Darkfield Illumination, an Alternative Technique for Photomicrography

The cover depicts a cross-sectional photomicrograph of a root from peach treated with a soil application of paclobutrazol. The 14-μm section was taken about 2 mm from the root apex. The tissue was fixed and embedded by conventional methods similar to those described by Sass (8), then double-stained with safranin and fast green. Photomicrographs were taken at ×180 with Ektachrome film (Kodak) using a Leitz Dialux 20 research microscope equipped with an Orthomat automatic microscope camera.

The photomicrograph resulted from investigations of the effects of paclobutrazol on peach root growth. Paclobutrazol recently has been shown to alter root growth and development of several plant species (1, 9, 11). The cortex parenchyma cells of peach roots are markedly enlarged and misshapened following soil and foliar applications of paclobutrazol (11).

The dramatic high contrast of the cover photomicrograph was achieved by darkfield (or darkground) illumination. Darkfield illumination is one of several techniques commonly used for image contrast enhancement. This technique differs from the conventional brightfield microscopy in that specimens appear as bright objects against a dark or black background. The darkfield phenomenon is achieved because only the light reflected or scattered by the specimen enters the objective. Darkfield diaphragm stops or specially designed condensors illuminate the specimen with a hollow cone of light. These rays of light are too oblique to enter the objective unless reflected or scattered by the specimen (2, 3, 5). The result is a bright image of the specimen against a very dark or black background.

Darkfield illumination is by no means a new technique. Although not commonly used until the early 20th century, there is little doubt that 17th century microscopist Robert Hooke was familiar with the technique (2). Another 17th century microscopist, Antoni Van Leeuwenhoek, may have known of darkfield illumination. Unfortunately Leeuwenhoek was very secretive in his writings about technique (2, 3). By 1905, darkfield illumination was used commonly to detect and study pathologic microorganisms, especially *Spirochaeta pallida* (5).

Darkfield illumination is well-suited for viewing transparent and semi-transparent specimens that appear 'washed out' when viewed by conventional brightfield microscopy (4, 6, 10). Darkfield aids in the observation of subtle contrasts, especially when staining is either impossible or undesirable. Increasing contrast does not increase the resolving power of the microscope. However, visualization of fine line structures, such as flagellae, cilia, cracks, and other low-contrast structures scarcely visible under brightfield, is greatly enhanced.

Increased contrast and minimum detecting power necessitate the need for special considerations in the preparation of specimens. Slides must be completely free from dust and scratches. It is advisable to use only new slides. Special care must be taken to use nonabrasive materials for cleaning and drying slides. It is especially important to ensure that all optical surfaces in the light path are clean and free from inclusions, such as air bubbles that can cause out-of-focus patches of light destroying the image contrast (4). The low light levels inherent to the darkfield method require either high-speed film or lengthy exposure times when producing photomicrographs. Fortunately, these problems largely can be eliminated with modern automatic photographic equipment.

Newer methods of contrast enhancement (i.e., phase contrast and Nomarski interference) are used more commonly than darkfield illumination. However, darkfield illumination is used routinely in several fields of science such as neurology, plant pathology, and mycology. Low cost is a major advantage of darkfield illumination. Most conventional microscopes not equipped with darkfield can easily be modified for darkfield illumination. The darkfield microscope seems applicable for the teacher or practitioner of horticulture when sophisticated laboratory equipment is not required. In certain situations, the darkfield technique has greater visual appeal than conventional brightfield microscopy or other methods of contrast enhancement. Darkfield illumination is well-suited for oral presentations of photomicrographic data because the black background produces no glare. Although darkfield photomicrographs are usually taken with black and white film, the investigator is free to use a variety of stains and filters which, in combination with color film, can produce striking results, such as in the cover photo. These "attention-getting" photomicrographs may capture the attention of a fatigued audience long enough to express an important idea.

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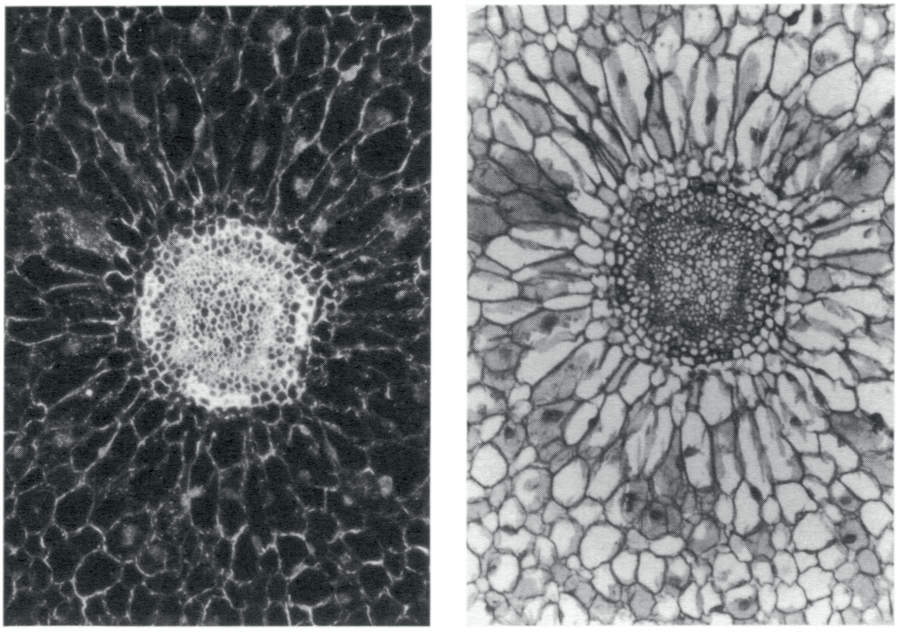

Fig. 1. Root tip cross sections of paclobutrazol-treated peach root tips taken under darkfield (**left**) and brightfield (**right**) illumination ×180.

Trade names are used in this publication solely for identification purposes and do not suggest endorsement of any product. We gratefully acknowledge Earlene A. Rupert and Renee Keese for their technical assistance and Jere A. Brittain for his support and encouragement.

Front cover: Cross-sectional photomicrograph of paclobutrazol-treated peach root.

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