NUMBER 7
FEBRUARY 12, 1979

This Week's Citation Classic

Feder N & O'Brien T P. Plant microtechnique: some principles and new methods. *Amer. J. Bot.* **55:**123-42, 1968.

Some structures of plant cells are easily seen with the light microscope when the cells are examined alive, but are destroyed or badly distorted by many customary fixatives and embedding media. We discuss the basis of these damaging effects and propose specific methods for minimizing the damage and achieving excellent structural preservation in fixed and embedded material. The methods include the use of non-coagulant fixatives, suitable dehydration techniques, and embedding in plastics, particularly glycol methacrylate. [The *SCI*® indicates that this paper has been cited 298 times since 1968.]

T. P. O'Brien
Botany Department
Monash University
Clayton, Victoria
Australia

April 28, 1978

"In the fall of 1963 Keith Porter and several of his staff initiated a one-semester course in cell biology at the Harvard Biological Laboratories Margaret McGully and I, graduate students of one year's standing, were selected as the "botanical" Teaching Fellows for the lab part of the course, and we met Ned Feder, who was in charge of the lab. Ned had always been interested in histological methods and had already produced an impressive collection of microscope slides from animal tissues fixed in acrolein and sectioned in a water-miscible plastic, glycol methacrylate (GMA). These specimens were so far superior to anything else available at that time that McCully and I were both inspired to try his procedures out on plant specimens. She was studying the histology of the brown alga, *Fucus vesiculosus*, and I was looking at photosensitivity of oat coleoptiles in the (vain!) hope of finding an anatomical basis for blue perception. I remember clearly the pleasant shock I got when I first compared a coleoptile apex done *a la Feder* with an apex treated with traditional botanical fixatives and sectioned in wax Early encounters with the traditional methods applied to bracken fern rhizomes had convinced me that these methods were art, not science, and that the final product was hardly worth the effort, a view shared by many plant physiologists.Suddenly, here were sections that even a dedicated physiologist would have to admit produced a reasonably faithful image of the living state Working in Ned's lab one weekend, McCully and I rediscovered the value of the dye toluidine blue as a polychromatic stain for plant tissue, both in GMA sections and in fresh hand-cut sections

"Armed with a set of slides I gleefully ap-, proached Emeritus Professor Ralph Wetmore for his reaction to the new techniques, for he had spent his life as a developmental anatomist.The reaction was not long in coming.Within weeks Ned and I received an invitation from a colleague of Wetmore's, Charles Heimsch, Editor of American Journal of Botany. He wanted us to prepare a special paper on the methods for that journal. The paper had a long gestation period due largely to the fact that Ned wanted to be sure of the techniques we were about to recommend. The paper, with a color plate, appeared in 1968. and can only be described as a sellout We had anticipated interest: we did not expect to go through 1,000 reprints. The great bulk of a second reprint ing is also gone. In 1969 McCully and I produced *Plant structure and development,* a book illustrated profusely with GMA sections, in a further effort to popularize these procedures. The out-come was not completely satisfactory. Despite obvious interest, relatively few groups around the botanical world actually adopted these techniques (or others based on epoxy resins) with the gusto we believed they deserved. Partly the explanation is scientists' conservatism, and partly it is that there are difficulties in handling plastic sections.The former is natural enough, and time takes care of it. The latter still needs solution. Initially we recommended acrolein as a fixative, but it is so nasty to handle that it has largely been replaced by glutaraldehyde alone or in mixtures or sequences with formaldehyde and osmium tetroxicle Ribbons of sections are hard to obtain with GMA, yet ribbons are often a great convenience Alcoholic stains were the preferred stains of the past, but alcohol in low concentration badly wrinkles GMA, and aqueous stains are best. Perhaps the worst problem is fading In our hands truly permanent stains have been hard to find, and many of the old classics (H and E) are rather pale on GMA sections of well fixed material. Partly this is because the sections are so thin (0 5 - 2υm).

"GMA can be sectioned for electron microscopy, but only with difficulty, and there is no doubt that today most of us use epoxy sections for all ad|acent light and electron microscopy and, in softened versions of Spurr's resin, for much of our light microscopy But the epoxy resins have several defects — toxicity of all components, wrinkling of thicker sections (1.0 υm) and lack of success with many histochemical and digestion procedures. Consequently there is still a place for the GMA procedures as well as a critical need for a resin combining the good features of GMA and the epoxy resins."