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DEATH IN THE LODGEPOLE PINE

VACATIONERS IN NATIONAL FORESTS of the western United States (Figure 1) now routinely see thousands of lodgepole pines standing as mausoleums of death (Figure 2). The beetle that is responsible for the devastation is called the mountain pine beetle (*Dendroctonus ponderosae* Hopk.) (Figure 3).

Figure 1. Tree in Cache National Forest killed because of mountain pine beetle infestation and/or blue-stain fungi infection. Beetles prefer large healthy trees with thick bark.

Figure 2. Research site at head of Logan Canyon near Swan Flat Road. Six 24 inch diameter trees in this area serve as experimental organisms in a cooperative (contractual agreement) study involving researchers at the Plant Anatomy Research Labs at Utah State University and the USDA Forest Service in Ogden, Utah.

Figure 3. Adult beetle (*Dendroctonus ponderosae* Hopk.). Average beetles measure 5 mm in length. Beetle shown was probably chewing its way out of a pupal chamber. During this process, fungal spores adhere to beetle body and mouth parts and are carried to new trees where they become inoculated.

Figure 4. Bark of previously infested lodgepole pine (*Pinus contorta*). Note holes where beetles make exit and fly to new uninfested trees.

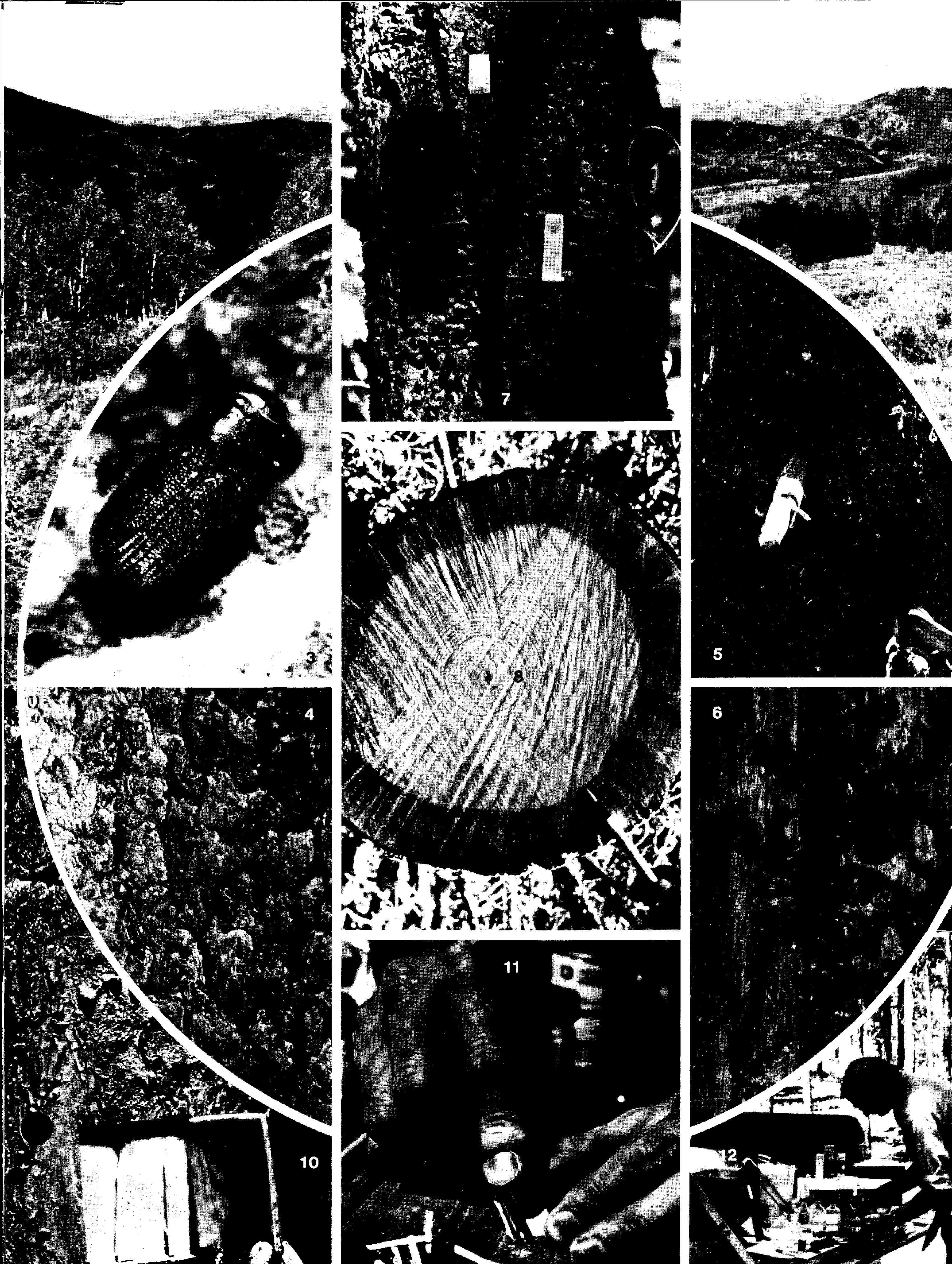
Figure 5. Trees where beetles bored into bark causing "pitch tubes." Boring action of beetle causes release of resin from canals in the bark and wood. Sometimes beetles are entrapped in resin, so that pitch tube formation possibly represents a defense mechanism.

Figure 6. Bark removed to show beetle galleries. Egg galleries are formed vertically and larval galleries around the circumference of tree. Gallery formation can have a girdling effect on inner bark (phloem) and thus downward flow of sugars is interrupted.

Figure 7. Healthy trees are "baited" using special insect attractant called pheremone. Chemical is introduced into "tygon" tubing wrapped around tree. Red area represents previous sampling site. Paint helps identify tree and protects tree. White area is where bark has been removed and xylem is currently being sampled for comparison with tissue sampled after attack.

Figure 8. Segment of fungal infected tree. Outer blue-black discoloration (hence the name blue-stain fungi) represents sap-wood where blue-stain fungi have grown and caused an enzymatic degradation of phenolic substances to occur. This is the area of wood involved with water conduction. If blockage resulted because of extensive hyphal development, tree would soon die.

Figures 9-12. Procedure used in sampling tissue. **9.** Broad chisel used to rapidly extract large tissue segments. **10.** Bark is pulled back and wood (xylem) is exposed. **11.** Electron microscopy on normal and infected tissue requires tissue be cut into small segments to facilitate rapid killing and fixation. **12.** Tissue is taken to nearby field laboratory operations. Graduate student researcher, Richard Ballard, prepares tissue samples for microscopical analysis. Further processing takes place at Plant Anatomy Research Labs.



The mountain pine beetle and the lodgepole pine (*Pinus contorta* Douglas var. *latifolia* Engelmann) have evolved an unprecedented relationship over eons of time. Despite all our information about the insect, its life history, preference for *P. contorta* and dynamics of attack, little can be done *directly* to save vigorous pine specimens from devastation. Forest management strategies to prevent and/or minimize these losses are now being tested over varied situations with some success.

Beetle Biology

Each year around the last week in July, young adult beetles (Figure 3) chew their way out of trees (Figure 4) that were attacked the previous year. They fly to other healthy, uninfested trees where they begin boring into the bark (Figure 5). Here they mate, beginning the next year's generation of beetles. Female beetles bore vertical tunnels called egg galleries (Figure 6) into the inner bark (phloem). The eggs deposited on alternate sides of the tunnel soon hatch, and first instar larvae begin their own feeding activities by chewing horizontal larval galleries around the circumference of the trunk.

As they work, the adult beetles bring the blue stain fungi into the tree with them and thus inoculate the tree. These blue stain fungi include possibly three species of *Europhium* and at least three species of *Ceratocystis: C. montia, C. minuta,* and *C. minor* isolated from, or associated with, mountain pine beetle attacks. The *Ceratocystis* fungi are similar to fungi associated with Dutch Elm Disease and a disease of pines in the southeastern United States. Because the fungi are consistently observed in association with the beetles, it is presumed a mutualistic relationship exists.

Local Research Scene

How the fungi function in the deaths of lodgepole pines is of major concern to investigators at the Plant Anatomy Research Laboratories of Utah State University. A research site has been established at the head of Logan Canyon (Figure 1) and cooperative study is underway with scientists at the USDA-Forest Service in Ogden, Utah.

To facilitate study, beetles are attracted to designated trees by using a "bait." Tygon tubing, impregnated with an attractant chemical (pheromone) is tied to selected trees (Figure 7). After beetles attack a tree, tissues obtained from the tree are subjected to preliminary treatment at the study site (Figures 9-12). They are then transported to the laboratory for further processing and microscopical analysis.

Following beetle infestation of a tree, fungal hyphae grow into the sapwood via living cells of the wood rays (Figures 16 and 17). The "blue-stain" discoloration of the sapwood (Figure 8) is caused by a metabolic by-product of fungal enzyme action on phenolic substances of host tissue. The stain marks the limits of inward fungal growth. The blue stained wood is rendered nonfunctional and as discoloration proceeds inward toward the pith, the tree shows signs of increased stress until finally its transpiration stream is totally disrupted and the tree dies.

A Fungus Among Us

It has been reported that the fungi are confined to living cells of the wood, namely the ray parenchyma cells (Figures 16 and 17). In our laboratory, however, we have obtained evidence (Figures 18 and 19) that the growth and distribution of fungal hyphae are greater than reported, especially in tissues attacked the previous year. This is especially significant because trees do not succumb until spring or early summer in the year following a beetle attack. Upon close inspection, we have seen hyphae throughout axial water conducting cells (tracheids) of the xylem (Figures 18 and 19). In many cases, the hyphae have occluded the bordered pits (Figure 19) through which water must pass in its ascent up the tree. In short, the fungi plug the tree's plumbing.

Such physical blockage, however, may only account for part of the observed disruption of water flow and subsequent tree death. For example, hyphal growth may affect resin ducts and induce a release of resins and gums into the water conducting pathway. Fungal spores and large molecules may also occlude bordered pits to some extent.

It is also possible that ray parenchyma cells, which are destroyed by the fungi, are somehow essential to normal sap ascent. Uninfested pine trees store great quantities of chemical energy in living ray cells. The energy (sugar) is mobilized in the spring and is essential to renewed growth each year. If the tree is deprived of this material by the fungus, it would certainly be weakened and hence susceptible to stress for other reasons. We have also wondered if structures called tyloses (wall ingrowths of neighboring parenchyma cells) are formed because of blue stain fungi. Potentially damaging embolisms or tiny gas bubbles in tracheids might be associated with fungi respiration. Also, production of toxin(s) by the fungi cannot be discounted. And finally, one cannot overemphasize the devastating effect of phloem girdling by beetles. This not only increases the potential for water loss, but also interrupts the flow of sugars to roots. In other words, we are still groping among unknowns that must be solved.

Future Prospects

Since the beetle is the indispensable vector in fungal inoculation, numerous attempts have been made to control the insect. Spraying, clear cutting, selective harvesting, burning, and even breeding programs have been implemented. However, successes have been only occasional, mainly due to environmental variables, the vastness of the area occupied by susceptible trees, the short time when beetles are vulnerable, and the total numbers of trees involved. Although one population of beetles can sometimes be eliminated, the trees remain highly susceptible to a new invasion.

In our laboratory, we are seeking data that will relate the physiological events of increased water stress to temporal aspects of radial growth of fungi into host trees. Our continuing studies of the seasonal development of xylem and phloem in lodgepole pines should help clarify fungal growth and development processes. We are now fairly sure of how to answer the question, "What's killing the lodgepole pines?" Now we must define the mechanism that actually causes death. Only then can we hope to stop the devastation.

ABOUT THE AUTHORS

Richard G. Ballard is a graduate student in the Biology Department. Mr. Ballard's training is primarily in botany, specializing in anatomy, microbiology, and mycology.

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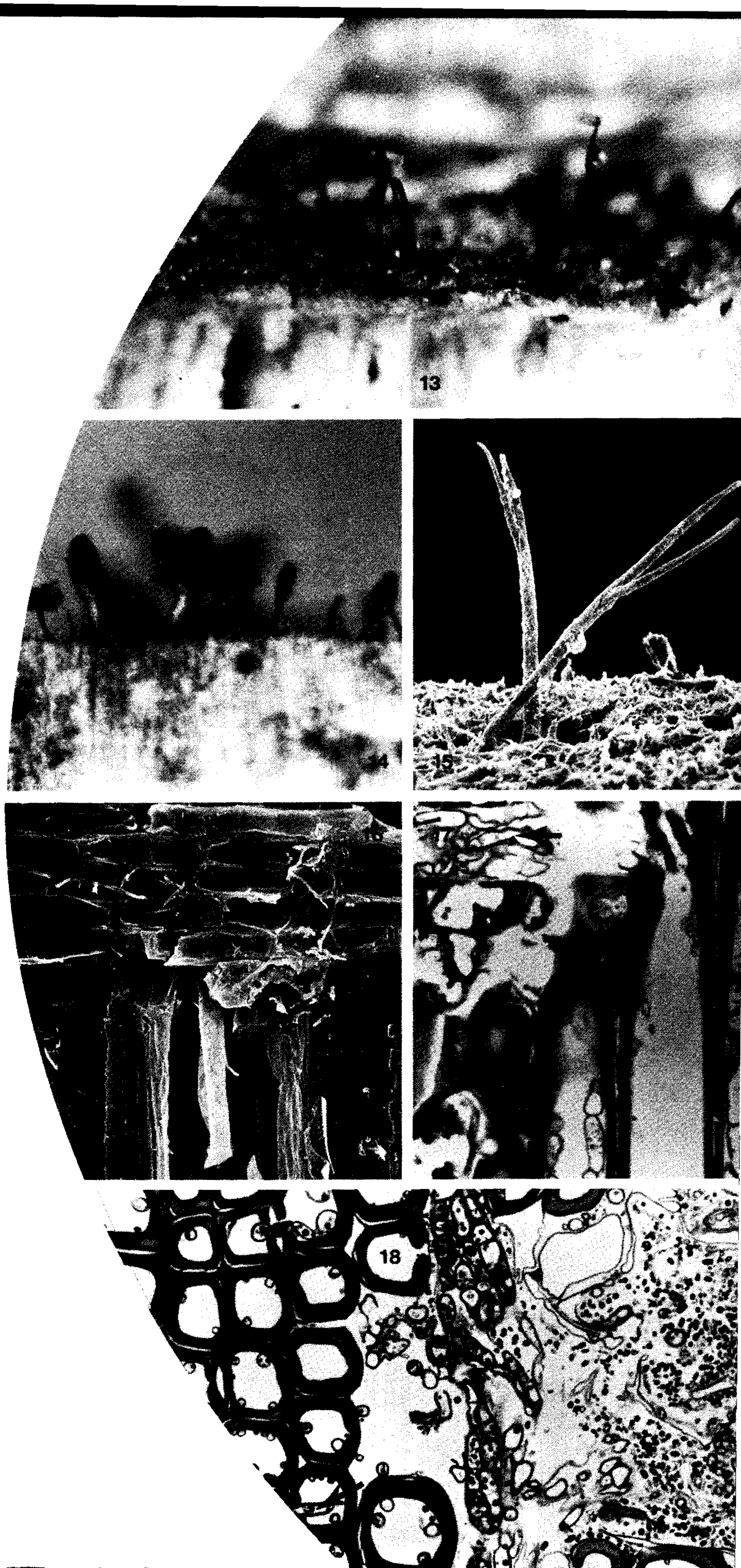

Figures 13 and 14. Fungal "fruiting" bodies as viewed with a compound stereo light microscope. Since several species of fungi inhabit wood, different "fruiting bodies" are present. Figure 13 shows one type, while Figure 14 shows another.

Figure 15. Scanning electron micrograph of part of a "fruiting body" similar to those shown in Figure 13. Fruiting bodies contain many sac-like structures that contain reproductive spores. Thousands of spores are contained within a single "fruiting body."

Figure 16. The hyphae (arrows), or "vegetative" part of the fungi, are shown in this scanning electron micrograph. They appear throughout the radial and axial water conducting system of the wood.

Figures 17 and 18. Light microscope views of wood (xylem). Fungal hyphae appear in wood rays (Figure 17, single arrow) where lateral transport of water occurs, also in axial tracheids (double arrows) where vertical transport occurs. Closer inspection (Figure 18, arrow) reveals hyphae plugging bordered pits between water conducting cells.

Figures 3 and 8 courtesy of W.E. Cole and G. Amman. All other figures by R. Ballard and M. Walsh.