

## A NEW METHOD FOR THE PREPARATION AND EXAMINATION OF PERFORATED RAY CELLS

by

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### SUMMARY

This paper describes a simple technique borrowed from paleopalynology to produce increased concentrations of perforated ray cells and to isolate them from fibers and vessel elements. The technique provides very clear slides with only parenchyma cells and perforated ray cells. The perforated ray cells are easily distinguished and occur in frequencies of up to 10 perforated cells per square centimeter. The methods described includes the maceration of wood material, separation of cells with granulometrical sieves and measurements of cell dimensions. After separation of parenchyma cells from vessels and fibers the suspension is concentrated by centrifugation. With these methods, perforated ray cells may be more easily compared among plant groups.

**Key words:** Perforated ray cells, cell isolation, microtechnique.

### INTRODUCTION

Perforated ray cells (PRCs) are ray cells that exhibit a perforation plate connecting two vessel elements. PRCs were originally described by Chalk and Chattaway (1933), and the occurrence of PRCs is reported in more than 30 plant families (McLean & Richardson 1973; Giraud 1983; Eom & Chung 1996 and references therein).

PRCs are relatively uncommon, and therefore often difficult to observe or quantify. For example, Flacourtiaceae and Saurauaceae contain from 0–15 PRC cm<sup>-2</sup> (Nagai et al. 1994). Only 0–2 PRC cm<sup>-2</sup> are usually observed in preparations which require substantial time and effort to prepare. Localization is surely one reason why these cells are poorly understood. To understand the role of perforated ray cells in water transport requires that these cells and their perforation plates be precisely measured. These measurements would allow analysis of their role in sap flow resistance. In the species that have been studied, interpretations of PRC conductivity diverge. Nagai et al. (1994) observed PRC resistance to sap flow much greater than that observed by Ceccantini & Angyalossy-Alfonso (2000), who suggest that *Bathysa meridionalis* has much lower resistance.

Although the IAWA Committee (1989) showed that PRCs may be the same size or larger than other ray cells, PRCs are usually larger (Chalk & Chattaway 1933; Rudall

1985; Nagai et al. 1994; Otegui 1994; Eom & Chung 1996; Ceccantini & Angyalossy-Alfonso 2000). PRCs may be separated from other cells because they are larger than other ray cells and much smaller than other vessel elements, and this combination of characteristics allows their separation through maceration techniques. Here we describe how a technique used often in paleopalynology (Traverse 1988) allows separation of PRCs on slides, which permits their identification among other cells. This technique provides a long-needed tool for better analysis and quantification of PRCs than has hitherto been possible.

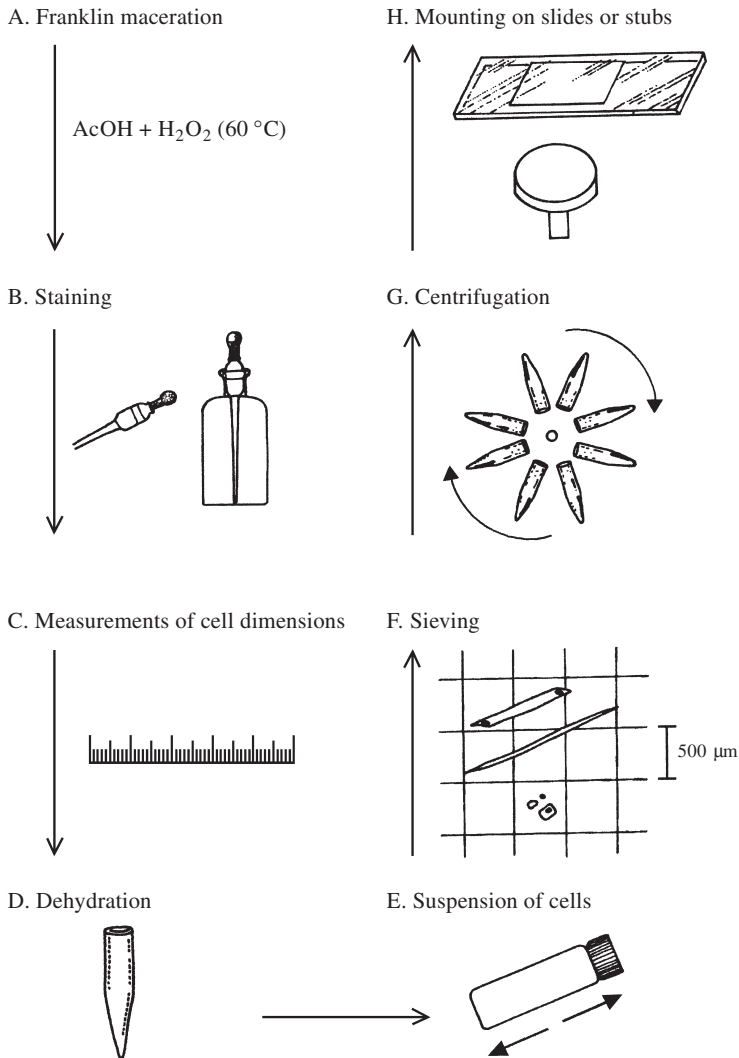


Fig. 1. Procedure to concentrate perforated ray cells from maceration of wood material.

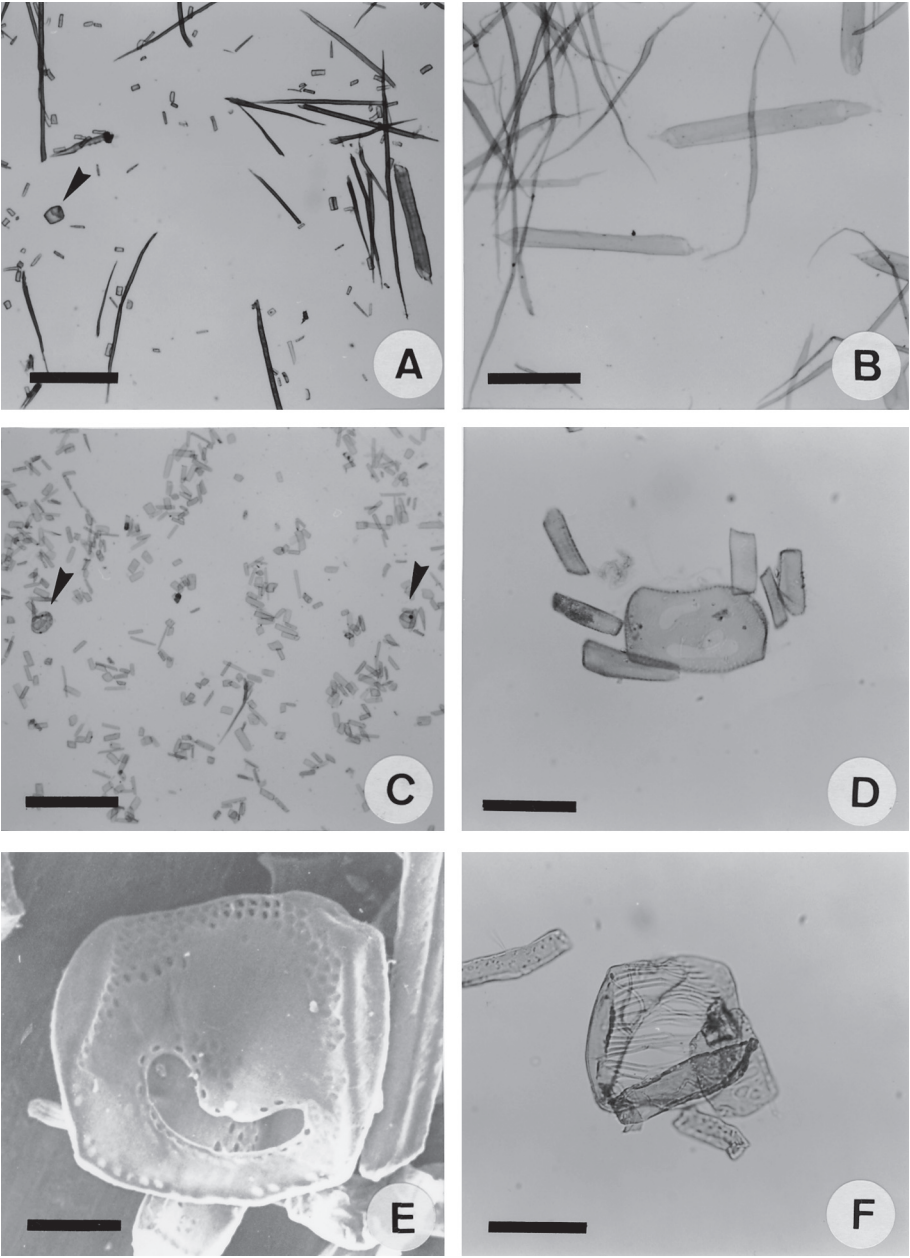


Fig. 2. Maceration of wood material in different steps of separations. — A: *Xylosma brasiliensis*, maceration with all cell types (arrow indicates PCR). — B–E: *Bathysa meridionalis*; B: vessels and fibers only; C–E: PCRs and parenchymatic cells only (arrows in C indicate PCRs); E = SEM. — F: *Sloanea monosperma*, PCRs and parenchymatic cells. — Scale bar for A–C = 500  $\mu\text{m}$ , for D, F = 100  $\mu\text{m}$ , for E = 50  $\mu\text{m}$ .

## PROCEDURE

Preparation of PRCs requires the usual tools for maceration, flasks, water, centrifuge, glass stir-rods, sieves of several mesh sizes (700–100  $\mu\text{m}$ ) and Ependorf flasks. Wood samples were taken from the collection at the Institute of Biosciences at the University of São Paulo and include *Bathysa meridionalis*, *Xylosma brasiliensis* (Flacourtiaceae), and *Sloanea monosperma* (Elaeocarpaceae).

Preparation of PRCs begins with conventional Franklin maceration methods, using acetic acid and hydrogen peroxide (Berlyn & Miksche 1976) (Fig. 1A, 2A), and the staining the researchers wish to use is added to the solution (Fig. 1B). Next, macerations are mounted on slides which are then used to measure and choose the cell types for further preparation (Fig. 1C). Based on these measurements, the sieve-sizes are chosen. Approximately 1  $\text{cm}^3$  of material is then suspended in 50 ml of water (Fig. 1E) in which 10–20 g of sucrose is added to keep the cells separated. Then the suspension is passed through the first sieve for separation of the fibers and vessel elements (Fig. 1F). This sieve should have pores of slightly smaller diameter than the smallest lengths of vessels and fibers (500 or 700  $\mu\text{m}$ ). For extremely small vessel elements (e.g. lianas and some Leguminosae) the sieve mesh size may be 200–300  $\mu\text{m}$ . The filtrate then contains PCR and axial and radial parenchyma cells (Fig. 2C–F) and the residue in the sieve is a mixture of vessel elements and fibers (Fig. 2B). The filtrate is then centrifuged for 2 minutes at 3,000 rpm (Fig. 1G), after which the supernatant is discarded, leaving the residue which contains the PCRs.

Some species contain PCRs much larger than the other ray cells (e.g. *Bathysa meridionalis*), and these species may be filtered again using a 100  $\mu\text{m}$  sieve size. This filtrate is again centrifuged, and the solid material (approximately 1–2 ml) collected and treated as previously described.

The resulting materials can then be mounted on temporary slides (with glycerine), permanent slides (Fig. 2C, D, F), or stubs for scanning electron microscopy – SEM (Fig. 1H, 2E). Mounting is done by first shaking the Ependorf flasks with the centrifugate to suspend the material, and then placing a drop of the centrifugate on the slide or stub. To prepare permanent slides, a dehydrating agent (alcohol, acetone, etc.) should be used as in traditional preparations and slides may be resin-mounted. Prior to viewing the material in SEM, conductive adhesive tape is placed on the stub, and a drop of centrifugate is placed on the tape and allowed to dry so that the cells adhere to the tape.

Preparations of up to 10 PCRs  $\text{cm}^{-2}$  (5 times the usual concentration) were obtained with this method. Thus, the resultant high-quality slides permits better observation of perforation plates free of parenchyma cells, and greater quantification of PRCs and their characteristics. Sometimes it is possible to find up to 40 PCRs in a single slide depending on the frequency in each species. This method, in principle, may be used for any xylem elements that are of a consistent size, different from that of other cells with which they co-occur. Some possibilities are latex-producing cells, fat-storage cells, and so on.

Perforated ray cells are poorly understood today, partly due to difficulties in producing consistent and high-quality concentrations of cells for analysis. The method presented here provides one way in which to concentrate and analyse PRCs, thus making a review of perforated ray-cell structure and function possible.

#### REFERENCES

- Berlyn, G.P. & J.P. Mikshe. 1976. Botanical microtechnique and cytochemistry. The Iowa University Press, Iowa.
- Ceccantini, G.C.T. & V. Angyalossy-Alfonso. 2000. Perforated ray cells in *Bathysa meridionalis* Smith & Downs (Rubiaceae). *IAWA J.* 21: 77–82.
- Chalk, L. & M.M. Chattaway. 1933. Perforated ray cells. *Proc. Roy. Soc. London B* 133: 82–92.
- Eom, Y.G. & Y.J. Chung. 1996. Perforated ray cells in Korean Caprifoliaceae. *IAWA J.* 17: 37–44.
- Giraud, B. 1983. Les cellules perforées de rayons ligneux chez les Euphorbiacées. *Bull. Mus. natn. Hist. nat., Paris, 4<sup>a</sup> sér., 5, section B, Adansonia*, no 2: 213–221.
- IAWA Commitee. 1989. IAWA list of microscopic features for hardwood identification, with an appendix on non-anatomical information. *IAWA Bull. n. s.* 10: 219–332.
- McLean, J.D. & P.E. Richardson. 1973. Vascular ray cells in wood stems. *Phytomorphology* 23: 59–64.
- Nagai, S., J. Ohtani & K. Fukuzawa. 1994. SEM observations on perforated ray cells. *IAWA J.* 15: 293–300.
- Otegui, M. 1994. Occurrence of perforated ray cells and ray splitting in *Rapanea laetevirens* & *R. lorentziana* (Myrsinaceae). *IAWA J.* 15: 257–263.
- Rudall, P. 1985. Perforated ray cells in *Hyptis hagei* – a new record for the Labiatae. *IAWA Bull. n. s.* 6: 161–162.
- Traverse, A. 1988. *Paleopalynology*. Unwin Hyman, Boston.