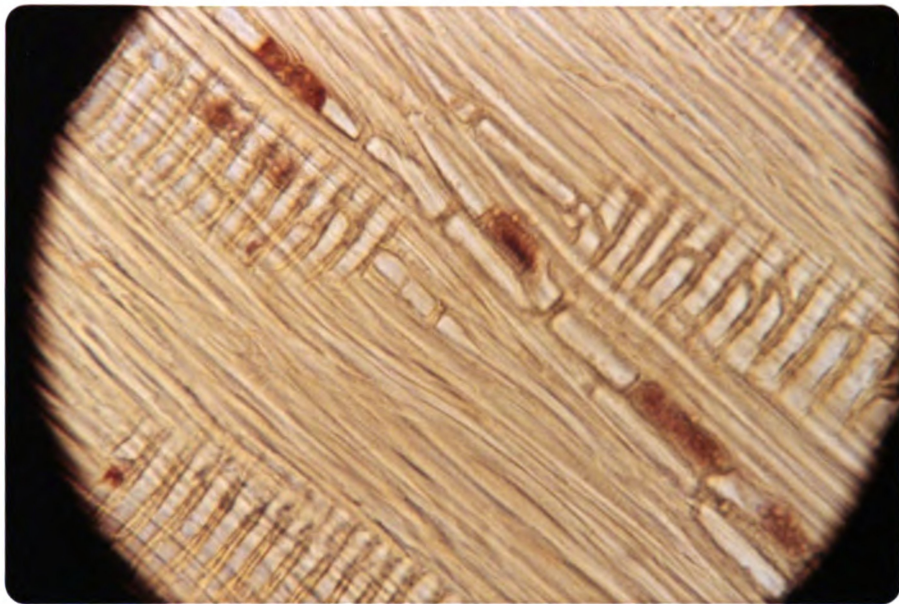


Figure 20.--Comparisons between cytological changes in cells in the transformation of sapwood to heartwood and discolored sapwood.

Solid lines and dots indicate positive results.

- (1) Lipids
- (2) Starch grains
- (3) Parenchyma reduce TCC
- (4) Nuclei
- (5) Catechol or its derivatives
- (6) 1% NaOH gives a cherry-red color to parenchyma
- (7) Amorphous deposits
- (8) Tannins

COMPARISONS BETWEEN CYTOLOGICAL CHANGES IN CELLS IN THE TRANSFORMATION OF SAPWOOD TO HEARTWOOD AND DISCOLORED SAPWOOD

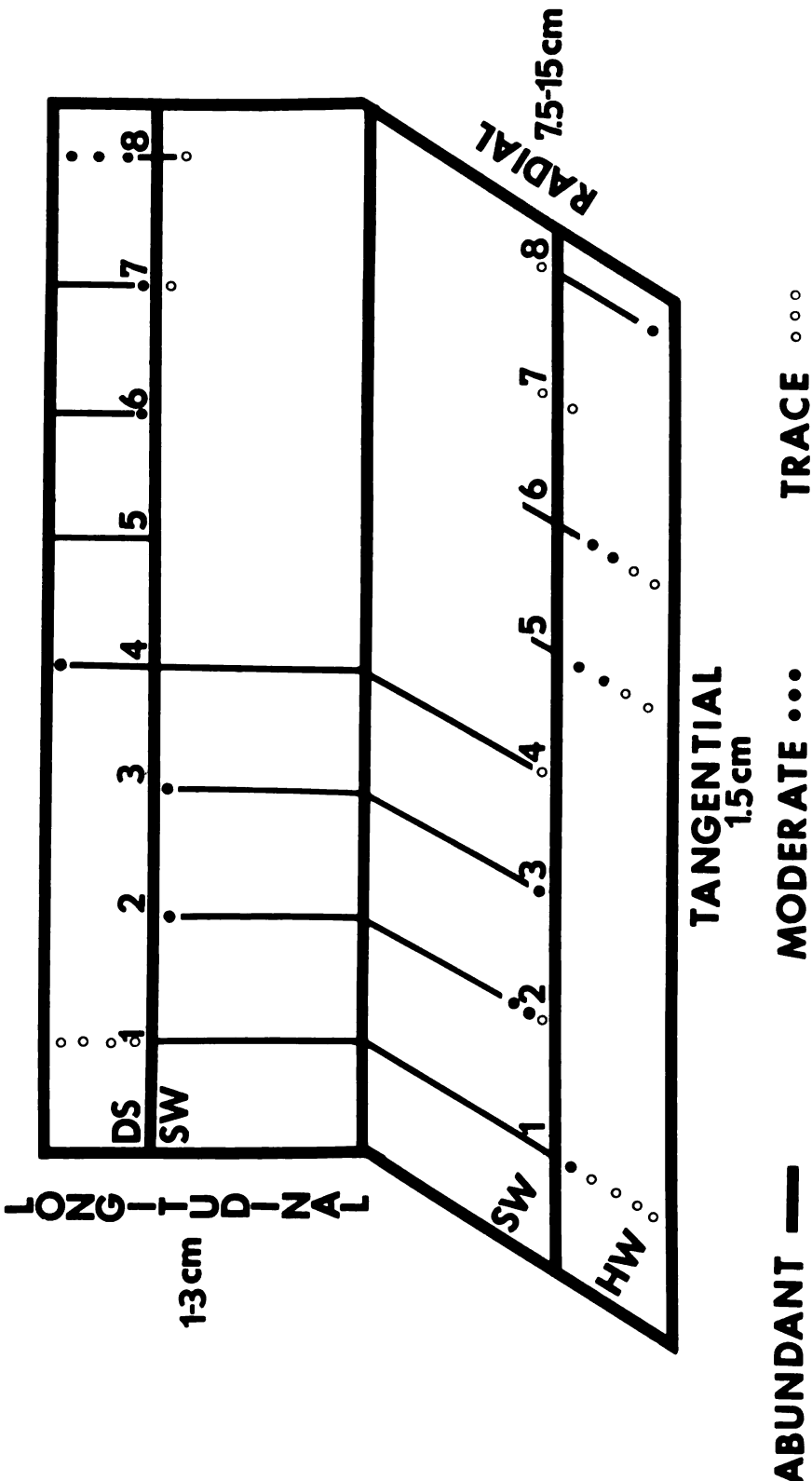


TABLE 2.--Comparisons between the sequence of events in the transformation of sapwood to heartwood and discolored sapwood.

Item Tested	Heartwood	Discolored Sapwood
Tyloses appear in springwood vessels	1 ^a	1
Starch grains disappear	2	2
Parenchyma cells are unable to reduce TTC	3	2
Nuclei disintegrate	4	5
Lipids decrease to trace amounts	5	4
Catechol and catechol derivatives undergo polymerization	5	4
Amorphous deposits appear	5	3
Tannins accumulate	5	3

^aSequence of events proceeding from the first event (lowest number) to last event (highest number).

Measurements of Oxygen Consumption

High variations of oxygen consumption between replications of tissue in individual trees were least frequent in heartwood and most frequent in discolored sapwood (Table 3). No consistent pattern was observed when comparing mean values of oxygen consumption of discolored sapwood with those of sapwood using a standard "t" test (Table 4). Mean values of oxygen consumption for discolored sapwood were higher and lower than mean values for sapwood (Figures 21 and 22).

TABLE 3.--Measurements of oxygen consumption.

Time ^a	SW ^b	SW	SW	HW ^c	HW	HW	DS ^d	DS	DS
Oxygen consumption of sapwood, heartwood, and discolored sapwood for series A. ^e									
0	.125	.155	.288	.050	.050	.039	.173	.140	---- ^f
2	.202	.214	.224	.048	.018	.005	.372	.315	.118
4	.159	.173	.154	.053	.087	----	.180	.174	.175
6	----	.244	.206	.031	.027	----	.388	.154	.208
8	.174	.150	.140	.057	.042	.045	.173	.139	.119
10	.215	.219	.178	.067	.079	.041	.177	.289	.221
12	.124	.284	.197	.017	.020	.017	.409	.450	.177
14	.219	.296	.272	.054	----	.009	.170	.218	.145
Oxygen consumption of sapwood, heartwood, and discolored sapwood for series B.									
0	.179	.142	.137	.030	.029	.013	.104	.138	.137
4	.185	.254	.264	.035	.037	----	.262	.260	.104
8	----	.091	.131	.004	.013	.026	.068	.090	.069
12	.099	.091	.131	.004	.013	.026	.068	.090	.069
16	.150	.140	.141	.021	.022	.031	.022	.096	.130
20	.130	.112	.140	.043	.050	.033	.250	.220	.184
24	.132	.109	.113	.022	.021	.026	.138	.053	.081
28	.196	.127	.102	.032	.032	.032	.138	.106	.091

^aDays after wounding tree was felled.

^bSapwood

^cHeartwood

^dDiscolored sapwood

^eValue expressed as oxygen consumed per mg tissue (dry weight).

^fSample contaminated with KOH.

TABLE 3.---Continued.

Time ^a	SW ^b	SW	SW	SW	HW ^c	HW	HW	DS ^d	DS	DS	DS	DS
Oxygen consumption of sapwood, heart, and discolored sapwood for Series C. ^e												
0	.128	.167	.115	.077	.014	.050	----	.188	.196	.146	.084	.105
4	.035	.119	----	.251	.007	.016	----	.092	----	.251	.280	.096
8	.073	.076	.059	.141	----	.022	.014	.047	.079	.095	.127	.090
12	.083	.156	.082	.047	.010	.006	.007	.228	.067	.283	.190	.127
16	.310	.253	.369	.286	.005	.000	.017	.298	.456	.209	.324	.387
20	.104	.121	.088	.084	.005	.008	.004	.136	.364	.554	.160	.157
24	.076	.074	.090	.092	.006	.013	.021	.119	.136	.222	.110	.158
28	.079	.099	.074	.090	.000	----	.015	.190	.129	.139	.159	.169

^aDays after wounding tree was felled.^bSapwood^cHeartwood^dDiscolored sapwood.^eValue expressed as oxygen consumed per mg tissue (dry weight).^fSample contaminated with KOH.

TABLE 4.--Significant deviations of mean values of oxygen consumption of discolored sapwood and heartwood when compared with mean values of oxygen consumption of sapwood.

Series	Tissue	Levels of Significance		Direction of Value
		.01	.05	
A	Discolored Sapwood		4 ^a	H ^b
		14	14	L
	Heartwood		0	L
		2	2	L
			4	L
		6	6	L
		8	8	L
		10	10	L
		12	12	L
			14	L
B	Discolored Sapwood		20	H
	Heartwood	0	0	L
			4	L
		12	12	L
		16	16	L
		20	20	L
		24	24	L
			28	L
C	Discolored Sapwood		12	H
		24	24	H
		28	28	H
	Heartwood		0	L
			12	L
		16	16	L
		20	20	L
		24	24	L
		28	28	L

^aDays after wounding that tree was felled.

^bL--mean value of oxygen consumption was less than that of sapwood

H--mean value of oxygen consumption was greater than that of sapwood.

Figure 21.--Oxygen consumption expressed as per cent of the sapwood (100%) for series B.

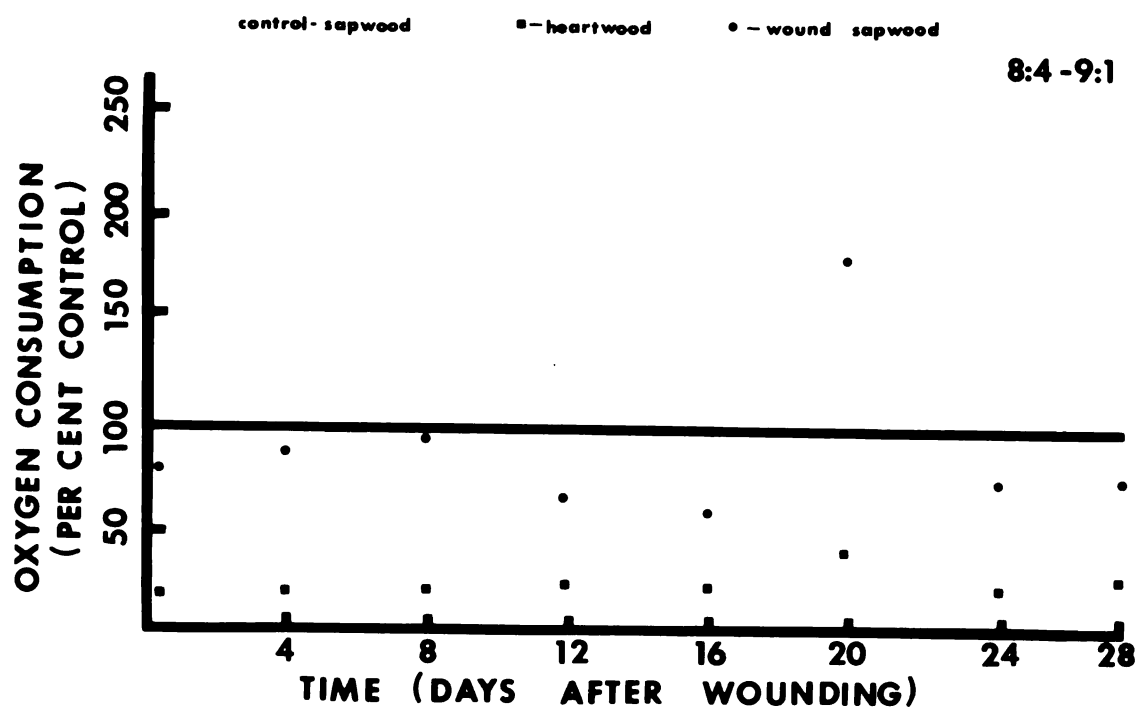


Figure 22.--Oxygen consumption expressed as per cent of the sapwood (100%) for series C (top) and series A (bottom).

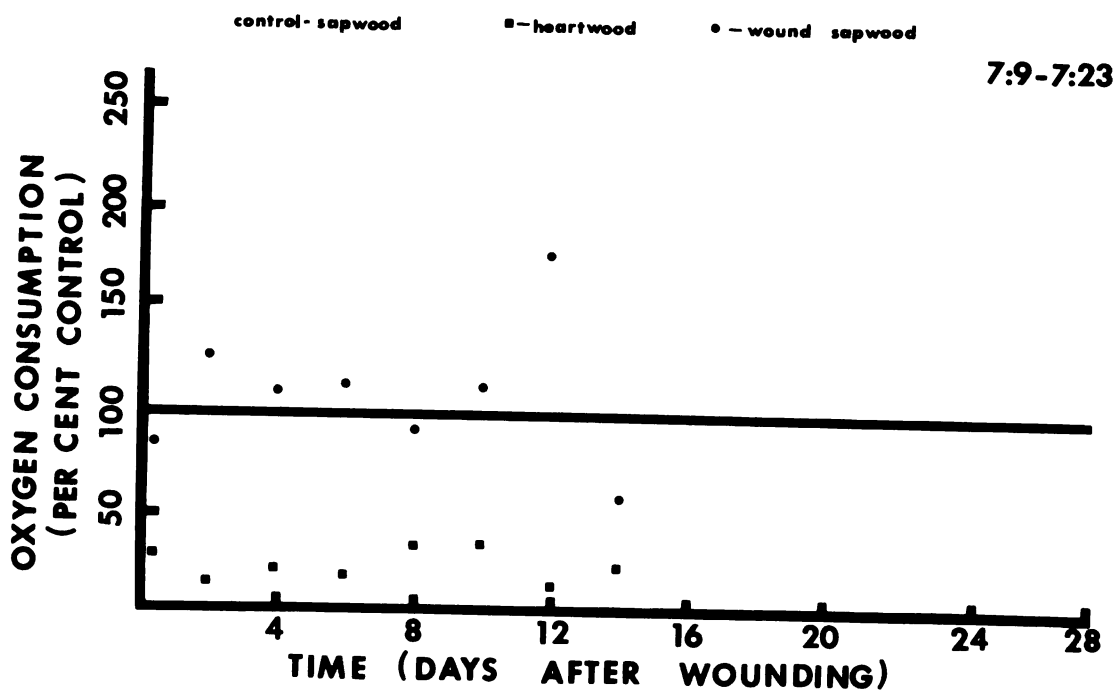
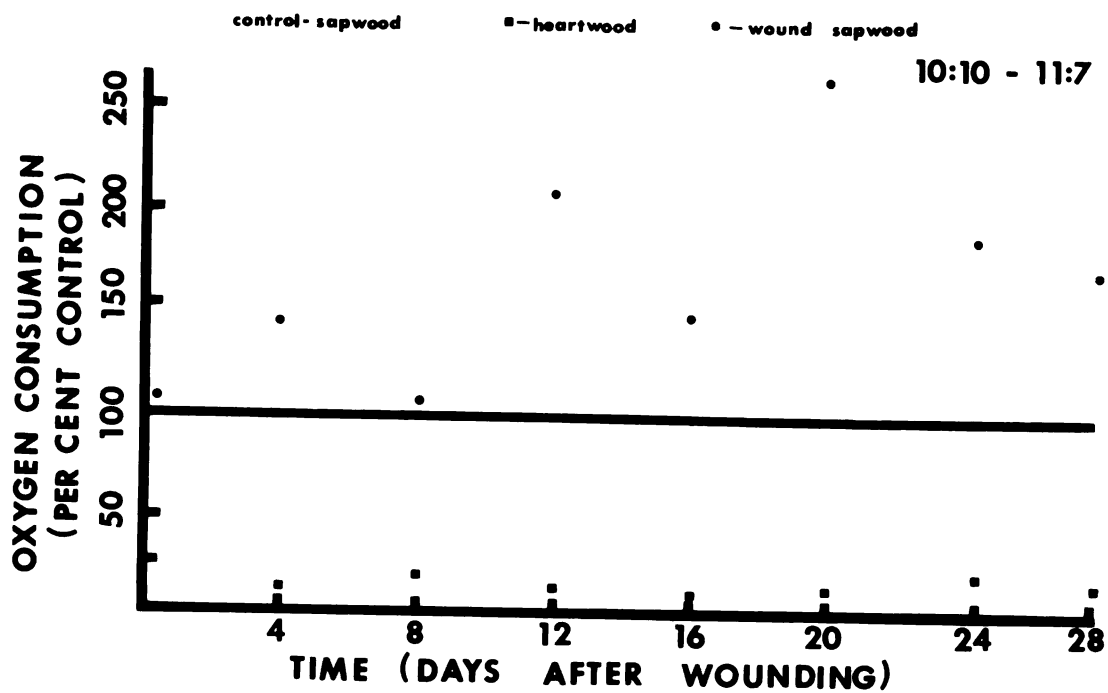
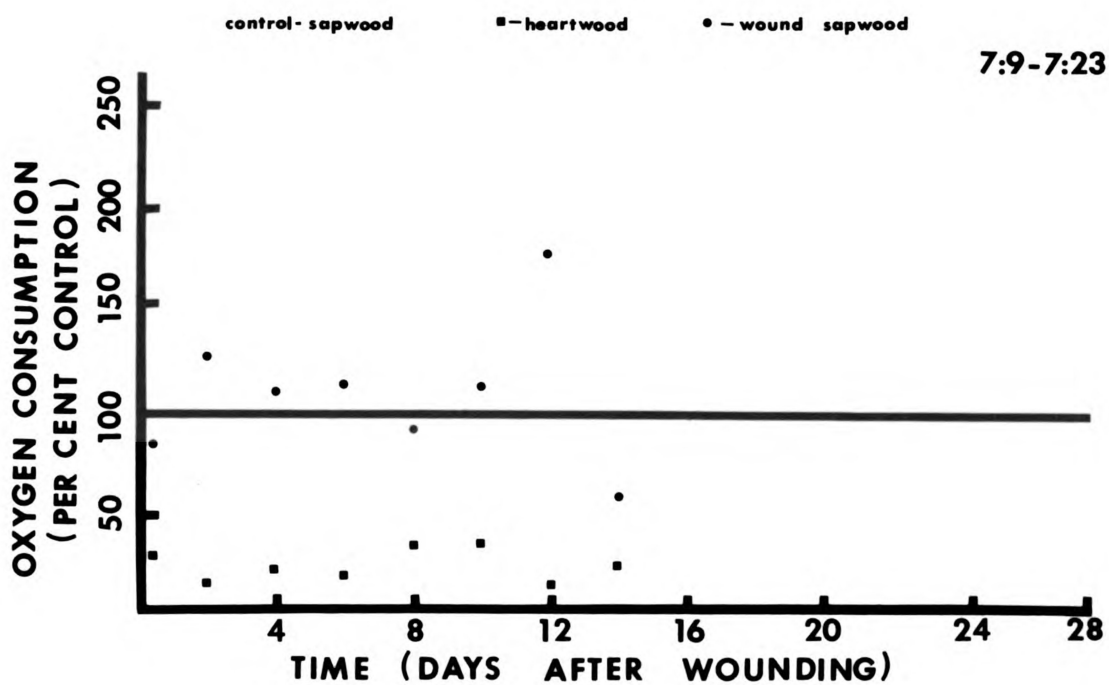


Figure 22.--Oxygen consumption expressed as per cent of the sapwood (100%) for series C (top) and series A (bottom).



DISCUSSION

This is the first investigation which studies the conversion of sapwood to discolored sapwood immediately after wounding and compares these changes with the conversion of sapwood to heartwood in a single species of tree. On the basis of cytological, anatomical, and chemical characteristics used in this investigation, the conversion of sapwood to heartwood and the conversion of sapwood to discolored sapwood appear similar in most aspects but differ significantly in a few aspects.

The disappearance of starch grains appears to be the first step in the initiation of heartwood and discolored sapwood. Starch content decreased inward from the inner sapwood towards the heartwood boundary and had disappeared before reaching the heartwood boundary. This is in agreement with previous investigations (Frey-Wyssling and Bossard, 1959; Fahn and Arnon, 1963). Starch grains disappeared from all parenchyma in the discolored sapwood. They decreased in living cells of the sapwood immediately adjacent to the interface between the sapwood and discolored sapwood. This suggests the mobilization of storage materials immediately adjacent to the wounding stimulus.

Inactivation of dehydrogenases of parenchyma in the sapwood, indicated through the inability of living cells to reduce TTC to formazan, was restricted to the annual ring of sapwood adjacent to the heartwood boundary. Cells within the sapwood remained alive until all their visible storage material had disappeared. Similarities were observed in the formation of discolored sapwood. Loss of dehydrogenase activity in parenchyma in the discolored sapwood did not occur until the starch had completely disappeared.

In heartwood formation, the nucleus was oblong to egg-shaped in the zone of the sapwood-heartwood transformation. The surface area of the nucleus increased and the nucleolus became more distinct when compared with nuclei found in the middle sapwood. These observations are in agreement with those of Hugentobler (1965) for oak. All cytoplasmic material appears to be absent and some of these cells would be considered dead because they were unable to reduce TTC. If the size of the nucleus was used as an indicator of cellular activity, increased metabolism would be occurring in these cells, perhaps the site of the sapwood-heartwood transformation (Hugentobler, 1965; Boss-hard, 1968). Chattaway (1952) considered the site of increased cellular metabolism to be the zone of the sapwood-heartwood transformation.

In the discolored sapwood, the nucleus appeared to become oblong after the disappearance of starch and after

parenchyma lost their ability to reduce TTC. In some cells, nuclei persisted in an oblong condition from 2 weeks after wounding until termination of the investigation. Nucleoli were quite evident in these nuclei. In other instances, degeneration of nuclei was observed. This degeneration of the nucleus was similar to that discussed for the heartwood boundary for oak by Hugentobler (1965). There was no apparent increase in the surface area of the nucleus in the discolored sapwood. Accordingly, this would suggest very little increase in cellular metabolism in the discolored sapwood.

Lipids appeared to decrease to trace amounts in the discolored sapwood following wounding but the lipid content of sapwood varied considerably between trees. In the sapwood, lipids occurred in moderate amounts but in trees felled in the autumn appeared to be almost absent. Apparently there are "fat" trees and "thin" trees in nature. In 6 of 8 trees removed after 20 days after wounding, lipids occurred in equal amounts in the sapwood and heartwood.

Sucoff et al. (1967) reported the disappearance of lipids in the discolored sapwood in trembling aspen within 10 days after wounding. Lipids rarely disappeared completely from the discolored sapwood in swamp white oak. Trees have been classified as to their predominant storage material into "fat" trees (Populus sp.), intermediate trees (Populus sp.), and "starch" trees (Quercus sp.) (Kramer

and Kozlowski, 1960). Storage materials are mobilized in response to wounding (Shain, 1967). This results in the disappearance of starch in swamp white oak and perhaps leads to the disappearance of lipids in trembling aspen. Until further evidence is assembled, one should view with caution the statement that "lipids disappear in the discolored sapwood of trembling aspen" (Sucoff et al., 1967) as being applicable to all trees.

Lipids occurred in moderate quantity in the zone of the sapwood-heartwood transformation and decreased to trace amounts in the heartwood. Fatty acids prevail in the heartwood of European timbers as opposed to neutral lipids (Frey-Wyssling and Bosshard, 1959). These lipids are not reserve material, rather they are compounds eliminated from aging cells. Further investigations are necessary to determine if a similar situation occurs in the discolored sapwood. Studies using Japanese timbers indicated considerably more oil and fats in the older sapwood after starch began to decrease (Higuchi et al., 1965). An analogous situation may occur in the formation of discolored sapwood where starch disappears prior to the decrease in lipid content.

Cytological criteria indicated one major difference between the conversion of sapwood to discolored sapwood and heartwood. Accumulation of amorphous deposits is quite apparent in the discolored sapwood and almost non-existent in the heartwood.

Catechol or its derivatives were present in moderate amounts in the zone of the sapwood-heartwood transformation and in the discolored sapwood between 12 and 16 days after wounding. They were not present in the sapwood and were present in the heartwood in trace amounts. The application of 1% NaOH to remove gum deposits frequently resulted in the appearance of a light brown to cherry-red color in cells of the discolored sapwood, heartwood, and the zone of the sapwood-heartwood transformation. The distribution and time of occurrence of the light brown to cherry-red color resembled the distribution and time of occurrence of catechol or its derivatives.

Polymerization of catechol or its derivatives appears to take place in the conversion of sapwood to heartwood or to discolored sapwood. In the discolored sapwood, the polymerization takes place immediately prior to the accumulation of amorphous deposits and extractives in large amounts. In formation of heartwood, an increase in tannins occurs at the heartwood boundary, most likely through condensation reactions (Hillis, 1967). A significant difference is that polymerization appears to precede the disintegration of the nucleus in the discolored sapwood and follows the disintegration of the nucleus during the conversion of sapwood to heartwood. Current evidence indicates that conversion of sapwood to heartwood is preceded by the disintegration of nuclei and consequently "before extractives are formed in large amounts" (Hillis and Inoue, 1966).

Catechol may undergo 3 possible reactions: an acid polymerization reaction, a reaction under alkaline conditions, and autoxidation (Hillis, 1967). Heartwood conditions would favor the first reaction and conditions in the discolored sapwood would favor the latter 2 reactions. The gas and moisture content and pH values of the heartwood are lower than those of the discolored sapwood (Hart, 1965). This suggests that different chemical reactions occur during the conversion of sapwood to discolored sapwood and heartwood and results in formation of different products. Evidence indicates that chemical reactions and constituents deposited in the heartwood are different from those deposited in the discolored sapwood (Hart, 1965; Hillis, 1967).

Oxygen consumption of the heartwood was much less than that of the sapwood. Oxygen consumption of the discolored sapwood exhibited considerable variation. In certain instances, it was much higher than the sapwood, in other cases much lower. Zelawski (1962) believed that increased respiration was not the result of wounding, but was caused by external factors which entered the tissue through the wound. In series C, cells of the discolored sapwood began to lose their ability to reduce TTC 10 to 12 days after wounding. As this test has been used to indicate cell death, and since oxygen consumption of the discolored sapwood was greater than that of the sapwood,

this may indicate that micro-organisms contributed to oxygen consumption of the discolored sapwood after this time. These micro-organisms would most likely be bacteria (Shigo, 1967).

Further research in this area may proceed along 3 avenues: (1) isolation of micro-organisms to determine when invasion first takes place, (2) determine the succession of micro-organisms, if any, during initial stages of discoloration and the contribution they make to discoloration processes, and (3) develop adequate techniques to measure oxygen consumption in discolored sapwood less the contribution of micro-organisms. The basic Warburg technique is capable of providing useful data on oxygen consumption by the sapwood and heartwood.

Based on cytological and anatomical characteristics, the conversion of sapwood to heartwood or discolored sapwood, with one exception, appears similar. Histochemical tests indicated important differences. Further elucidation of the chemical reactions occurring during the formation of heartwood and discolored sapwood and the nature of compounds deposited in the respective tissues is important to the understanding of these changes.

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