INTERVASCULAR PIT PLUGS IN THE TRANSITION ZONE BETWEEN SAPWOOD AND WETWOOD OF ULMUS AMERICANA L.

by

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Summary
Scanning electron microscopic analysis of freeze-dried American elm wood revealed the presence of unique plugs in bordered pit chambers of vascular elements. Non-dispersive X-ray analysis of these plugs demonstrated the presence of high levels of calcium and potassium. Some pits contained only fragmentary deposits or bacteria. An hypothesis is proposed to account for the precipitation of CaCO₃ in the bordered pit chambers of vascular elements at the sapwood-wetwood boundary, resulting in the formation of amorphous plugs that fill the pit chamber.

Key words: pit deposits, vessels, elm, wetwood, scanning electron microscopy, X-ray analysis, calcium, potassium.

Introduction
Wetwood, an abnormal wood which often appears water-soaked, occurs in many species but its characteristics vary, especially between hardwoods and softwoods (Bauch & Tiedemann, 1976). In American elm (Ulmus americana L.) wetwood replaces normal heartwood and is characterised by the presence of bacteria, fermentative odours, dark brown discoloration, elevated pH, and high moisture content (Murdock, 1981). Wetwood capillary liquid in elm has a higher osmotic potential than sapwood expressate and contains higher concentrations of potassium, calcium, and magnesium (Murdock, 1981). A scanning electron microscope (SEM) study was initiated to investigate the possibility of a structural barrier zone between wetwood and sapwood. This paper reports preliminary results of that study.

Materials and Methods
Increment cores were obtained from four American elms at approximately breast height on May 26, 1981. The borings were clamped and a radial surface was exposed using a razor blade. Specimens, approximately 1/4" in length were cut to include the sapwood-wetwood boundaries in each core. Specimens were quick frozen in a slurry of hexanes (m.p. = -94°C) which was cooled in liquid nitrogen. Wood specimens were individually transferred to pre-cooled glass vials, which were capped and stored in liquid nitrogen. The samples in the vials were placed under vacuum in a freeze-drier overnight, and capped and stored in a desiccator.

Specimens to be viewed in the SEM were mounted on carbon stubs with a small amount of tacky Duco cement (a glue free of inorganic ions). Liquid graphite was spread around the sides of the specimen leaving the shaved surface open for viewing. Specimens were coated with carbon in a Denton DV-515 vacuum evaporator and stored in a desiccator.

The wetwood-sapwood boundary of each sample was examined using a Cambridge S4 Stereoscan SEM. Elemental analysis was performed on an ORTEC EDDS II system.

Results
In the wetwood-sapwood boundary region, cells were examined for evidence of structural barriers. Most fractures revealed inner cell wall surfaces (cell lumen side). Occasionally, however, cell fracture followed the middle lamella and in these fracture planes paraboloid-shaped deposits or plugs were observed in bordered pit chambers of vessels (Figs. 1, 2, 3). The plugs fill the pit chambers and often bear the negative impressions of pit apertures (Figs. 3, 4). The deposited material, which occurs on both sides of the pit membrane (Fig. 5), appears amorphous rather than crystalline. Occasional cracked plugs (Fig. 6) indicate that the material is a brittle solid when freeze-dried. Similar, but more irregular, deposits appeared in the smaller vessel to parenchyma pit pairs (Fig. 7). X-ray analysis demonstrated that these deposits had a composition similar to the paraboloid plugs.

Spot scans with energy dispersive X-ray analysis (EDXA) demonstrated that the pit plugs contained potassium and calcium (Fig. 8). Line scans outlined the higher concentrations of these elements in pit plugs, with calcium...
Fig. 8. Spot scan of a pit plug using energy dispersive X-ray analysis. Calcium is the dominant peak. — Fig. 9. EDXA line scan (dots = calcium, solid area = potassium). Peaks correspond to concentrations of these elements in pit plugs along the line in Fig. 1. — Fig. 10. Position of scan line for EDXA shown in Fig. 11; x 3323. — Fig. 11. The shapes of two pit plugs in Fig. 10 are outlined due to the higher concentrations of calcium.

being the most concentrated (Figs 9, 10, 11). Other vessels scanned in the wetwood lacked pit plugs but had bordered pit membranes with particles on their surfaces. Some of these particles have the form of rod-shaped or rounded bacteria (Figs. 12 & 13) and compare with bacteria in wood depicted in previous studies (Greaves, 1969; McGinnes et al., 1974; Sachs et al., 1974). Other donut-shaped particles on the membranes do not resemble bacteria previously reported (Fig. 14). The membranes have a coated appearance, which may be bacterial slime or other deposited material. Careful examination of pit plugs occasionally revealed bacteria-like particles. In addition small depressions, which could be the negative molds of bacteria, were found where the plug had pressed against the over-arching pit border (Fig. 15).

Discussion

Pad-like structures on the bordered pit membranes of Daphne, Osmanthus, Ribes, Prunus, Pyrus, and Ligustrum have been noted in other studies (Parameswaran & Liese, 1973, 1981; Ohtani & Ishida, 1978; Parameswaran & Gomes, 1981). The pads described in previous studies, except those found in Ribes, were ‘torus-like’ although often laterally eccentric on the mem-

Fig. 1. Earlywood area near sapwood/wetwood boundary containing pit plugs (PL). The scan line (SL) is shown for the energy dispersive X-ray analysis in Fig. 9; x 82. — Fig. 2. Area outlined in Fig. 1. Plugs (PL) can be seen filling the pit chambers of some bordered pits where outer cell walls have been exposed; x 234. — Fig. 3. Area outlined in Fig. 2. The pit plugs conform to the shape of the pit chambers and bear imprints of the pit apertures (arrow); x 3298. — Fig. 4. Bordered pit plug bearing an imprint of a pit aperture (IA). In the background is a bordered pit with evidence of a torn-away plug (arrow); x 3410. — Fig. 5. A plug is partly lifted from the pit chamber exposing the aperture below (AP). Torn pit membrane can be seen (arrow); x 3962. — Fig. 6. Cracked plugs indicate that the material is a brittle but amorphous solid; x 3352. — Fig. 7. The vessel pit to the left (arrow) is smaller and contains an irregularly shaped deposit; x 3324.
branes (Parameswaran & Liese, 1981). The plugs in elm, however, cover the entire membrane and fill the pit chambers. The pads observed in the fibre-tracheids of Ribes have the greatest morphological concurrence with the pit plugs of elm but the pads in Ribes form and disappear during cell maturation (Parameswaran & Liese, 1973) whereas the plugs in elm are found in mature cells at the wetwood-sapwood boundary.

All the pads described in previous studies are reported to contain plasmodesmata. The fractured plugs in the present study failed to reveal plasmodesmata but sectioned material examined by TEM would be required to substantiate their absence or presence in pit plugs. Elemental analyses were not performed in the previous studies so chemical comparisons are not possible.

The most common calcium-containing deposits found in wood are calcium oxalate and calcium carbonate. Calcium oxalate is usually reported as crystalline whereas calcium carbonate has been reported as a white solid that fills some vessel lumens and pit chambers of discoloured heartwood in many hardwoods inclu-
ding American elm, Calcium carbonate has especially been noted around cracks and other wounds (Dadswell & Hillis, 1962). Epstein (1965) states that calcium carbonate can be deposited at the boundary between vessels, which contain calcium in solution, and mesophyll cells, which contain endogenous carbonate in solution.

Conditions at the wetwood-sapwood boundary in American elm could initiate precipitation of calcium carbonate. Carbonates, although water-insoluble, can be dissolved in carbonated water (CO₂ dissolved in water). Bacterial respiration may release CO₂, the solubility of which would be enhanced under the high pressures found in elm wetwood (Murdock, 1981). When the pressure is released and CO₂ is liberated the insoluble carbonates precipitate out (Brescia et al., 1974). The most likely site for this to occur is the wetwood-sapwood boundary. Bacteria on pit membranes in this region could concentrate the precipitate in the pit chambers.

The difficulty we encountered in locating pit plugs may be, in part, a reflection of their evanescent nature. As the wetwood-sapwood boundary progresses outward from the pith, pit plugs should be dissolving in some vessels and reforming in others. We observed different levels of development of pit plugs, which may be a reflection of this dynamic process. Although we did not investigate seasonal variation, this may also be occurring, as was reported for the encrustations on bordered pit membranes in *Fraxinus americana* (Wheeler, 1981).

References