A Staining Anomaly of the Wood of Crossosoma (Crossosomataceae)

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An unusual staining reaction has been encountered in histological studies of *Crossosoma bigelovii* Wats. and *C. californicum* Nutt., the only species in the Crossosomataceae. The xylem and phloem were studied as a part of an examination of the total morphology and anatomy of the family. The study was undertaken to clarify the phylogenetic position of the family, whose affinities are a matter of doubt.

The genus *Crossosoma* is limited to the southwestern part of the United States and adjacent Mexico, *C. bigelovii* being found about the margins of the Sonoran desert, and *C. californicum* on the islands off the shores of California and Baja California. Both species are small shrubs.

During microscopic study of the wood it was found that xylem was difficult to stain with safranin, the usual wood stain, which acts on the lignin in the walls of wood cells. The wood gives a strong lignin reaction with phloroglucinol so it would be expected to stain with safranin. A normal staining period in safranin-alcohol-Methyl Cellosolve (Johansen, 1940) left the tissue a very light pink. Uniform safranin staining of the wood was achieved only after the material was also stained with iron-alum-mordanted safranin (Gray and Pickle, 1956).

For study of the phloem, lacmoid stain was used. Lacmoid is reported to be specific for callose (Eschrich and Currier, 1964), a substance found in heavy concentrations on the end walls of sieve plates of the conducting elements or sieve tube members in the phloem. Callose is a beta-1,3 linked glucose polymer which is deposited on sieve plates as a wound response. Since the distribution and structure of these conducting cells was under investigation, the callose served as an identifying feature. It makes little difference that callose is a fixation artifact (Evert and Derr, 1964). Its presence as a histological feature is often used in phloem tissue study.

In a study of callose, Currier (1957) surveyed tissues in many organs of numerous plant species. He found callose in various tissues but reported that it was rare in xylem, occurring only in occasional cells.

The phloem slides used in the current study were prepared from whole-stem preparations since the largest stems examined were about 2 cm in diameter and most stems were a very convenient size for microtoming.

When the sections were stained it was observed that phloem parenchyma and cortical parenchyma cells did not stain with lacmoid. Callose depositions, but not cell walls, took the stain in sieve elements of the phloem. Walls of xylem cells, however, took a dense blue stain.

Since the walls of the xylem stained well with lacmoid and were difficult to stain with safranin, it is possible that the walls of these cells were impregnated with callose which interfered with safranin staining. Callose in xylem tissue in significant amounts must be considered unusual in view of Currier's (1957) work. A possible alternative explanation is that lacmoid is not completely specific for callose, and that it acted upon some other substance in *Crossosoma* xylem cells. This seems less likely than the first hypothesis.

In summary, in *Crossosoma* the walls of cells in tissues other than the xylem do not stain with lacmoid, a stain reportedly specific for callose. The callose in protoplasts in the phloem does stain with this material. In the xylem all types of cells have walls which stain readily with lacmoid but not with safranin, a stain for lignified walls. It is therefore possible that the walls of xylem cells are callose-impregnated, a previously unreported phenomenon.

Literature Cited

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