5-1982

The Pathogenicity of Blue-stain Fungi on Lodgepole Pines Attacked by Mountain Pine Beetle

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THE PATHOGENICITY OF BLUE-STAIN FUNGI ON LODGEPOLE PINES ATTACKED BY MOUNTAIN PINE BEETLES

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Biology
(Plant Anatomy)

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1982

ACKNOWLEDGEMENTS

During the time I have been enrolled at Utah State University I have incurred many "debts". I would like to take this opportunity to thank all of those individuals who have worked "overtime" to keep me on an even keel. First and foremost, thanks to my parents for their encouragement and gentle prodding: "When are you going to be done with your work, Rick?". Thanks are also due to Dr. Michael A. Walsh for his oftimes underappreciated support and brainstorming sessions; to Dr. E. A. Rechel for his technical assistance and comaradarie on all those days and nights in the Plant Anatomy Research Laboratories; to Big and Little Juanita and Juan, Major Marc and the other "staff" members - a Salud to you all.

Acknowledgement is due Walt Cole and the Intermountain Forest and Range Experiment Station, Ogden, Utah for material and financial support in the early phases of research. Acknowledgement is also due Dr. Gene Miller and the Department of Biology at Utah State University for material and financial help during the later phases of my program.

I thank my committee members: Drs. Sherm Thomson, Ray Brown, Mike Jenkins for their valued help; and especially Drs. Ron Lanner and Donald Davis for their help in "getting it done right". I take this final opportunity to thank Dr. Hermann Weibe for his advice, material support, and patience for a slow learner.

Spike

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ABSTRACT

The Pathogenicity of Blue-stain Fungi

on Lodgepole Pines

Attacked by Mountain Pine Beetle

by

Richard Grant Ballard, Doctor of Philosophy

Utah State University, 1982

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In the western regions of North America, mountain pine beetle, Dendroctonus ponderosae Hopk., infestations take a tremendous toll of pines , especially lodgepole pine, Pinus contorta Dougl. var. latifolia Engelm.. Mass attack by the beetles is a devastating event for the trees. As well as girdling the tree, a massive inoculation of blue stain fungus "complex" (composed of several species of Ceratocystis, numerous yeasts and other mycelial fungi) is made beneath the bark. These fungi colonize and destroy the parenchyma tissue system of the host sapwood, primarily the ray parenchyma and resin duct epithelium.

A blue stain is produced in the sapwood as a consequence of destruction of the sapwood parenchyma. The stain develops inward through the sapwood, and the transpiration stream is cut

off. As more and more sapwood is stained, foliar water stress begins to increase. Foliage however, remains green and apparently healthy for up to 10 months after inoculation. When spring bud break begins the year following beetle attack, terminal buds of blue-stained trees begin to expand, then abort. Soon after, the needles of these trees fade to a reddish brown color.

Transpiration stream disruption was not caused by penetration of tracheids by fungal hyphae; tyloses were not observed; nor was microconidial blockage of bordered pits seen. Though resin duct epithelium was eventually destroyed, little resin soaking was observed in the initial blue stained regions. Many bordered pits of tracheids in stained regions appeared to be aspirated, suggesting introduction of embolisms.

(89 Pages)

INTRODUCTION

In the western regions of Canada and the United States, mountain pine beetle, Dendroctonus ponderosae Hopk. epidemics are devastating to lodgepole pine, Pinus contorta Dougl. var. latifolia Engelm. forests. Mountain pine beetles initially attack the largest trees in the forest stand and as the epidemic progresses, smaller and smaller trees are also attacked.

In their feeding activities beneath the bark, beetles inoculate a "blue-stain fungus complex" into the trees. The fungus complex, composed of several species of Ceratocystis and Europhium, grow via the wood rays into the sapwood of these trees. In the process the transpiration stream of these trees is disrupted. The final expression of tree death, which occurs during the following spring, is a spectacular, and for many people, an unsettling sight.

Economic, watershed, wildlife habitat, and scenic losses resulting from mountain pine beetle attack on lodgepole pine are great. A massive effort has been underway for many years to learn of the underlying causes of the problem and develop a solution. Much is now known about the biology and ecological role of the beetles in the forest. Inroads have been made on understanding the physiology and ecological place of lodgepole pine. However, comparatively little effort has been expended to learn about the pathogenic effects of the blue-stain fungus on the host trees.

Four objectives were proposed to study the effect of blue-stain in the onset of water stress and eventual death of lodgepole pines attacked by mountain pine beetles. They were: 1) Determine the tissue systems colonized by blue-stain fungi. 2) Correlate the temporal aspects of development of blue-stain, tissue drying and onset of water stress in mountain pine beetle attacked trees. 3) Determine the mechanism of transpiration stream disruption in blue-stained sapwood. 4) Determine if the girdling action of beetles and larvae is responsible for the decline and death of attacked trees.

LITERATURE REVIEW

An understanding of lodgepole pine ecology, anatomy, and physiology is important in determining the role that blue- stain fungi and mountain pine beetles play in the decline and death of the trees after attack. Knowledge of the processes in lodgepole pines is incomplete, but generalizations can be made by incorporating studies of related tree and beetle species.

Ecology

Pinus contorta Dougl., or lodgepole pine, is one of the most widespread pines in North America. The genus is composed of four geographical subspecies: latifolia, contorta, murrayana, and bolanderi. They are found in very diverse habitats; latifolia is found in the Rocky Mountains from Colorado north to the Yukon; contorta is found in British Columbia along the coast; murrayana occurs in the Sierra Nevadas and north into the southern Cascades; bolanderi is restricted to a narrow coastal region in northern California. Lodgepole pine reaches its greatest distribution in British Columbia (Mirov 1967). They grow in habitats from bogs to dry mineral soils. Lodgepole pines grow best on moderately acid, sandy or gravelly loams that are moist and well drained. They are often established as a fire climax species, but may become established as a permanent climax species on some sites (Mirov 1967).

Anatomy - Functional Histology

Secondary Xylem

Conifer wood is considered to be relatively homogeneous, in that it is made up of one basic conductive cell type, the tracheid (Esau 1965). Tracheids are elaborately pitted elongate cells that taper at both ends. The wood of pines is laid down in annual increments or growth rings and is produced by the meristematic activity of the vascular cambium. In the spring of the growing year the vascular cambium produces thin walled cells, or earlywood tracheids that have broad lumen and are primarily responsible for conduction of water. Late in the summer the vascular cambium produces thicker walled cells or latewood tracheids, these cells are destined for supportive functions (Esau 1965; Bannan 1964; Carlquist 1975). At the overlap regions of these cells, bordered pit pairs occur on the radial walls and provide cell to cell connection. Water passes from cell to cell via these pit pairs (Kramer and Kozlowski 1979; Milburn 1979). A general relationship found amongst conifers is that the smaller, slower growing tree forms tend to have shorter elements than do the larger, more rapidly growing forms (Bannan 1965). Greater tracheid length results in improved water conduction, there are greater overlap areas (with more pits). Greater tracheid length provides for fewer tracheids per unit length of wood, therefore less impedance to conduction as water passes from cell to cell (Kramer and Kozlowski 1979). In lodgepole pine it was observed that a reduction in ring width below

1mm resulted in production of narrow tracheids. There are definite differences in tracheid size between young, vigorous trees and older more suppressed trees (Bannan 1965).

The Bordered Pits

The pit membrane of a bordered pit pair is composed of an inner region of closely interwoven cellulose microfibrils, termed the torus, and an outer margin of comparatively loosely woven microfibrils, termed the margo (Esau 1965). Water is believed to pass relatively freely through the pit membranes (Milburn 1979), primarily through the margo. An important feature of the bordered pit membranes is their ability to seal off cavitated (embolized) tracheids from other functioning tracheids. Stresses placed on the pit membrane can be very great, up to -100 bars from adjoining cells (Milburn 1979; Liese and Bauch 1967), therefore they can be considered quite sturdy entities. The sealing mechanism is due to an unequal attraction by water surface tension on the pit membrane by a functioning cell. In contrast, the adjoining cavitated cells do not exert any tension on the pit membrane (Liese and Bauch 1967). Bordered pit sealing can take place when air penetrates the pit chamber and disrupts the adhesion forces between the pit membrane and the water in the tracheid. The surface tension in the adjoining functional tracheid draws the membrane against the aperture of the border, thus tightly sealing the bordered pit (Liese and Bauch 1967). The pit membane remains an effective barrier to diffusion of gas only as long as it remains saturated with water (Milburn 1979).

The Ray Parenchyma Tissue System

The living or parenchyma system comprises roughly 10% of the cells in conifer wood (Carlquist 1975). The vast majority of these parenchyma cells are contained in the horizontal or radial system. Two types of rays occur in most conifers including lodgepole. One type is the narrow, uniseriate rays one cell layer wide, and the other is the fusiform rays that are several cell layers wide that contain horizontal resin ducts. The parenchyma cells that make up the rays are thin walled cells possessing living protoplasts (Balatinecz and Kennedy 1967; Thomas 1968; Fengel 1970; Imamura and Harada 1973). Ray tracheids are usually located on the upper and lower margins of the rays. These cells are procumbent, thick walled, nonliving cells that possess, in lodgepole, unique wall ingrowths. They are thought to be involved with radial transport of water because they are provided with bordered pit connections with axial tracheids. Both ray parenchyma and ray tracheids are derived from the vascular cambium (Esau 1965). They are arranged in files with the cell's long axis aligned along the radial axis of the trunk. Ray parenchyma cells possess cytoplasmic components that characterize living cells: nuclei, mitochondria, endoplasmic reticulum, dictyosomes, plastids, and vacuoles. They are metabolically active near the younger differentiating vascular regions, but activity gradually decreases away from these regions (Frey-Wyssling and Bosshard 1959). Depending upon the season, both starch and lipids are found as storage products. The rays almost exclusively supply the fusiform initials of the cambium with carbohydrates and other nutrients during tracheid differentiation. This may

be an effective feedback mechanism for regulating the arrangement and distribution of ray cells (Zimmermann and Brown 1977). A relative paucity of parenchyma in conifer wood (compared to angiosperms) may relate to the tendency for slower conductive rates in tracheids (compared to vessels) demanding less parenchyma for conductive requirements (Kramer and Kozlowski 1979). The outermost ray parenchyma is a totipotent cell system. Cells have nuclei, and several characteristics of undifferentiated cells and are thus capable of dedifferentiation, proliferation and redifferentiation into specialized functional forms, i.e. wound callus.

Ray parenchyma cells nearest the heartwood which may be 40-60 years old are in the process of senescence. In the process of aging, cell nuclei become deformed and lose chromatin, while mitochondria gradually cease their function (Frey-Wyssling and Bosshard 1959). Cell walls often thicken and become lignified at the sapwood/heartwood interface. Fengel (1970), examining the wood of Pinus sylvestris L. with the electron microscope, observed that heartwood forming compounds within parenchyma cells passed through the parenchymatous pit membrane into tracheid lumina. These compounds became deposited in tracheid cell walls and bordered pit membranes causing a decrease in permeability characteritics of heartwood. Bordered pits become encrusted, then experience a reduction of conductivity, and eventually aspirate (Bauch et al. 1974).

The Contact Cells

Window pits and pinoid pits connect ray parenchyma cells with tracheids. Window pits are characteristic of haploxylon pines while most diploxylon pines possess pinoid pits. Pinoid pits differ from window pits primarily in size, however both are composed of cellulose of the primary cell walls of predifferentiated tracheids (Thomas and Nicholas 1968). In pines, pinoid pits and window pits are of such large size and abundance as to suggest that ray parenchyma may function as "contact cells" (Braun 1970). Contact cells are thought to be specialized cells that actively secrete (or exchange) substances with tracheary elements (Braun 1970; Zimmermann and Brown 1977). Carlquist (1975), observed that all tracheids, because of their great length, make contact with at least one ray. This is accomplished via cross field pitting. With this close association between tracheids and ray parenchyma via pinoid pits, rays probably augment tracheid function in some way.

Physiology

Water Flow Through Conifer Wood

Though Pinus contorta is a much studied tree, the knowledge of its physiology and functional anatomy is still quite limited. Swanson (1967), studying seasonal flow of water in the sapwood, found springtime sap velocities to be a relatively slow, 3.8cm/hr. Flow of sap in succeeding months became progressively slower as soil dried out. Carlquist (1975) stated that conduction by any given tracheid is not isolated from conduction by adjacent tracheids. The entire sapwood of

secondary xylem in a conifer stem conducts water as a continuous cylinder. Kozlowski and Winget (1963) have shown that water flow is spread over many layers of annular growth rings. In the sapwood, the velocity profile increases with depth, reaching a peak at 15mm with declining velocities to 35mm (Swanson 1967). Thomas (1968) studying southern yellow pines, and Mark and Crews (1973) studying lodgepole pines demonstrated that bordered pit membranes in the outer regions of the sapwood had smaller perforations than did bordered pit membranes located in somewhat deeper rings. However, perforations in pit membranes closer to the heartwood were encrusted with minerals, tannins, and phenolic compounds that would tend to increase resistance to flow compared to pit membranes in younger rings. Rudinsky and Vite (1959) conducting dye injection studies have shown that ascent of sap occurs in a spiral fashion, possibly reflecting spiral wood pattern or radial location of bordered pits. They claimed that the spiral pattern of sap ascent provides more effective distribution of water to all parts of the crown than direct vertical ascent.

Control of Water Flux

Spomer and Stoszek (1978) found the osmotic potential of needles to be consistently higher than the osmotic potential of stems. Values for both were much more negative in August than September, with September values being more variable. Their interpretation of the higher needle values compared to those of stems is that it is advantageous to the tree for continued photosynthetic activity . As a consequence higher water potential in needles than stem would insure sufficient

flow of water for physiological processes. They reported further that trees exposed to water stress became acclimated by osmotic adjustment in the needles.

Axial water flow in wood depends upon maintenance of a continuous water column from roots to needles. Tracheids must have water saturated cell walls to maintain their function (Milburn 1979). The lignified cell walls of tracheids are quite porous. When saturated the pores are filled with water (up to 50% dry weight). Relatively little is known about the water uptake processes in roots, but intimate environmental penetration by root tips is known to be a requirement. Flow of water upward from the roots, through the trunk, and out to the branch tips is controlled ultimately by the rate of water evaporation from needles via the stomata.

Recent studies (Lopushinsky 1969; Running 1976 and 1980) have shown that trees can control transpirational water loss when suffering water stress. In winter when trees are under extreme moisture stress the transpiration rate of conifers is less than 1% of the summer rate (Kramer and Kozlowski 1979). Stomata open and close in response to changes in turgor of guard cells relative to subsidiary cells, and general needle water status. Turgor is a major factor influencing stomatal behavior. When needle water potentials of -16 to -18 bars are reached, stomates closed. Stomatal response is quite rapid, with closure occurring within a rather narrow range of leaf turgidity and water potential. Stomata of pines are therefore quite sensitive to water stress. This explains the ability of most species of pine to tolerate drought conditions. In studies on conifer photosynthesis,

leaf water potential was shown to influence photosynthesis mainly through its effect on mesophyll resistance to carbon dioxide exchange (Hillerdal-Hagstrome et al. 1982). The needles still have high water content which is maintained for a period of time after stomate closure.

Beetle-fungus Symbiosis

Though the evidence may be circumstantial, it is believed by most researchers studying mountain pine beetles that the mountain pine beetle/blue-stain fungi/pine triad has evolved as a unit. The best evidence for co-evolutionary relationships is the beetle/fungus interaction. A guilt-by-association argument, though inclusive, is a reasonable foundation on which to base further studies. Bark beetles and fungi are commonly found together. Ambrosia beetles, closely related beetles, have evolved a total dependency (nutritional) on the fungal symbiont, with the host tree simply a container to provide suitable environmental conditions for beetle/fungus survival (Rudinsky 1962;Franke-Grossmann 1967; Graham 1967).

From the early days of bark beetle research, many studies have been conducted on micro-environmental conditions necessary for beetle brood survival. It has been an accepted fact since these early studies that blue-stain fungi are associated with beetle attacked trees (Berryman 1972). It has been shown that in trees developing blue-stain, sapwood dries out rapidly and trees succumb (Nelson 1934; Reid 1961; Mathre 1964).

More recent research has suggested that the beetle/fungus symbiosis is a mutualistic relationship. Barras and Perry (1972) reported on the direct association of mycangial fungi and feeding Dendroctonus beetles in host trees. In later studies Barras (1973) showed that absence of normal complement of symbiotic fungi caused significant reductions in brood production, survival, and delayed emergence of progeny by up to 24 days. Webb and Franklin (1978) reported that a higher moisture content in rearing bolts (a segment of tree trunk) resulted in lower brood survival and longer larval mines. An earlier report by Barras (1970) suggested that an equilibrium exists between larvae and fungi must be maintained for brood success. In preinoculated pine bolts, normal larval development was inhibited, larvae constructed atypically long mines and progeny numbers were lowered.

High moisture content of host tissues retards growth of blue-stain fungi and is detrimental to brood development and survival (Caird 1935). Brood survival is directly related to the final moisture content of the outer sapwood (Safranyik 1978). Since the average diameter of the infested trees decreases during the course of epidemics, and since smaller diameter trees tend to dry at a faster rate than large diameter trees, the moisture factor during brood development would seem to play an important role in the population dynamics of mountain pine beetles (Cole et al. 1976).

Pathology

Though lodgepole pines are subject to numerous root and foliar diseases (Boyce 1938; Bega 1978) and numerous insect pests (Graham and

Knight 1965), none are quite so destructive as mountain pine beetle and blue-stain fungus epidemics.

Craighead (1928) reported the invariable association of certain wood staining fungi, the blue-stain, with Dendroctonus (bark beetle) attacks. He believed that fungi were introduced under the bark by beetles. He suggested their combined role in the demise of host trees. Nelson and Beal (1929) reported circumstantial evidence to support the symbiosis theory . The symbiotic relationship of beetle and fungus is now an accepted theory by most workers in the field (Graham 1967; Franke-Grossmann 1967; Berryman 1972; Coulson 1979; Birch 1978). Craighead (1928) noted the close association between development of bark beetle broods and presence of blue-stain fungi. He was first to suggest a symbiotic relationship between blue-stain fungi and Dendroctonus spp. In his 1928 paper he speculated that the fungi were instrumental in conditioning the tree so that it became a proper medium for normal development of beetle broods. The blue-stain fungi appear mainly to affect the moisture content of host trees (Nelson and Beal 1929; Nelson 1934; Caird 1935; Bramble and Holst 1940; Mathre 1964; Amman 1972). Several researchers have reported that with development of blue-stain in the sapwood, the transpiration stream of bark beetle infested trees became restricted to the inner portions of sapwood and eventually stopped altogether (Nelson 1934; Caird 1935; Bramble and Holst 1940; Mathre 1964; Basham 1970). Concomittant with flow disruption, tissue drying was noted in blue-stained regions. All researchers reported that trees experienced water stress and death only

when the entire cross sectional area of the sapwood was stained blue.

Nelson (1934) and Basham (1970) looked at sapwood tracheids and reported a high percentage of bordered pits of these cells to be aspirated. Unattacked trees showed a much lower percentage of aspirated bordered pits.

Nelson (1934) suggested that aspiration of bordered pits of blue-stained sapwood was due to encrustation products from dead ray parenchyma cells lodged against pit membranes. As blue stain-fungi destroyed ray parenchyma cells, Nelson suggested cytoplasmic contents leaked through pinoid pit membranes into the lumen of tracheids and were drawn to bordered pits. As mentioned earlier, bordered pit membranes are made up of two regions: the central torus, a tightly woven pad of of microfibrils and a peripheral region, the margo, composed of a loosely woven array of microfibrils. In Nelson's theory the cytoplasmic byproducts resulting from ray parenchyma cell death would lodge against the margo region of the pit membrane and slow the flow of water . Eventually so much build-up of encrusted material would occur that water flow would be completely disrupted, resulting in tracheid cavitation. Bramble and Holst (1940) looked for this encrustation and found oleoresin globules. Mathre (1964) and Basham (1970) performing multiple histochemical tests found no encrustations in blue- stained wood of ponderosa pine.

Histological studies of blue-stained wood showed fungal hyphae essentially confined to medullary rays. Hyphal penetration into tracheids was rare (Nelson 1934; Rumbold 1941).

Nelson and Beal (1929) tested the hypothesis that girdling action of beetles and larvae in the phloem would starve roots of photosynthate and consequently reduce to flow of water upward to the crown. Their experimentally girdled trees did not dry out (or die) as rapidly as trees with blue-stain.

Nelson (1934), Caird (1935), Mathre (1964) all attempted artificial inoculation of trees with blue-stain fungi. Their results showed that moisture content of sapwood (and phloem) determined to a great extent the success or failure of inoculations and that tissue developing blue-stain had lowered water content. It was demonstrated that with successful inoculation, the blue-stain fungi grew readily and rapidly into the sapwood of living trees(Nelson 1934).

Nelson (1934), Caird (1935), Reid (1961), and Mathre (1964) showed that in the weeks following beetle attack, trees developing blue-stain had lowered water content. Nelson (1934) considered the most likely explanation for sapwood drying to be transpiration pull from the crown. Mountain pine beetles in their feeding attack on host trees inoculate, via specialized "maxillary mycangia", a blue- stain fungus complex into the tree (Whitney and Farris 1970). The "complex" is comprised of several species of Ceratocystis and Europhium as well as several other mycelial fungi and yeasts (Robinson 1962; Robinson-Jeffrey and Grinchenko 1964; Robinson-Jeffrey and Davidson 1963; Whitney 1971). The complexity of the fungal species infecting the wood suggests that a microbial succession occurs. Though controversy exists (Hetrick 1949; Barras 1970; Franklin 1970; Whitney 1971; Yearian et al. 1972), studies on southern pine beetle (Barras 1973)

have suggested a symbiotic beetle-fungus association to the extent that each partner is able to complete its life cycle more successfully in the presence of the other. Excellent reviews on this complex symbiotic partnership, between beetle and fungi, and their host trees are presented by Graham (1967), Franke-Grossmann (1967), Berryman (1972), Birch (1978) and Coulson (1979).

Although it is acknowledged that fungi benefit from transport to a food source and a site for new reproductive potential, it is not fully understood what benefit is derived by beetles in this relationship nor what role the fungus complex plays in the death of the host tree or in the predispositon of tree tissues for brood development and survival (Nelson and Beal 1929; Nelson 1934; Mathre 1964; Reid et al. 1967; Shrimpton and Whitney 1968; Berryman 1972; Shrimpton 1973 and 1978; Barras and Hodges 1974; Wong and Berryman 1977; Webb and Franklin 1978). Few detailed developmental/anatomical studies of the fungi within the host have been attempted. For example, only one study with a single illustrative micrograph showing fungal development in rays is available for lodgepole pine (Rumbold 1941). Shepherd and Watson (1959) reported on the presence of fungi in blue-stained wood in lodgepole pine, but no micrographs were included in their report. A few early reports are available for fungal development in southern pines (Nelson and Beal 1929; Nelson 1934). They also presented very few light micrographs showing hyphal establishment in ray parenchyma and in some tracheids. Micrographs of fungi in xylem of northern white cedar and a drawing showing hyphae of blue-stain fungi passing through bordered pits are contained in a book by Hubert (1931 citing von

Schrenk 1903). Liese and Schmid (1961) studied pines and spruce with blue-stain and showed passage of Ophiostoma sp. hyphae through bordered pits, mechanical destruction of pit membranes, and presence of hyphae in ray cells. This study seems to be the most comprehensive to date on hyphal development in blue stained wood.

MATERIALS AND METHODS

Histology

The first season of study, summer 1978, was used to work out cytohistological techniques and learning to recognize healthy and infected trees and tissues. Experimentation dealt with various concentrations of fixative, periods of fixation, post fixation, and infiltration to achieve optimum preservation of tissues in plastic. Suitable study sites were chosen close to roads to accommodate transport of oftimes bulky equipment to the study trees. Study sites were located near the head of Logan Canyon in Cache County, Utah (elevation 2300 meters, Fig.1). Sampling began late in June, when snow melted sufficiently for access to the study site (Fig. 2). This first field season continued through October. Initial tissue processing was done in the field (Fig.3). A data base study was deemed important to ascertain seasonal aspects of lodgpole pine phloem and xylem development (Fig. 4) and the temporal relation to beetle attack. Sampling was also undertaken from trees attacked the previous (1977) season. Trees were also baited with pheromone impregnated Tygon tubing to attract beetles to trees of our choosing (Fig. 5). Tissue was processed for light and electron microscopy in a sodium cacodylate buffered, full strength Karnovsky's (Karnovsky 1965) fixative and post-fixed for two hours in 1% (aq) osmium tetroxide. Tissue samples were dehydrated in ethyl alcohol, infiltrated and embedded in Spurr's low viscosity medium (Spurr 1969). For light microscope observation, sections 1-2um thick were taken with glass knives on a Sorvall MT-2 Port-

er-Blum ultramicrotome, stained with toluidine blue O, and viewed and photographed with a compound light microscope . For transmission electron microscopy, thin sections were taken with a diamond knife on the Sorvall ultramicrotome, stained with uranyl acetate and lead citrate, then observed and micrographed on a Zeiss EM-9 transmission electron microscope.

Fungus Developmental Studies

During the summer of 1980 a study was undertaken relating to hyphal colonization and development in sapwood. Increment borer cores were taken from trees attacked by mountain pine beetles (see Fig.8). Presence of bluestaining in tissues was determined visually. One centimeter square segments of wood at the frontal margin of blue stain were sliced along the radial axis into approximately 2mm squares and fixed in cacodylate buffered Karnovsky's fixative. Processing of tissue was the same as previous work (see Histology).

Scanning electron microscope investigation was also begun during the summer of 1980. Up to this point in time, the extent of colonization of wood rays and sapwood tracheids by blue-stain fungi had not been studied. It was determined, that in the early phases of fungal colonization of sapwood, fungi were difficult to observe. To compensate for low numbers of fungal hyphae, tissue samples from comparatively large areas were needed to localize them. Tissue for scanning electron microscope observation was obtained in much the same way as for the transmission electron microscope work; several increment borer samples were made from beetle attacked trees. Cores were removed and

sliced radially with a sharp blade (usually a double edged razor blade) and fixed in Karnovsky's fixative for two or three hours. Samples were then washed and taken through an ethyl alcohol dehydration series to remove much of the water in the tissue. The tissue was then taken to absolute dryness by the Critical Point method, on a Bomar Critical Point machine. Tissue was mounted on aluminum stubs and coated with a gold-palladium alloy using a Denton Sputter Coater. Tissue specimens were observed and micrographs obtained with an AMR 1000 Scanning electron microscope.

To test the hypothesis that direct fungal penetration of tracheids resulted in their inactivation, samples of sapwood were taken from near the blue stain margin and serially sectioned. For orientation of the sections a unique feature of that section was chosen, then utilized to achieve identical positioning for all serial section replicates. To insure hyphal counts, rays containing fungal hyphae were sought and used in orientation. Six counts were made per set of serial sections, this represented roughly .5mm tracheid length. This insured visualization of at least half the length of tracheids present.

Physiology

During the 1979 study season, water stress measurement studies were begun to determine the rate and degree of tissue drying that results from beetle attack. Screen and ceramic psychrometers were obtained from Wescor, Inc., Logan, Utah. They were implanted beneath the bark and in the outer sapwood of both control and experimental trees as suggested by H. Wiebe(personal communication). This was intended to show changes in water potential in the region of outer sapwood and inner bark of trees attacked by beetles, and also to act as a comparison to pressure bomb studies being conducted concurrently (Fig.6). Readings were attempted for several weeks but were discontinued for lack of consistent results. When psychrometers were removed they were found to be resin soaked. The pressure bomb study was continued. Terminal branches were cut from primary branches that originated on the main trunk usually about 5 meters above ground level. Sometimes these could be obtained from ground level, but more often a ladder was required or the tree was climbed. The water potential study was performed to determine water potential at a time of day when the tree had come the point nearest recovery from daily occurring plant water deficits. The study began at the end of July, just before beetle attacks began and continued through to the end of October.

Concurrent with the 1979 pre-dawn water potential study, tree girdling studies were undertaken to test the hypothesis that beetle larval mining activities alone induced water stress on host trees. The experiments tested the theory that girdling disrupts phloem tran-

slocation to the roots, causing stress and disruption of water uptake (Noel 1970). A narrow groove (1 cm)or a broad groove (15 cm) was cut into the bark of three trees, completely severing the phloem. Stem water potential readings were taken periodically for several months and ocasionally during the next two study seasons (1980 and 1981).

In early August (1981 season) another water potential study was begun on several trees attacked by beetles (compare Figs. 9,10, and 11). Along with the pressure bomb work, a sapwood water content study was conducted to correlate sapwood drying with onset of water stress in foliage of host trees. Using an increment borer, a core was removed from various locations around the trunk (between 3 and 6 feet above ground level). These cores were placed in screwcap test tubes and transported back to the laboratory. In the laboratory, cores were sectioned into 2cm segments and placed in tared weighing vials. Fresh weights were determined and recorded then tissue samples were dried for 24hrs. at 100 degrees centigrade, dried samples were reweighed. These readings were made on a weekly basis. Presence of blue-stain was determined visually. The percentage moisture content was calculated on an oven dry weight basis by the standard method as follows:

$$\frac{\text{Fresh Weight - Dry Weight}}{\text{Dry Weight}} \times 100 = \text{Percent Dry Weight}$$

RESULTS

General Observations

In northern Utah at an elevation of 2300m, young mountain pine beetles emerged from their host, lodgepole pine, near the end of July or the first of August. Female beetles flew to large, green trees where they bored into the bark to establish nuptial chambers. After mating, the female bored a vertical egg gallery through the inner bark, cambium, and outermost sapwood tissues (Fig. 7).

Several weeks before beetle attack (sometime in June), cambial activity was renewed in lodgepole pines. Cambial growth in this species appears to proceed much the same as in other pines (Alfieri and Evert 1968) except for temporal differences related to the higher elevations at which they grow.

In the spring of the year following beetle attack, in the midst of healthy trees (Fig. 9), needles of beetle infested trees began to show decreased chlorophyll content (Fig. 10). Within a month these trees were completely brown (Fig. 11).

Histology - Vascular Cambium

By mid August, at the peak of beetle attacks, vertical resin ducts (which occurred only in the late wood) had differentiated, though were not yet functional entities (Fig.12). Horizontal resin ducts (Fig.13) appeared as continuously differentiating tissues from the vascular cambium to the regions of maturity in either phloem or xylem. Those cells that composed the epithelium of the differentiat-

ing horizontal resin ducts were richly provided with cytoplasmic content.

Histology - Phloem

The functional phloem appeared as a distinct layer of cells (usually 10-15 cells thick) immediately outside of the vascular cambium (Fig.14). The layer was composed of alligned radial files of sieve cells interspersed with rays. The cells to the outside of this innermost layer of phloem appeared to be in various stages of deformation. Also, the radial files of cells became irregular and were no longer in straight files. This tissue, though possibly not functioning in translocation of photoassimilate may instead be involved in storage of starch and lipids. The ray parenchyma cells appeared to continue to possess cytoplasmic content. These cells formed a continuous, though irregular file of cells (Fig. 14 arrows) out to the cork cambium. Resin cysts, reported to be continuous with horizontal resin ducts and containing oleoresin under pressure, occurred frequently enough to provide hazard for mining beetles and larvae (Fig. 15).

To the inside of the vascular cambium, the secondary xylem cells had become differentiated as thick walled latewood tracheids (Fig. 15 arrows). These cells, being so thick walled,

would appear to be a barrier to beetle or larval feeding activity. This layer was 4-5 cells thick within the annual increment of tracheids. These cells were laid down during late August or early September.

Histology - Rays

The uniseriate rays typical of pines were composed of thin walled parenchyma cells whose function is thought to be storage and radial translocation of sugars and thick walled horizontal tracheids whose function is radial transport of water (Kramer and Kozlowski 1979). The parenchyma cells were rich in cytoplasmic contents (Fig. 16,17,18). Storage products appeared to be primarily lipidic in nature at the time of beetle attack (Fig. 17 asterisk). The tangential walls were uniform in thickness and possessed numerous plasmadesmata. The radial walls in contrast possessed comparatively few plasmadesmata. Simple pits or pit fields were essentially lacking and seen only on transverse walls. Pit fields when they occurred possessed many plasmadesmata. Ray tracheids were located either at the top or bottom margins of the uniseriate ray or interspersed with parenchyma within the ray (Fig.18).

The fungal spores (or propagules) carried into the host tree germinate in the frass (material that has passed through the gut of adults) within the egg galleries. They must live saprophytically for a short time (Whitney 1971). With hatching of eggs and subsequent feeding activity by larvae (Fig.7), new

routes for lateral growth are opened to fungi in the bark region. The fungi soon find their way to the now exposed outer portions of the many uniseriate rays of the sapwood and inner bark. In this way many infection courts are made available to invading fungal hyphae.

Histology - Fungal Development in Rays

As the fungal hyphae of the blue stain fungus complex penetrated the rays, they grew radially toward the center of the tree. The hyphae penetrated ray parenchyma cells and grew within the lumen of the once living cells. Hyphal proliferation within the ray parenchyma cells appeared to be extensive, with much branching (Figs. 19 & 22). In some cases (8 slides observed) it appeared that fungal hyphae, upon reaching the inner tangential cell walls of colonized cells, proliferated greatly, then pushed through to the next cell (Figs. 19 & 22 arrows). These fungal hyphae passed to the next cell by again penetrating the next inner tangential cell wall of the attacked cell, and passed from cell to cell in a similar fashion (Fig. 20).

The mechanism of cell penetration by fungal hyphae is still in doubt. The fungal hyphae of the blue-stain fungal complex appeared to utilize penetration pegs. Note the deformation of cell wall layers near the peg (Fig.21). Fungal hyphae also grew between the ray parenchyma cells (Figs. 23 & 24). It appeared that these hyphae pushed their way between cells via the middle lamella region. The hyphae that grew intercellularly were not restricted to the middle lamella region but passed through walls of ray parenchyma cells (Fig.23 arrow). Hyphae appeared to be of two morphological types. There was

a large, darkly pigmented type (approx. 9um dia.) and a small, hyaline type (approx. 3um dia.). It has been suggested that these small, hyaline hyphae are a young phase of the large, pigmented hyphae. Indeed upon careful observation of cultures it was seen to be so.

In the course of fungal development in rays, fungal hyphae encountered resin ducts. Fungal attacks on the thin walled epithelial cells surrounding the ducts appeared to be similar to attacks on thin walled ray parenchyma cells (Fig. 27).

Histology - Hyphal Growth in Tracheids

Hyphae appeared (in 24 slides observed) to be essentially confined to the rays (Fig.25). Penetration into the neighboring tracheids was very infrequent. Hyphal colonization of tracheids near the leading margin of the developing blue stain was rare. Counts of tracheids occupied by fungal hyphae showed that anywhere from 5-18% of the tracheids at the blue stain front were actually occupied by hyphae (Table I). The small number of tracheids penetrated cannot account for the complete disruption of the transpiration stream at the hyphal front. Sapwood normally has a small percentage of embolized tracheids with minimal reduction of conductive capacity (Kramer and Kozlowski 1979). Hyphal proliferation within tracheids in blue-stained sapwood

TABLE I. Tracheids occupied by fungal hyphae in blue-stained sapwood of lodgepole pines attacked by mountain pine beetles

Sampling	Tree	% Tracheids occupied	Standard deviation
FBB-32(B)	1	18.5	±4.4
(9/16/80)	1	15.5	±5.1
FBB-32(B)	1	5.3	±1.3
(9/26/80)	2	9.8	±2.3
	2	9.3	±1.6

however, appeared progressively more extensive the further away from the leading edge of the developing blue-stain. Fungal hyphae entered tracheids adjoining rays via pinoid pits only (Figs. 25& 28). The hyphae must pass (mechanical or enzymatic digestion) through the cellulose fibrils of the primary cell wall of the pinoid pit. As mentioned above, this event was rare, much searching was required to find even one such passage for micrography.

The hyphae, after entering the tracheids grew in a longitudinal fashion through the tracheid (Fig. 29). Hyphal branching to occurred infrequently in occupied tracheids. An occasional branch found its way to a bordered pit and passed through (Figs. 27, 30, 31).

The hyphal tip does not appear to differentiate between thin or thick areas of the bordered pit membrane and appears to pass through via the torus (Fig.31). The margin of the aperture through which the hyphae passed appeared almost peeled back. More penetrations of bordered pits were observed in late stage infections than early.

Eight to ten weeks after beetle attack, the sapwood became completely stained by blue-stain fungi. This indicated nearly complete colonization of the ray parenchyma by fungal hyphae and total disruption of the transpiration stream in the host tree by fungal attack. On very rare occasions , host trees did not develop blue-stain in the season of beetle attack. Two trees never developed stain; while in three other trees, stain developed only slowly. This may have been an indication of inoculum size, the rate of development of the fungi in host tissues, or the relative health or resistance of the host trees.

One must speculate that as winter approaches, fungal growth probably slows (also substrate may become a limiting factor) and as cold temperatures set in growth probably ceased altogether. It is not known in what form blue-stain fungi overwinters, but they do survive and resume growth the following spring.

By June of the following year, fungal growth and succession had gone so far that many colonized tissues had been completely destroyed or rendered non-functional (Figs. 32,34,36,38). Rays were packed with hyphae. Ray parenchyma cells had collapsed and were no longer recognizable as such (Figs. 33,34,38). Ray tracheids however, remained intact and comparatively uncolonized. This may reflect their greater resistance to fungal penetration, because they possess thick lignified cell walls. Resin ducts were extensively colonized, not only by fungal hyphae but also by yeast-like (or microconidia-like) bodies (Figs.32 & 36). Any resin that may have been present, apparently did not adversely affect growth and development of microbes. Epithelial cells lost their integrity and surrounding tissues became resin soaked.

Tracheids, though only lightly colonized by fungi in the early phase of the disease cycle, became heavily colonized in the later phase of the disease cycle (Figs. 35 & 37). Hyphae occasionally (5 slides observed) became tightly packed within the tracheids, filling the entire lumen of the cells (Fig. 37).

Water Stress

During the summer of 1979 a series of experiments was begun to determine onset of stress symptoms in beetle attacked trees. Using pre-dawn water potential studies, several trees were sampled over a period of two months (Fig.39). Over the course of the season water potential of the control trees declined gradually. This reflected gradual drying of the soil and the tree's increasing difficulty in water uptake. At no point did water potential of these trees reach the critical level (-16 to -18 bars) where stomatal closure usually occurs in this species. The water potential of beetle attacked trees however changed significantly. Initially, the trees showed no sign of stress induced by beetle attack or stain development in the sapwood; and water potential of these trees paralleled the water potential of the unattacked control trees. In the fifth week one of the attacked trees had begun to show decline of water potential. By the eighth week all trees were exhibiting severe water stress (approx. -26 bars). Subsequent tissue sampling showed that all trees developing water stress symptoms possessed stained sapwood. This lended credence to the concept that blue stained wood does not conduct water to the crown. In June, 8 months later, these same trees were checked by pressure bomb and showed water potentials of

between -30 and -40 bars (one tree = -40 bars). The trees appeared to have initiated spring bud break, the terminal buds appeared to have expanded somewhat, but the bud expansion soon ceased with subsequent fading of needles.

During the summer of 1981, another series of pressure bomb studies was initiated. These studies were conducted mid-day to demonstrate daytime transpirational stress. Three trees with pitch tubes were selected. There was some variation in the timing of the onset of water sterss shown by these trees (Fig.40). Water potentials were quite variable from tree to tree as each tree responded differently to beetle attack and fungal colonization. After seven weeks one tree showed a high level of water stress. Another tree showed water stress after twelve weeks. All blue-stained trees showed stress by the twelfth week.

Tissue samples were collected and percent water content determined to show a correlation between development of blue-stain in sapwood, drying of sapwood, and onset of water stress of foliage of beetle attacked trees (Fig. 41, 42, 43). Tissue samples were collected from around the circumference of the tree trunk at breast height. In late August, approximately 2 weeks after beetle attack, during early stages of the disease cycle, the sapwood was unstained and quite moist. As blue-stain developed inward, those regions developing stain showed rapid drying. Moisture content declined to 30-40% as more and more sapwood became stained, and increasing percentage of conductive area inactivated, foliar water stress increased. Finally by the first week of October, the entire sapwood was stained, dry, and foliar water stress was quite high (Fig.41).

There was a great deal of variation between trees. Moisture content appeared to vary unaccountably between the various increments taken from around the tree trunk. Also, width of sapwood seemed variable between trees and within each tree. One tree possessed sapwood 10 cm thick while a nearby tree had only 6 cm of sapwood. There were always differences in moisture content between stained and unstained sapwood.

However, in at least one sampling (Fig. 42 - 7 Oct.), moisture content of sapwood was low without the appearance of blue-stain. In spite of the variations during the weeks following beetle attack, by 11 November all trees showed complete sapwood stain and foliar water stress.

Water content of another tree (D), showed delay of about 6 weeks before development of any stain or moisture change in the sapwood. Width of sapwood and moisture content varied from the various samplings. In the third tree studied (tree E), Fig.43, a delay in development of blue-stain in sapwood was also seen. Development of stain was not uniform, nor was water content of sapwood cores taken from locations around the tree trunk. The lower moisture value, 23 Sept., was probably due to an error in sampling or moisture determination.

The control tree (Fig.44), sampled toward the end of the season, showed no water stress and no evidence of sapwood tissue drying.

To test the role of beetle/larval girdling on tree water stress six trees were girdled. Bark was removed, either in a narrow strip (2-4cm) or a broad strip (15-20cm). Over a period of time the trees were checked for water stress using a pressure bomb. Two years later the trees had not developed the same severe stress symptoms as seen in bee-

tle attacked/blue-stained trees. Three years later they were still alive, but showing needle yellowing and other signs of decline.

Fig. 1. Overview of Logan Canyon study site.

Fig. 2. Accessibility to study site by motor vehichle was a necessary consideration. Several pieces of equipment were quite bulky.

Fig. 3. Tissue collections were initially processed at the study site and transported back to the laboratory for further processing.

Fig. 4. Many tissue collections were made using hammer and chisel. Samples for vascular cambium periodicity studies were collected in this way.

Fig. 5. Several trees were pheromone baited each year to attract beetles to trees selected for study.

Fig. 6. Water potential studies were made using a portable pressure bomb unit. Terminal branches were cut with a razor blade from branches that originated from the main trunk about 6 meters above ground level.

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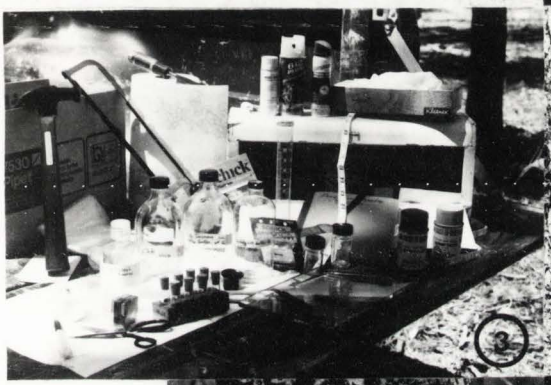

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Fig. 7. Section of bark removed from beetle attacked tree. Vertical egg gallery (about 3 mm wide) with numerous larval galleries extending away laterally. All galleries are filled with frass (feces). Fungal hyphae readily colonize this material.

Fig. 8. Increment borer with core removed to show sapwood stain (very light in this photograph). Stain intensifies with exposure to air. Stain develops along radius of sapwood until entire cylinder of sapwood is stained. At this stage the transpiration stream has been completely disrupted.

Fig. 9. Stand of apparently healthy Pinus contorta.

Fig. 10. Early June: trees attacked the previous year show signs of needle decline.

Fig. 11. July: chlorophyll in needles has been completely destroyed. All parts of the tree are dead.


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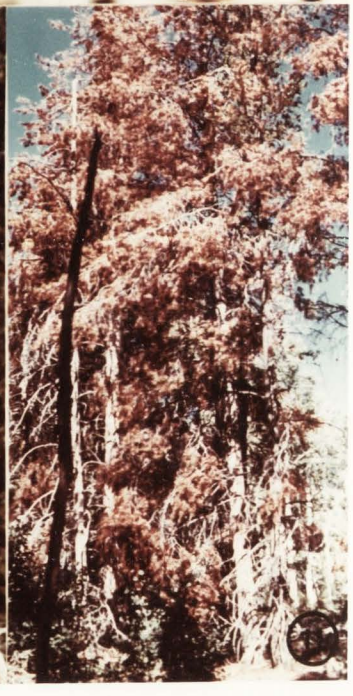
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Fig. 12. Transverse section of P. contorta showing inner bark, vascular cambium (VC) and young sapwood. Axial resin ducts (RD) of the late wood have differentiated. SC=sieve cell. Sample taken 21 Aug., 1978. X750.

Fig. 13. Transverse section of P. contorta showing inner bark. Horizontal resin duct visible in upper third of figure. Epithelial cells (indicated by e-arrows) rich in cytoplasmic content show schizogenous formation of duct at right margin of micrograph. Sample taken 2 Sept., 1978. X400.

Fig. 14. Transverse section of P. contorta inner bark showing functional phloem (FP) as a zone of radially arranged cells. Non-functional sieve cells become deformed, but are retained. Note phloem ray (arrows). Sample taken 15 Sept., 1978. X120.

Fig. 15. Transverse section of P. contorta inner bark. Resin cyst (RC) presents hazard to mining adult beetles and larvae. Resin cysts are always connected to horizontal resin canals. Resin contained therein is always under positive pressure. Sample taken 29 Sept., 1978. X330.

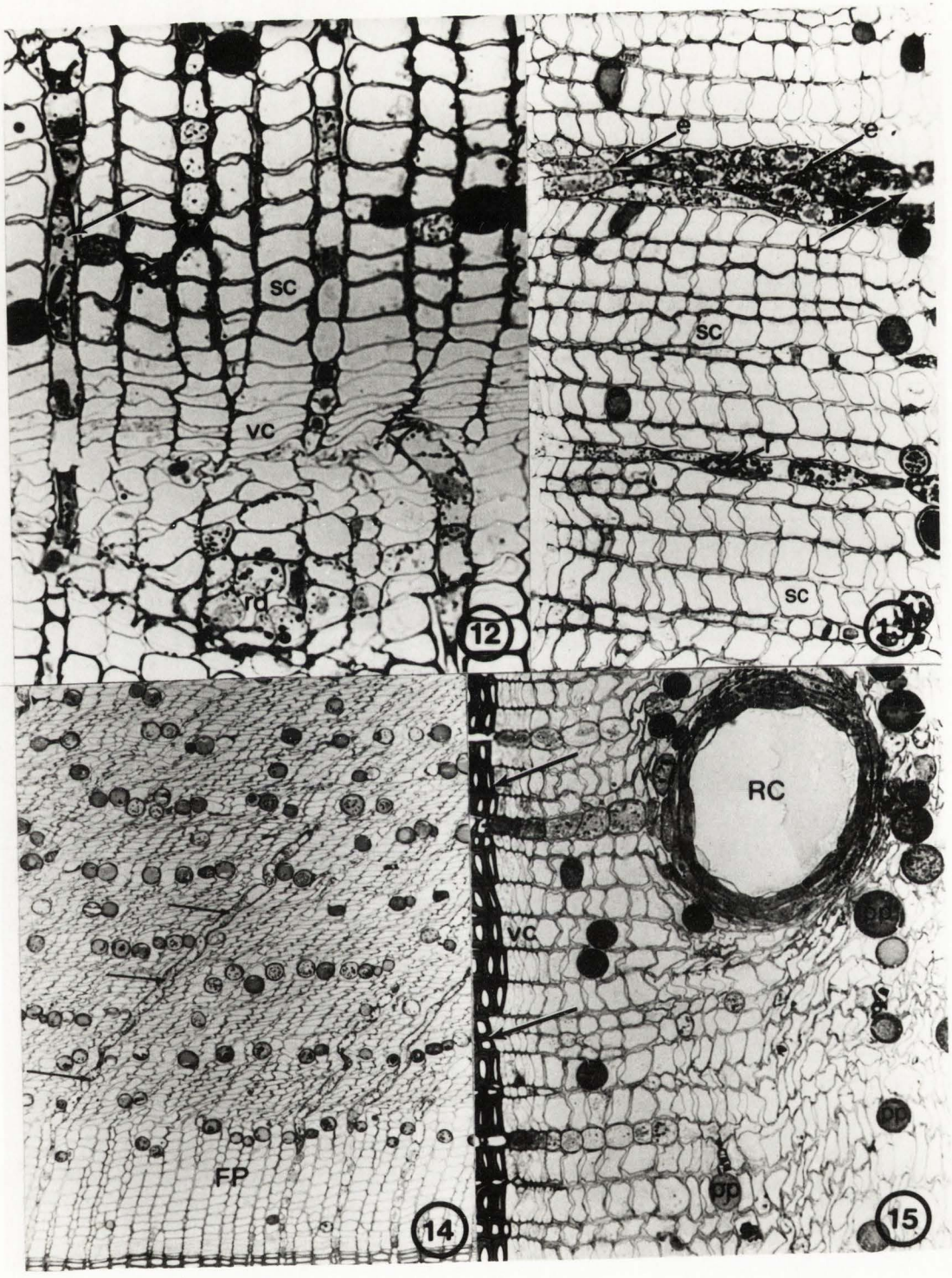
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Fig. 16. Transmission electron micrograph of transverse section of P. contorta showing phloem rays near vascular cambium. Parenchyma cells possess dense cytoplasmic content. Storage product within cells appears to be composed primarily of lipid. Arrows indicate plasmadesmata connections in primary cell wall. Sample taken 21 Aug., 1978. X11155.

Fig. 17. Transmission electron micrograph of radial section of P. contorta showing wood ray parenchyma cells. Note storage product is in the form of lipid containing plastids (asterisks). Thin primary cell walls possess numerous plasmadesmata. X9800.

Fig. 18. Light micrograph of radial section of P. contorta sapwood showing arrangement of ray parenchyma (RP) and ray tracheids (RT). Note the procumbant nature of cellular arrangement and presence of storage product in plastids scattered throughout parenchyma cells. X700.

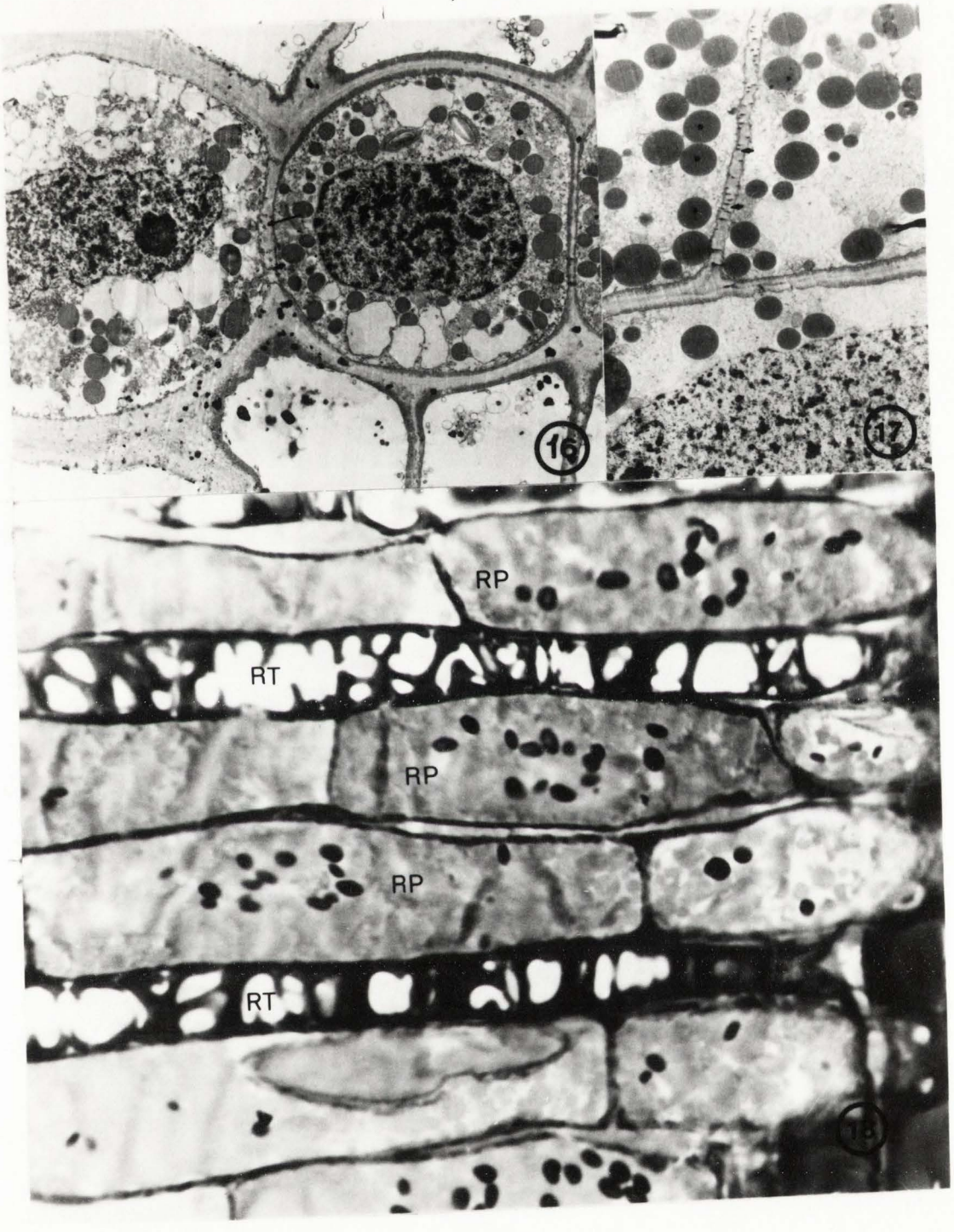
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Fig. 32. Tangential view of phloem ray possessing horizontal resin duct. Epithelial cells of duct have been destroyed by fungi. Sample taken 29 Sept., 1978. X800.

Fig. 33. Tangential view of sapwood showing extent of hyphal development (arrows) through living parenchyma of rays. Ray tracheids (RT) are intact and show considerably less colonization by fungi. Sample taken 8 July, 1979, the year following beetle attack. X330.

Fig. 34. Light micrograph of radial section of xylem ray showing extensive colonization by fungal hyphae (FH) in region of former ray parenchyma cells. Sample taken 8 July, 1979. X1000.

Fig. 35. Light micrograph of transverse section of sapwood showing extensive colonization of tracheids and rays late in the disease cycle. Note passage of fungal hyphae through bordered pit. Sample taken 8 July, 1979. X800.


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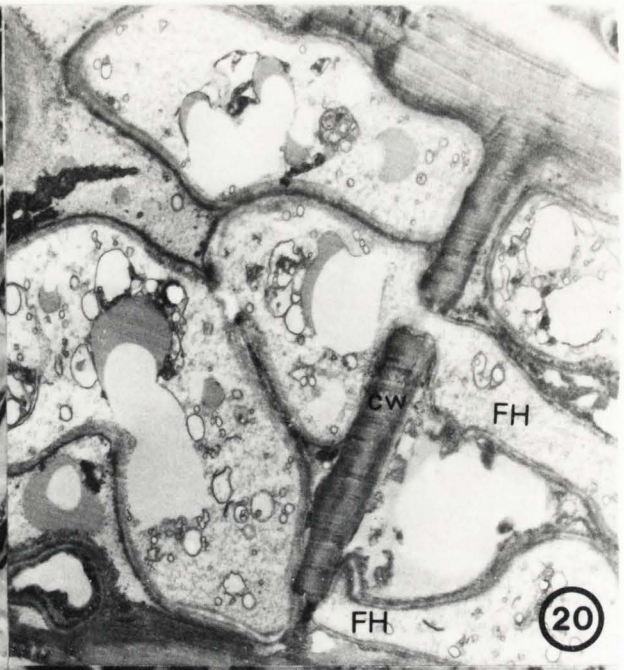
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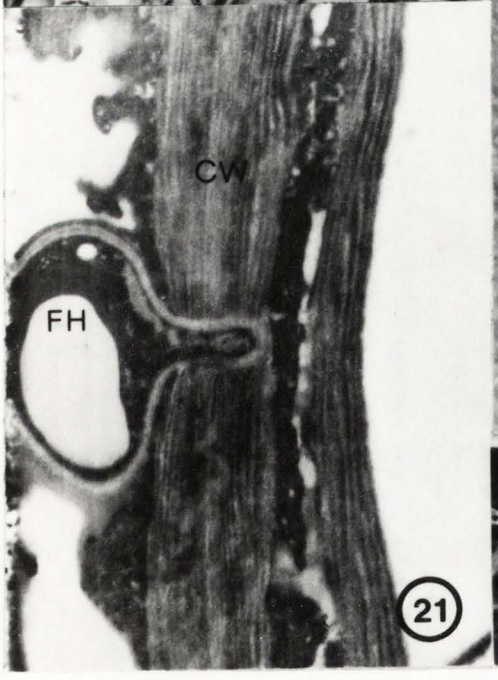
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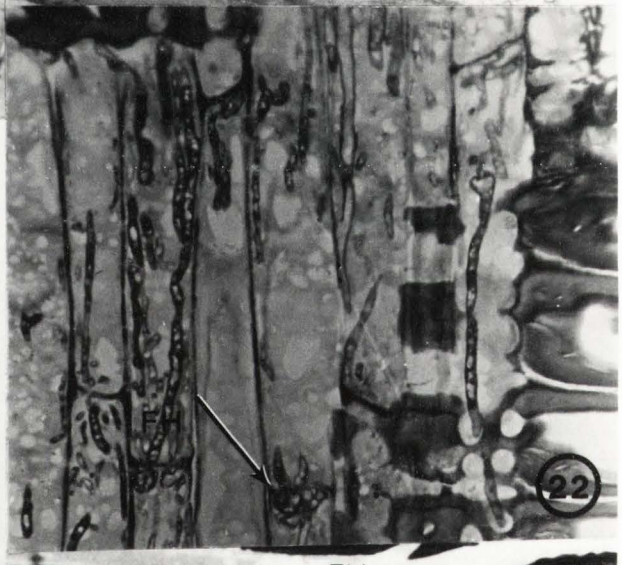
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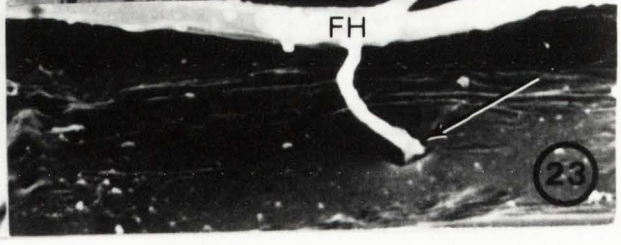
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Fig. 28. Scanning electron micrograph showing fungal hyphae emerging from pinoid half bordered pit into lumen of axial tracheid. In early phases of infection this is a rare event. X5520.

Fig. 29. Scanning electron micrograph showing longitudinal growth of fungal hyphae in lumen of axial tracheid. At top of micrograph branch penetrates bordered pit aperture. X1000.

Fig. 30. Scanning electron micrograph showing detail of fungal hypha branch penetrating bordered pit of tracheid. X5175.

Fig. 31. Scanning electron micrograph of fungal hypha penetrating torus of a aspirated bordered pit membrane in an axial tracheid. Note the peeled back nature of the torus where the hypha pushed its way through. X7400.

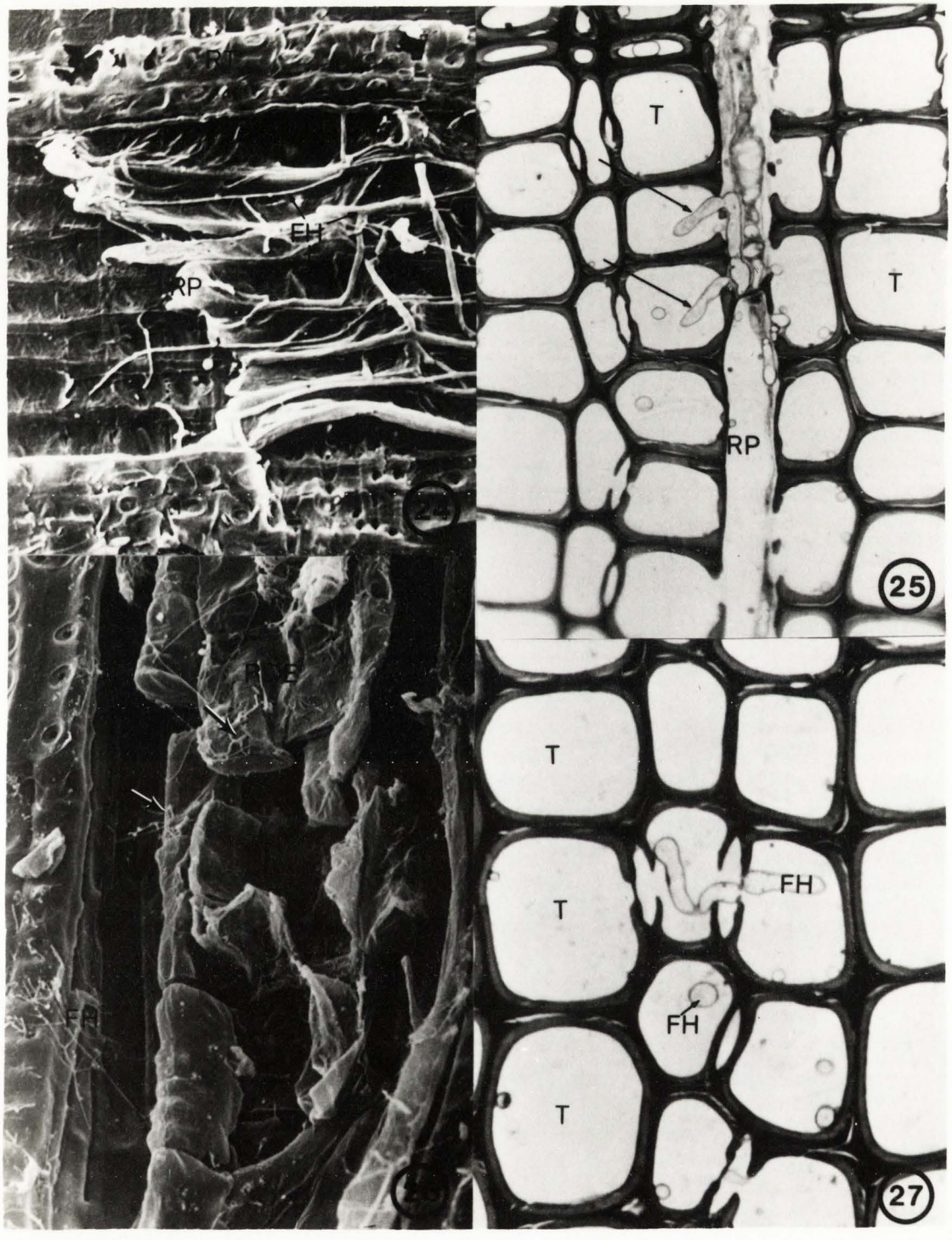
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Fig. 24. Scanning electron micrograph of hyphal (FH) proliferation in the middle lamella region of the rays. Note intact, though slightly collapsed ray parenchyma cells. Sample collected Sept., 1980, near inner margin of developing blue stain. X8000.

Fig. 25. Light micrograph of transverse section of blue-stained sapwood. Arrows indicate penetration of axial tracheids by fungal hyphae via pinoid half bordered pits. Sample taken in Sept., 1980 behind the margin of developing blue-stain. X1000.

Fig. 26. Scanning electron micrograph of radial section of blue-stained sapwood. Arrows indicate fungal hyphae growing on the surface of vertical resin duct epithelial cells. Sample taken behind the margin of developing blue-stain. X700.

Fig. 27. Light micrograph of transverse section of blue-stained sapwood. Fungal hyphae pass from tracheid to tracheid via bordered pit pairs. Sample was taken near the inner margin of blue-stain, relatively few hyphae occupy tracheids. X1500.


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Fig. 19. Light micrograph of radial section of infected P. contorta sapwood showing proliferation of fungal hyphae within ray parenchyma cells. Note clumping of hyphae at tangential walls (arrows). In lower right hand region of micrograph observe hyphae penetrating cell walls. Sample collected Sept., 1980. X2250.

Fig. 20. Transmission electron micrograph of fungal hyphae passing through cell wall of ray parenchyma cell. Note restriction of hyphal cell diameter at passage point. Note simple pit with plasmadesmata at upper margin of micrograph. Sample taken Sept., 1980. X3800.

Fig. 21. Transmission electron micrograph of fungal penetration peg, pushing through cell wall of ray tracheid (on left) into axial tracheid (on right). Note slight deformation of cell wall lamellations near penetration peg. X12,000.

Fig. 22. Light micrograph of radial section of infected P. contorta sapwood rays. Note hyphal cell wall passage at lower margin of micrograph. X2250.

Fig. 23. Scanning electron micrograph of hyphal penetration of radial wall of ray parenchyma cell. Note dimorphism of hyphae, large segment is probably pigmented. X1875.

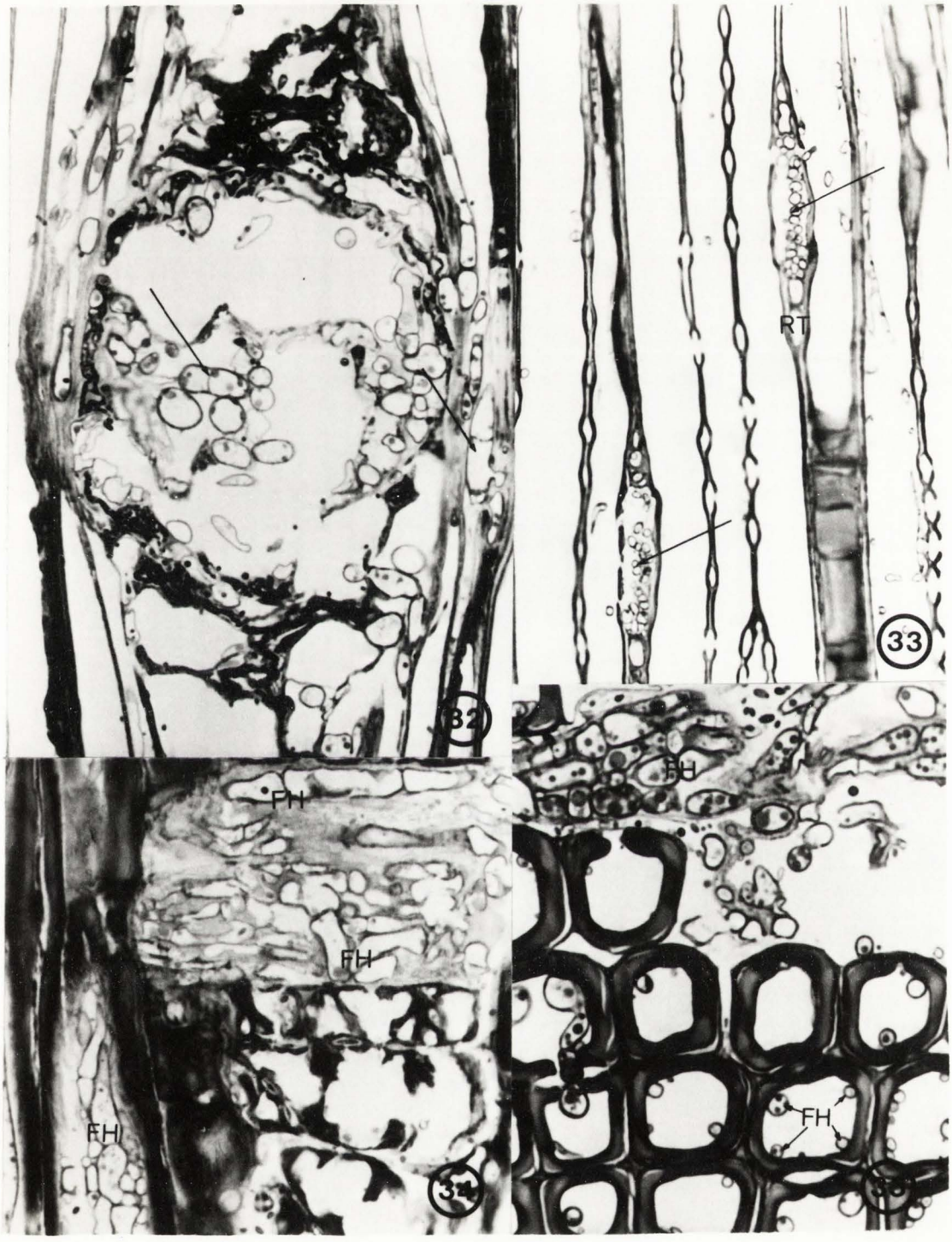
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Fig. 36. Transverse section of sapwood showing vertical resin duct. Extensive colonization of tissues and duct lumen is apparent. Resin duct epithelium has collapsed, leaving what may be axial parenchyma cells (?). Small black dots are probably yeast cells. Sample taken 8 July, 1979. X500.

Fig. 37. Radial view of xylem showing portions of two axial tracheids occluded with fungal hyphae (FH) late in the disease cycle. X750.

Fig. 38. Tangential view of xylem ray showing growth of fungal hyphae (arrows) through pinoid, half bordered pit pairs from extensively colonized ray. Note total collapse of ray parenchyma. Sample taken 8 July, 1979. X1000.


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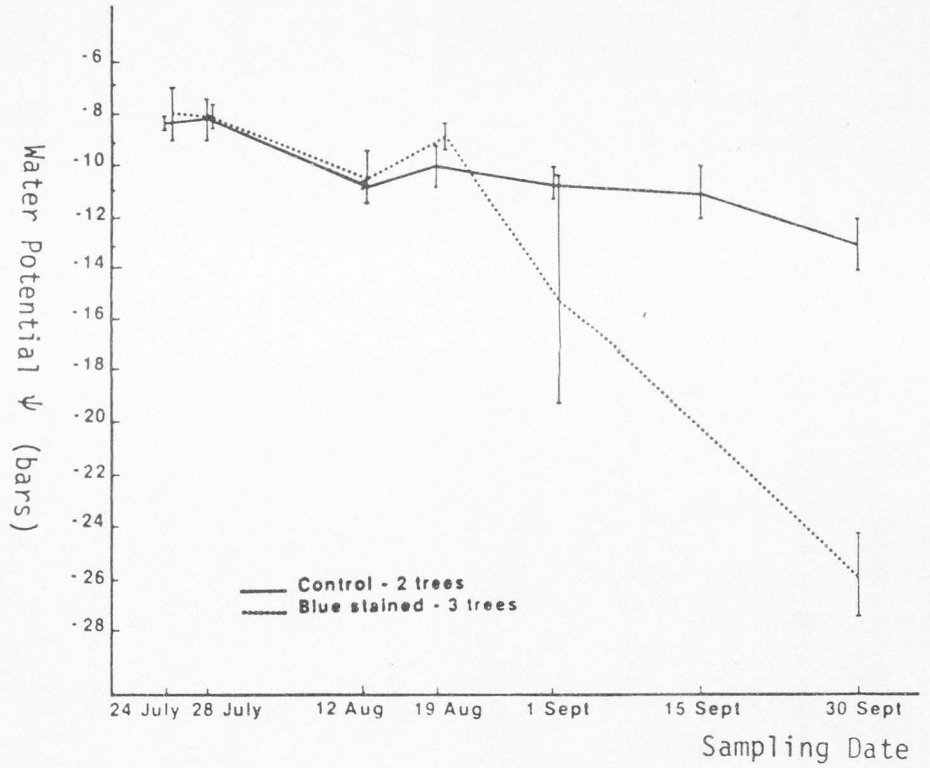

Fig. 39. Pre-Dawn water potential of lodgepole pines attacked by mountain pine beetles. Summer, 1979.

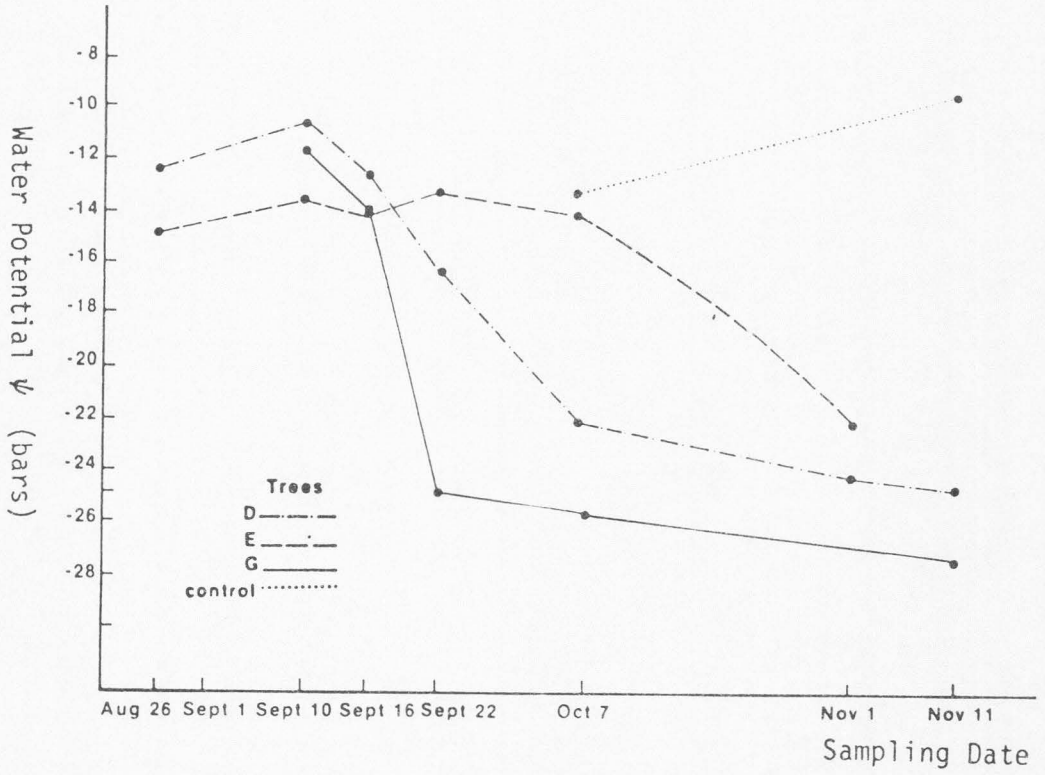

Fig. 40. Mid-Day Water potential of lodgepole pines attacked by mountain pine beetles. Summer, 1981.

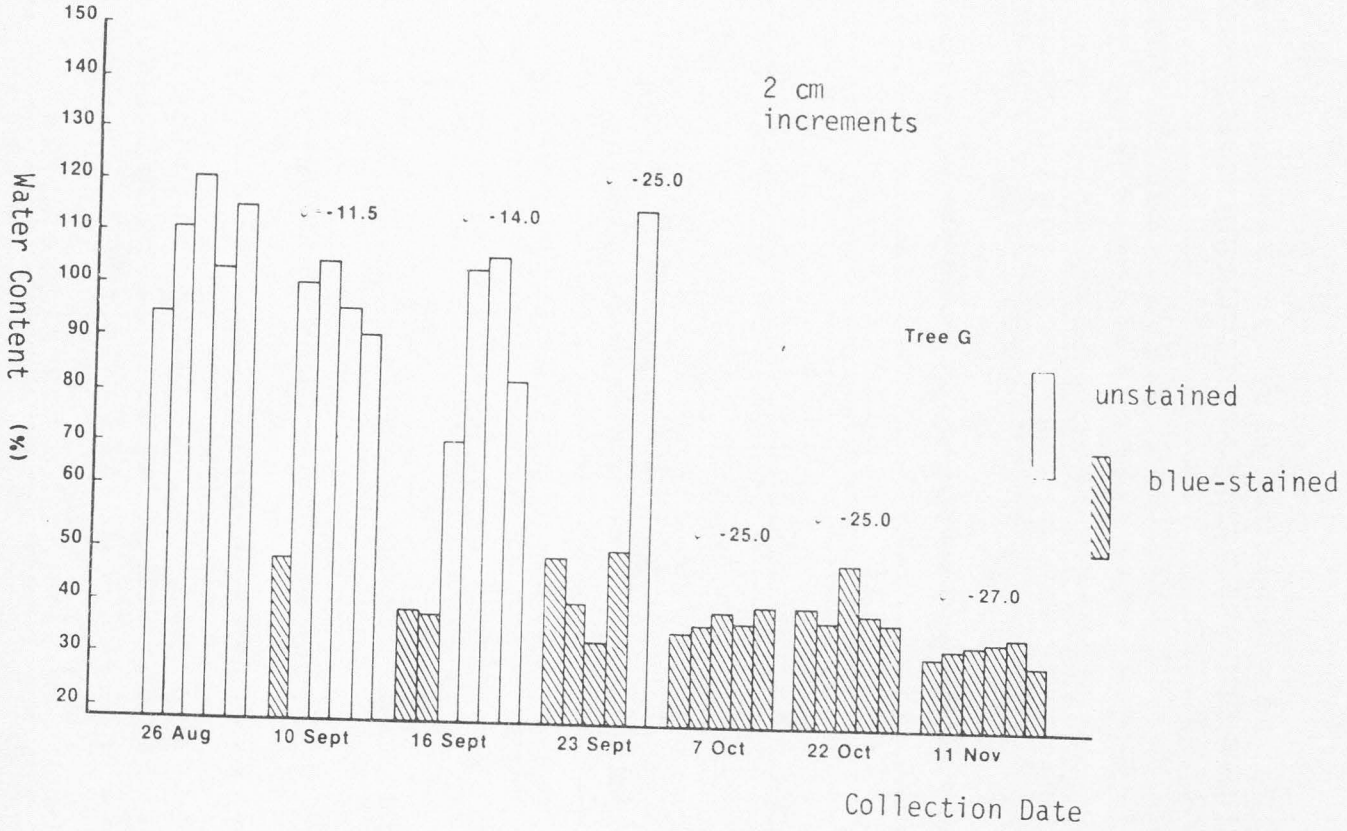

Fig. 41. Water content of sapwood of lodgepole pines attacked by mountain pine beetles. Summer, 1981. Tree G.

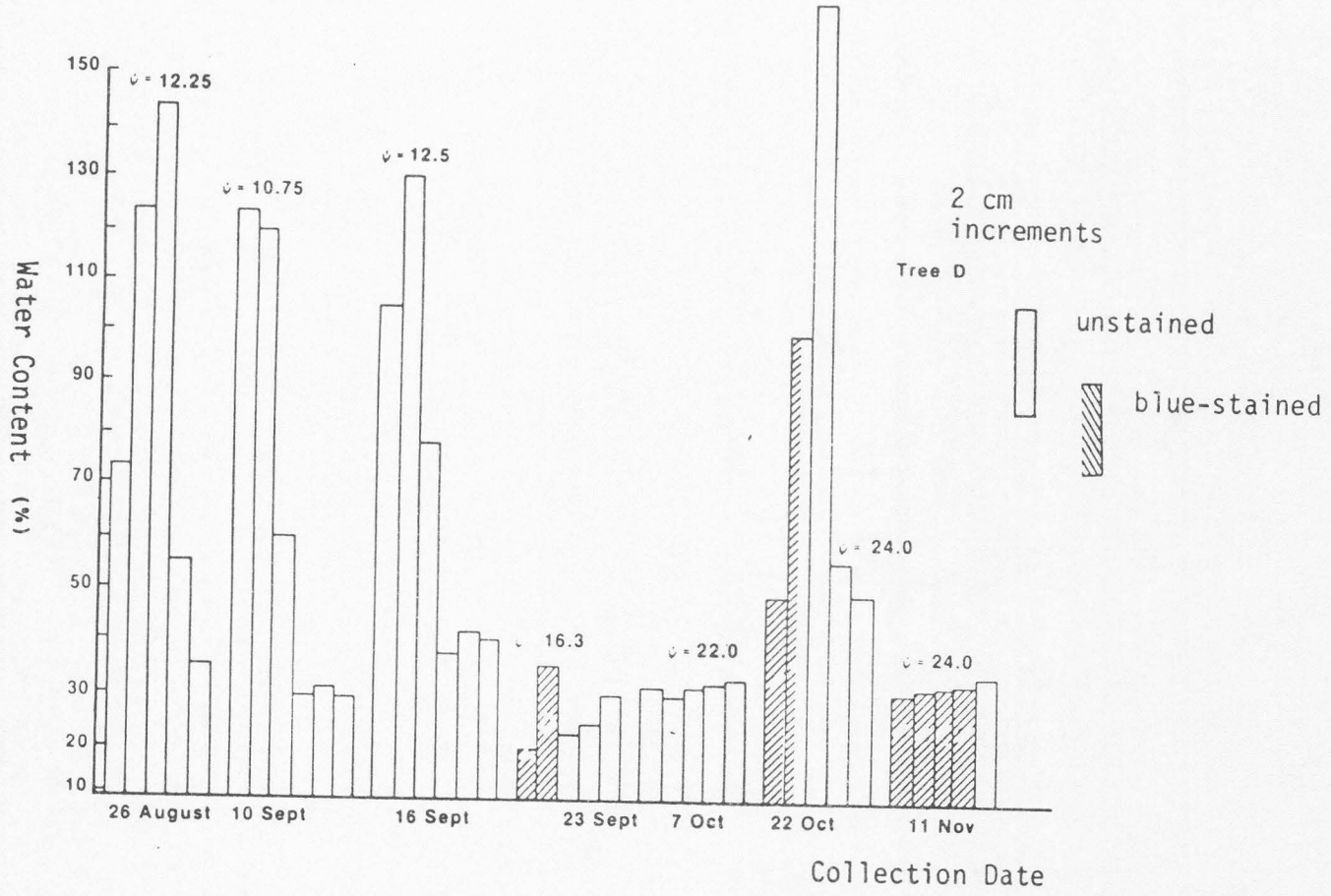

Fig. 42. Water content of sapwood of lodgepole pines attacked by mountain pine beetles. Summer, 1981. Tree D.

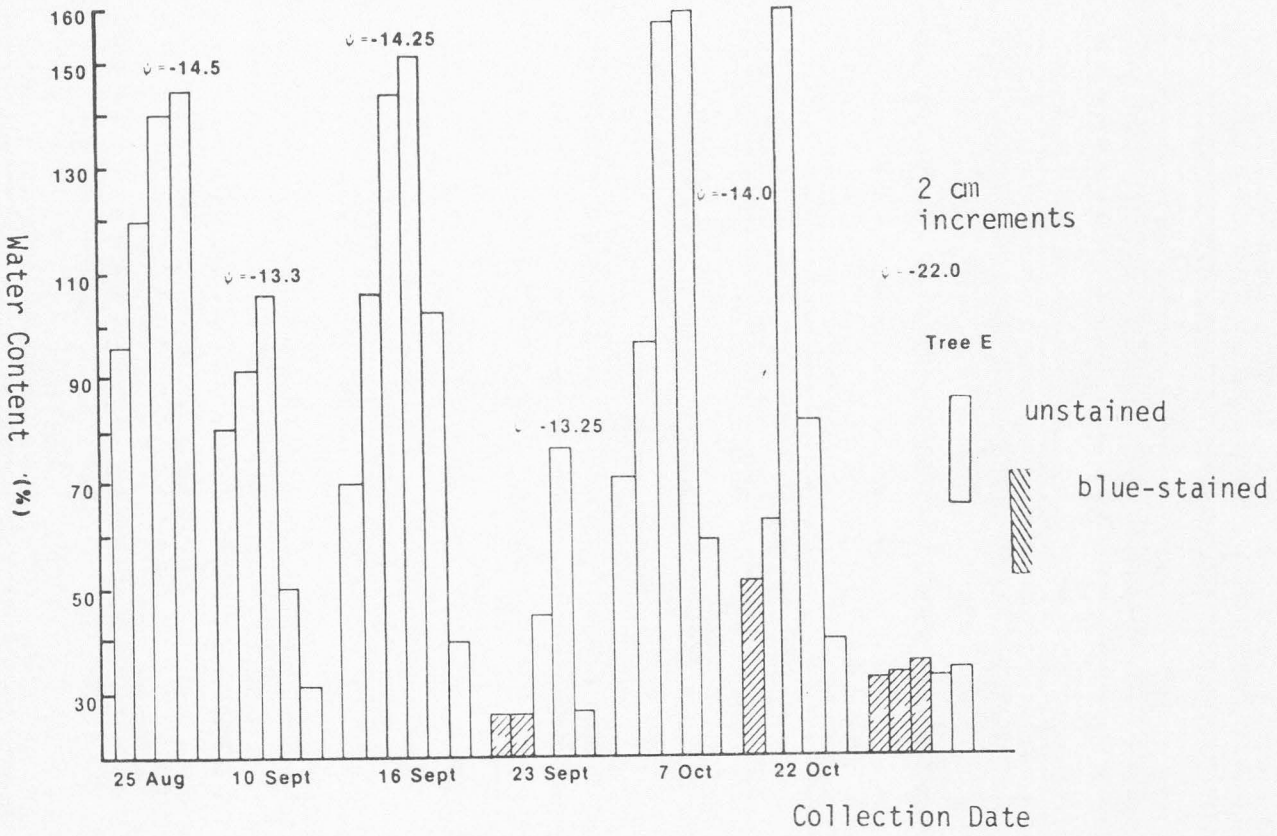

Fig. 43. Water content of sapwood of lodgepole pines attacked by mountain pine beetles. Summer, 1981. Tree E

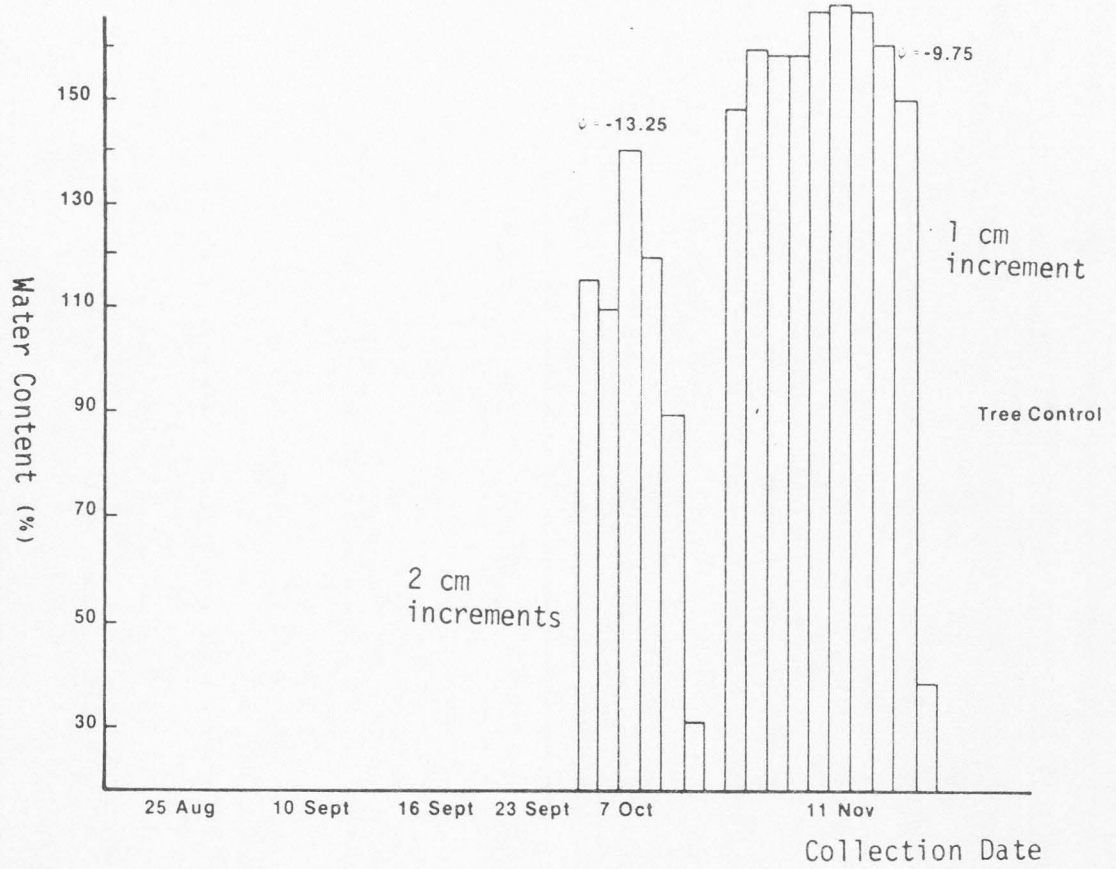

Fig. 44. Water content of sapwood of lodgepole pine control tree. Summer, 1981.

DISCUSSION

This study showed that in initial stages of blue-staining, fungal hyphae are confined to the symplastic system of the sapwood. Previous studies have shown disruption of water conduction at the leading edge of the developing blue-stain in the sapwood (Nelson 1934; Bramble and Holst 1940; Mathre 1964), a condition that should not develop because hyphae do no penetrate the tracheids. It is therefore important to put the temporal events of hyphal growth and transpiration stream disruption into perspective vis a vis one another.

From initial observations it was apparent that fungal hyphae were widespread in sapwood tracheids in late stages of the disease cycle. Also, approximately 90% of bordered pits of tracheids in blue-stained sapwood have been shown to be aspirated (Nelson 1934; Mathre 1964). Reflecting this, the hypotheses tested in this study presupposed that fungal penetration of tracheids were responsible for transpiration stream dysfunction. In this study only minimal colonization of axial tracheids at the leading edge of the inward developing blue stain was observed. It is possible therefore to rule out physical penetration of axial tracheids as a causal mechanism for introducing embolisms. Microconidia, produced by penetrating fungal hyphae, were not observed to lodge in tracheids (and thereby restrict water flow). Tyloses were not seen, though they have been reported in trees stressed in other ways (Peters 1974). Resin

soaking of sapwood, though reported by Bramble and Holst (1940), Shrimpton (1978) and hypothesized by Fares et al. (1980), was not demonstrated in this study. Fungal attack on resin duct epithelial cells was observed though no evidence of resin leakage into surrounding tissues in early disease stages was seen. Lodgepole pine, being a diploxylon pine (or hard pine), does not develop lignified secondary cell walls in ray parenchyma cells. Cell wall thickening in young ray parenchyma is a characteristic of haploxylon (or soft) pines. As a consequence, blue-stain fungi can penetrate cell walls quickly, and readily reach untapped food sources. Hyphal growth is directed along a path of least resistance in the sapwood. One result of this is that mycelia proliferate extensively within this less resistant tissue before penetration of more resistant tracheids.

Nelson (1934), Caird (1935), and Bramble and Holst (1940) all studied the effect of Ceratocystis minor on southern pine physiology while Mathre (1964) conducted similar investigations with ponderosa pine. They all found that in trees where inoculum was sufficient, water conduction up the tree was severely affected. Water was conducted around but not through stained regions of sapwood. The transpiration stream was also shown by Mathre to be disrupted a short distance in front of the advancing blue-stain. Mathre's results suggest the fungus' effect on the transpiration stream to be immediate. Histological examination of tissues immediately in front of the stain showed fungal hyphae essentially confined to rays. In this study histological

examination of tissues at the front margin of blue-stain shows ray parenchyma to be intact at this point in the infection, but cellular contents had begun to degenerate.

In this study immediate and extensive drying of stained sapwood was observed. The moisture content gradient of uninfected and blue-stained tissue was seen to be quite sharp. Nelson (1934) suggested and Caird's (1935) experiments confirmed that water was lost from sapwood via the transpirational pull by the crown of infected trees to be the major source of drying of stained sapwood.

The integrity of wood rays is destroyed by fungal attack. In histological examination of tissues it was observed that soon after infection of the parenchyma cells in the rays, the cell walls tended to pull away from adjacent tracheids. Late in the disease cycle total collapse of the parenchyma cells in the rays was observed. In late stages of the disease, fungal hyphae fill the space left by the collapse of ray parenchyma cells. In light of such evidence it is possible to view the destroyed rays as open passages to the outside of the tree. Water diffusing through cell walls of adjacent tracheids could find easy escape from the trees.

Another mechanism to explain transpiration stream flow disruption is pit membrane incrustation. Blue-staining and heartwood formation possess numerous parallel characteristics. In both, one finds death of ray parenchyma cells occurring. In both, phenolic compounds are produced and released into sur-

rounding tracheids. It has been suggested (Nelson 1934), but not proven, that gums and tannins (also released in heartwood formation) may be released in blue-stained sapwood. With destruction of the ray parenchyma cellular integrity, cytoplasmic contents of these cells would be free to diffuse into neighboring tracheids and into the sap stream (Fengel 1970). With entry into the sap stream viscosity of solution may increase, creating greater resistance to water flow. Also, such compounds may precipitate on the surface of microfibrils of pit membranes in bordered pits. This encrustation would tend to decrease the size of pit membrane perforations and thereby also restrict flow of water. Though both Mathre (1964) and Basham (1970) tried to demonstrate these plugging compounds in pines using histochemical approaches, they were unsuccessful. The question is by no means resolved.

Lodgepole pine at the time of attack by mountain pine beetles at the Logan Canyon study site were seemingly healthy, actively growing trees. These trees for the most part possessed thick phloem. Thickness of phloem has been utilized in the past as an indicator of tree vigor (Cole 1974). It is trees with thick phloem that mountain pine beetles seek out and attack. These trees are usually the largest trees in the forest in early phases of mountain pine beetle epidemics. Some controversy exists as to whether trees experiencing some undefined stress are the first to be attacked or trees entering physiological maturity (again undefined) are the primary hosts (Amman 1978). Thick

phloem indicates however that the tree is retaining more of the yearly increments of otherwise nonfunctional sieve cells noted by Cabrera (1978). This means that the tree trunk is not expanding as rapidly as in earlier years. As a consequence, physical pressure on the bark which causes crushing of older bark tissues, is not being exerted. Deformation and crushing of cells is slowed. The cork cambium, which is responsible for cutting off the crushed and deformed layers of nonfunctional phloem, appears less active in keeping the inner bark thin as is found in younger rapidly expanding trees.

The vascular cambium does not produce as many xylem cells as in earlier years of tree growth, and the cells appear to be smaller. Indeed many trees at the study site have produced very narrow growth rings for the past 15-20 years. Some rings were 1mm wide. Narrow growth rings tend to reduce conductive capacity of those rings (Bannan 1965). It is possible that as trees mature, growth or assimilate partitioning strategies within the tree change. The sampling area on the tree trunk was often somewhat removed from the photoassimilate source region, the crown. Also, proximity to the crown may have had a relationship to hormone levels in the meristematic regions of the lower trunk, thereby affecting size and numbers of differentiating tracheids and sieve cells.

A final comment needs to be made concerning the unresolved question of insect/fungus symbiosis. Without doubt, fungal activity causes the reduction of moisture content of the sapwood.

It has been demonstrated with southern pine beetles that excess moisture beneath the bark of beetle attacked trees is detrimental to brood survival (Barras 1973; Webb and Franklin 1978). It is also known that excessive drying of attacked trees is detrimental. Moisture content of the bark has no relation to its nutritional value to the larvae (W. E. Cole, personal communication). Moisture content of the bark in larval diet could conceivably have an effect on certain physiological processes of the larvae, as shown with southern pines (Barras and Perry 1972; Webb and Franklin 1978). It would be interesting, informative, and potentially valuable to see how well mountain pine beetle larvae survive in trees not developing blue-stain or water stress. Does blue-staining and subsequent moisture content reduction play a role in brood survival? Can larvae survive in trees not experiencing sapwood drying? A consideration would be that mountain pine beetles overwinter in the larval stage, normally the third instar. At some point during the fall of the year the larvae respond to environmental cues and begin to undergo as yet undefined physiological changes to prepare for winter. Part of this process probably involves elimination of water from the gut and hemocoel (Duman 1980) and part involves producing antifreeze compounds (Somme 1964). It seems reasonable and testable that the microenvironment in which larvae live mediates the physiological processes involved in cold tolerance. If the inner bark within which they live is very dry or very moist at the time the larvae are going through these processes,

then increased mortality may arise later on in the winter months. Host trees that do not develop blue-stain remain moist. Should the larvae not eliminate enough water from hemocoel or gut, then damaging ice crystals could form within their bodies (this should be tested).

It would also be interesting and informative to compare blue-stain fungal development in soft pines. In haploxylon pines, ray parenchyma develops secondary cell walls that could conceivably resist blue-stain fungus hyphal penetration. Further, a comparison study of the physiological processes of susceptible vs. nonsusceptible trees would help determine whether the trees are indeed stressed or overmature. Further, stress physiology studies would show how lodgepole pines can postpone needle death for up to 10 months after beetle attack while needles of southern pines often succumb within weeks after attack. More effort should be exerted to understand the host and fungal symbionts in the search for potential approaches to understanding and preventing mountain pine beetle epidemics in the West .

CONCLUSIONS

1. Fungal hyphae are initially confined to the rays of the sapwood where they proliferate greatly. Eventually the ray parenchyma collapses. Late in the disease cycle fungal hyphae completely occupy what was formerly the ray parenchyma and penetrate many axial tracheids.

2. Development of water stress is closely correlated with development of blue-stain and tissue drying in sapwood. These trees develop water stress 8-10 weeks after beetle attack. Tissue drying also closely accompanies blue-stain development.

3. Water conduction has been shown to be disrupted at the inner margin of the developing blue-stain. Microscopic observation of tissues in the vicinity of the blue-stain margin revealed no tylosis formation, no physical plugging by either fungal hyphae, microconidia, or resin. Presence of encrusting compounds on pit membranes was not seen, though microchemical tests were not conducted, so results here were inconclusive. Physical penetration of tracheids by fungal hyphae can account for only a small amount of tracheid dysfuncion, because only 10% of adjacent tracheids were actually occupied. None of the hypotheses can account for transpiration stream disruption.

4. Girdling of trees effectively cuts off flow of sugars and amino acids to the roots (phloem translocation is disrupted). Trees girdled (and not developing blue-stain) in the study rema-

ined alive for three years, foliage was not as healthy looking as control trees (possibly due to nutritional stress). Severe water stress did not occur within two years after girdling. Mining activity of beetles and larvae cannot account for early tree water stress.

LITERATURE CITED

Alfieri, F. J. and R. F. Evert. 1968. Seasonal development of the secondary phloem in Pinus. Amer. J. Bot. 55:518-528.

Amman, G. D. 1972. Mountain pine beetle brood production in relation to thickness of lodgepole pine phloem. J. Econ. Entomol. 65:138-140.

-----. 1978. Biology, ecology, and causes of outbreaks of mountain pine beetle in lodgepole pine forests. In Theory and practice of mountain pine beetle management in lodgepole pine forests. Edited byA. A. Berryman, G. D. Amman, R. W. Stark. Papers presented at a symposium held at Washington State University, Pullman, April 25-27, 1978. pp. 39-53.

Balatinecz, J. J. and R. V. Kennedy. 1967. Ray parenchyma cells in Pine. For. Prod. Jour. 17:57-64.

Bannan, M. W. 1964. Tracheid size and anticlinal divisions in the cambium of lodgepole pine. Can. J. Bot. 42:1105-1118.

-----. 1965. The length, tangential diameter, and length/width ratio of conifer tracheids. Can. J. Bot. 43:967-984.

Barras, S. J. 1970. Antagonism between Dendroctonus frontalis and the fungus Ceratocystis minor. Ann. Entomol. Soc. Am. 63:1187-1189.

-----. 1973. Reduction of progeny and development in the southern pine beetle following removal of symbiotic fungi. Can. Entomol. 105:1295-1299.

----- and J. D. Hodges. 1974. Weight, moisture and lipid changes during the life cycle of the southern pine beetle. U. S. For. Serv. Res. Note 20-178.

----- and T. Perry. 1972. Fungal symbiont in the prothoracic mycangium of Dendroctonus frontalis (Coleoptera Scolytidae). Z. Angew. Entomol. 71:95-104.

Basham, H. G. 1970. Wilt of lobolly pine inoculated with blue-stain fungi of the genus Ceratocystis. Phytopath. 60:750-754.

Bauch, J., W. Schweers, and H. Berndt. 1974. Lignification during heart wood formation: Comparative study of rays and bordered pit membranes in coniferous woods. Holzforschung. 28:86-91.

Berryman, A. A. 1972. Resistance of conifers to invasion by bark beetle-fungus associations. Bioscience 22:598-602.

Bega, R. V. 1978. Diseases of Pacific Coast conifers. U. S. Dep. Agric., Agric. Handb. 521.

Birch, M. C. 1978. Chemical communication in pine bark beetles. Amer. Sci. 66:409-419.

Boyce, J. S. 1938. Forest pathology. McGraw-Hill, New York.

Bramble, W. C. and E. C. Holst. 1940. Fungi associated with Dendroctonus frontalis in killing shortleaf pines and their effect on conduction. Phytopath. 30:881-899.

Braun, H. J. 1970. Funktionelle Histologie der sekundaren Sprossachse. 1. Das Holz. Handbuch der Pflanzenanatomie. Gebruder Borntraeger, Berlin. 190 pages.

Cabrera, H. 1978. Phloem structure and development in lodgepole pine. In Theory and practice of mountain pine beetle management in lodgepole pine forests. Edited by A. A. Berryman, G. D. Amman, R. W. Stark. Symposium held at Washington State University, Pullman, April 25-27, 1978. pp. 54-63.s

Caird, R. W. 1935. Physiology of pines infested with bark beetles. Bot. Gaz. 96:709-733.

Carlquist, S. 1975. Ecological strategies of xylem evolution. Univ. of California Press, Berkeley.

Cole, D. M. 1974. Estimation of phloem thickness in lodgepole pine Pinus contorta, Dendroctonus ponderosae vulnerability tree growth vigor basal area increment equations. U. S. For. Serv. Res. Pap. Int. 148:1-10.

Cole, W. E., G. D. Amman, C. E. Jensen. 1976. Mathematical models for the mountain pine beetle-lodgepole pine interaction. Environ. Entomol. 5:11-19.

Coulson, R. N. 1979. Population dynamics of bark beetles. Annu. Rev. Entomol. 24:417-447.

Craighead, F. C. 1928. Interrelation of the tree-killing bark beetles and blue-stain. J. Forestry. 26:886-887.

Duman, J. G. 1980. Factors involved in overwintering survival of the freeze tolerant beetle, Dendroides canadensis. J. Comp. Physiol. 136: 53-59.

Esau, K. 1965. Plant anatomy. Wiley, New York.

Fares, Y., J. D. Goeschl and P. J. H. Sharpe. 1980. Dynamics of bark beetle-fungus symbiosis. I. Pine tree anatomy and fungus growth pattern. In Proc. on modeling southern pine beetle populations. Edited by F. M. Stephen, J. L. Searcy and G. D. Hertel. USDA Tech. Bull. #1630.

Fengel, D. 1970. Ultrastructural changes during aging of wood cells. Wood Sci. Technol. 4:176-188.

Franke-Grossmann, H. 1967. Ectosymbiosis in wood inhabiting insects. pp. 141-205. In Symbiosis, Vol. II. Edited by S. M. Henry. Academic Press, New York.

Franklin, R. 1970. Observations on the blue stain-southern pine beetle relationship. J. Georgia Entomol. Soc. 5:53-57.

Frey-Wyssling, A. and H. H. Bosshard. 1959. Cytology of the ray cells in sapwood and heartwood. Holzforschung 13:129-137.

Graham, K. 1967. Fungal-insect mutualism in trees and timber. Annu. Rev. of Entomol. 12:105-126.

Graham, S. A. and F. B. Knight. 1965. Principles of forest entomology. McGraw-Hill, New York.

Hetrick, L. A. 1949. Some overlooked relationships of southern pine beetle. J. Econ. Entomol. 42:466-469.

Hillerdal-Hagstromer, K., E. Mattson-Djos and J. Hellkvist. 1982. Field stuies of water relations and photosynthesis in Scots Pine. II. Influence of irrigation and fertilization on needle water potential of young pine trees. Physiol. Plant. 54:295-301.

Hubert, E. E. 1931. An outline of forest pathology. John Wiley and Sons, New York.

Imamura, Y. and H. Harada. 1973. Electron microscopic study on the development of bordered pit in coniferous tracheids. Wood Sci. & Technol. 7:189-199.

Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmollity for use in electron microscopy. J. Cell Biol. 27:137A-138A.

Kozlowski, T. T. and C. H. Winget. 1963. Patterns of water movement in forest trees. Bot. Gaz. 124:301-311.

Kramer, P. J. and T. T. Kozlowski. 1979. Physiology of woody plants. Academic Press, New York.

Liese, W. and J. Bauch. 1967. On the closure of bordered pits in conifers. Wood Sci. Technol. 1:1-13.

----- and R. Schmid. 1961. Licht= und Elektronenmikroskopische Untersuchungen uber das Wachstum von Blauenpilzen in Kiefern= und Fichtenholz. Holz Roh Werkst. 19:329-337.

Lopushinsky, W. 1969. Stomatal closure in conifer seedlings in response to leaf moisture stress. Bot. Gaz. 130:258-263.

Mark, R. W. and D. L. Crews. 1973. Heat-pulse velocity and bordered pit conition in living Engelmann spruce and lodgepole pine tress. Forest Sci. 19:291-296.

Mathre, D. E. 1964. Pathogenecity of Ceratocystis ips and Ceratocystis minor to Pinus ponderosae. Cont. Boyce Thomp. Instit. 22:363-388.

Milburn, J. A. 1979. Water flow in plants. Longman, New York.

Mirov, N. T. 1967. The genus Pinus. Ronald, New York.

Nelson, R. M. 1934. Effect of blue-stain fungi in southern pines attacked by bark beetles. Phytopath. Zeit. 7:327-353.

----- and J. A. Beal. 1929. Experiment with blue-stain fungi in southern pines. Phytopath. 19:1101-1106.

Noel, A. R. A. 1970. The girdled tree. Bot. Rev. 36:162-196.

Peters, W. J. 1974. Tylosis formation in Pinus tracheids. Bot. Gaz. 135:126-131.

Reid, R. W. 1961. Moisture changes in lodgepole pine before and after attack by the mountain pine beetle. For. Chron. 37;368-403.

-----, H. S. Whitney and J. A. Watson. 1967. Reactions of lodgepole pine to attack by Dendroctonus ponderosae Hopkins and blue-stain fungi. Can. J. Bot. 45:1115-1126.

Robinson, R. C. 1962. Blue-stain fungi in lodgepole pine (Pinus contorta Doug. var. latifolia Engelm.) infested by the mountain pine beetle (Dendroctonus monticolae Hopk.). Can. J. Bot. 40:609-614.

Robinson-Jeffrey, R. C. and R. W. Davidson. 1968. Three new Europhium species with Verticicladiella imperfect states on blue staining pine. Can. J. Bot. 46:1523-1527.

Robinson-Jeffrey, R. C. and A. H. H. Grinchenko. 1964. A new fungus in the genus Ceratocystis occurring on blue stained lodgepole pine attacked by bark beetles. Can. J. Bot. 42:527-532.

Rudinsky, J. A. 1962. Ecology of Scolytidae. Annu. Rev. Entomol. 7:327-348.

Rudinsky, J. A. and J. P. Vite. 1959. Certain ecological and phylogenetic aspects of the pattern of water conduction in conifers. Forest Sci. 5:259-266.

Rumbold, C. T. 1941. A blue-stain fungus, Ceratostomella montium n. sp. and some yeasts associated with two species of Dendroctonus. J. Agric. Research 62:589-601.

Running, S. W. 1976. Environmental control of leaf water conductance in conifers. Can. J. For. Res. 6:104-112.

-----. 1980. Environmental and physiological control of water flux through Pinus contorta. Can. J. For. Res. 10:82-91.

Safranyik, L. 1978. Effects of climate and weather on mountain pine beetle population. In Theory and practice of mountain pine beetle management in lodgepole pine forests. Edited by A. A. Berryman, G. D. Amman and R. W. Stark. Papers presented at a symposium held at Washington State University. pp. 77-86.

Shepherd, R. E. and J. A. Watson. 1959. Blue-stain fungi associated with the mountain pine beetle. Can. Dept. Agr. For. Biology Div. bimonthly Progr. Report. 15:2-3.

Shrimpton, D. M. 1973. Age- and size-related response of lodgepole pine to inoculation with Europhium clavigerum. Can. J. Bot. 51:1155-1160.

-----. 1978. Resistance in lodgepole pine to mountain pine beetle infestation. In Theory and practice of mountain pine beetle management in lodgepole pine forests. Edited by A. A. Berryman, G. D. Amman and R. W. Stark. Papers presented at a symposium held at Washington State University, Pullman. pp. 64-76..

----- and H. S. Whitney. 1968. Inhibition of growth of blue-stain fungi by wood extractives. Can. J. Bot. 46:757-761.

Somme, L. 1964. Effects of glycerol on cold hardiness in insects. Can. J. Zool. 42:87-101.

Spomer, G. G. and K. J. Stoszek. 1978. Comparisons of water potentials in bole and needle tissues of lodgepole pine. Can. J. For. Res. 8:439-441.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.

Swanson, R. H. 1967. Seasonal course of transpiration of lodgepole pine and Engelmann spruce. In Forest hydrology. Edited by W. E. Sopper and H. W. Lull. Pergammon Press, Oxford.

Thomas, R. J. 1968. The development and ultrastructure of the bordered pit membrane in the southern yellow pines. Holzforschung 22:38-44.

----- and D. D. Nicholas. 1968. The ultrastructure of the pinoid pit in southern yellow pine. Tappi 51:84-88.

Webb, J. W. and R. T. Franklin. 1978. Influence of phloem moisture on brood development of the southern pine beetle (Coleoptera: Scolytidae). Environ. Entomol. 7:405-410.

Whitney, H. S. 1971. Association of Dendroctonus ponderosae (Coleoptera: Scolytidae) with blue-stain fungi and yeasts during brood development in lodgepole pine. Can. Entomol. 103:1495-1503.

----- and S. H. Farris. 1970. Maxillary mycangium in the mountain pine beetle. Science 167:54-55.

Wong, B. L. and A. A. Berryman. 1977. Host resistance to the fir engraver beetle. 3. Lesion development and containment of infection by resistant Abies grandis inoculated with Trichosporium symbioticum. Can. J. Bot. 55:2358-236

Yearian, W. C., R. J. Gouger and R. C. Wilkinson. 1972. Effects of the blue-stain fungus, Ceratocystis ips, on development of Ips bark beetles in pine bolts. Ann. Entomol. Soc. Amer. 65:481-487.

Zimmermann, M. H. and C. L. Brown. 1977. Trees: structure and function. Springer-Verlag, New York.

RESUME

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PUBLICATIONS:

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BALLARD,R.G. and D.E. Bianchi. 1979. The Effects of Cholesterol on Sporulation of Phytophthora cinnamomi. American Society for Microbiology. 4-8 May, 1979. Los Angeles, Calif.

Ballard,R.G. and M.A. Walsh. 1981. Development of Blue-Stain Fungi in Pinus contorta var. latifolia Engelm. and Subsequent Tree Water Stress. Bot. Soc. Amer., Bloomington, Indiana, Aug. 16-21, 1981.

Ballard,R.G. and M.A. Walsh. 1981. Blue-Stain Fungi in Xylem of Lodgepole Pine - A light microscope study. Spring meeting of Utah Academy of Sciences, Arts and Letters. 10 Apr., 1981. Provo, Utah.

Ballard,R.G., M.A. Walsh, and W.E. Cole. 1982. A Light- and Electron Microscope Study of the Growth and Development of Blue-Stain Fungus in Sapwood of Lodgepole Pine. Amer. Phytopath. Soc. 8-12 Aug., 1982. Salt Lake City, Utah.