

# This Week's Citation Classic™

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Currier H B. Callose substance in plant cells. *Amer. J. Bot.* 44:478-88, 1957.  
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Callose ( $\beta$ -1,3-glucan) adsorbs aniline blue (w.s.) to fluoresce yellow in 'blue,' or UV, light. Neither fluoresces alone. Callose forms in a variety of cells and tissues. Stimulation, e.g., injury, promotes synthesis, but some deposits are natural. Artifacts can occur. This reversible system may act to block translocation pathways. [The SCI® indicates that this paper has been cited in over 210 publications since 1957.]

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"I was privileged to spend a sabbatical during 1954-1955 at the Botanical Institute, University of Münster, Federal Republic of Germany, where S. Strugger<sup>1</sup> had laboratories well equipped for research in plant 'protoplasts'—the study of living cells and tissues microscopically, including fluorescence analysis. A Guggenheim Fellowship, which provided support, was helpful and much appreciated.

"One day, following a survey of callose locations in living *Vitis* phloem sections that used water-soluble aniline blue and bright light, the temporary slides were re-examined with 'blue' light before being discarded. Callose appeared yellow. Next, we made the dye solution with M/15 alkaline phosphate buffer. The results were striking. In addition to producing staining of sieve plates and lateral sieve areas in phloem, the parenchymal pits (thin areas of cell walls through which plasmodesmata pass) also appeared yellow. Thus the term 'pit callose reaction' arose. Using Strugger's elegant method of removing small inner epidermal squares from onion bulb scales, we determined that the pit callose appeared in the anticlinal walls, mostly as a wound stimulation effect.<sup>2</sup> My host remarked, 'I cannot believe my eyes.' However, in healthy, undisturbed rapidly frozen-thawed tissue, no callose could be seen, suggesting natural absence. Incubation of living tissue over-

night in nutrient containing 0.2M sucrose increased callose accumulations in the pits.

"What is dealt with in the callose studies is a sensitive, reversible, metabolic system in which the glucan forms and appears to seal, plug, and constrict cell-to-cell pathways in epidermis and parenchyma, and systemically, in sieve tubes. Artifacts of 'presence' and 'absence' potentially abound. Careful handling of plant material is essential. Killing by freezing is preferred over chemical fixation. Translocation studies to verify assumed blockages by pit callose remain to be done.

"During a visit to the University of Bonn, I met Walter Eschrich,<sup>3</sup> who was studying the chemical properties of cystolith callose and, moreover, was preparing a review of callose literature for *Protoplasma*. He told me about the paper by Arens,<sup>4</sup> of which I was unaware, that described yellow aniline-blue fluorescence of mycelia, pollen tubes within stigma and style, sieve plates, and cystoliths. The dye was used in dilute solutions of NaOH, KOH, and NH<sub>4</sub>OH.

"While tissue locations of the substance had been known for a long time, from aniline-blue and lacmoid staining, an important new location was shown to be pit areas of epidermal and parenchymal tissues. In healthy, undisturbed sections, rapidly deep frozen and thawed, no callose could be detected, but following injury, it quickly appeared. Ridgway's 1913 report<sup>5</sup> of root hair callose, and my finding of the substance in the hair tips, were correctly judged by Lerch<sup>6</sup> in Potsdam to be wound artifacts.

"Returning to Davis, I submitted a more complete report of the callose studies in Münster to the *American Journal of Botany*. Studies utilizing *Allium cepa* bulb tissues, *Vitis vinifera* stem, *Elodea densa* and *E. canadensis* leaves, yeast, pollen grains and tubes, *Ficus elastica* plugged sieve tubes and laticifers in petiole excision wounds were described.

"The high citation frequency may be due to the interests of researchers in many plant subfields, e.g., cytology, anatomy, pathology, physiology, biochemistry, and genetics. Also, new methodologies and surprising fluorescence phenomena were involved.

"Additional information on this subject may be found in Esau's<sup>7</sup> monograph."

1. Strugger S. *Praktikum der Zell- und Gewebephysiologie der Pflanze*. Berlin: Springer-Verlag, 1949. 225 p.
2. Currier H B & Strugger S. Aniline blue and fluorescence microscopy of callose in bulb scales of *Allium cepa* L. *Protoplasma* 45:552-9, 1956. (Cited 90 times since 1956.)
3. Eschrich W. Kallose. *Protoplasma* 47:487-530, 1956. (Cited 65 times since 1956.)
4. Arens K. Provo de calose por meio da microscopia a luz fluorescente e applicações do metodo. *Lilloa* 18:71-5, 1949.
5. Ridgway C S. The occurrence of callose in root hairs. *Plant World* 16:116-22, 1913.
6. Lerch G. *Untersuchungen über Wurzelkallose*. Jena: Gustav Fischer Verlag, 1960. 111 p.
7. Esau K. *The phloem*. Berlin: Gebrüder Borntraeger, 1969. 505 p.