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TECHNICAL NOTE

Forest Pest Management Institute

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AN INSECT CUTICLE PREPARATION METHOD FOR LIGHT MICROSCOPY

The shallow depth of field in light microscopy presents a major disadvantage for the topographic study of insect cuticle. This is particularly true if the micrographs are required to illustrate a specific feature since it is difficult to produce slides with the surface lying sufficiently flat so that it is entirely within the depth of field. As a consequence, the scanning electron microscope (SEM) has largely superseded light microscopy in these studies. However, since SEM facilities are not always available, it is useful to have a technique that will provide light micrographs of a quality suitable for publication and which will provide useful information when shown in conjunction with SEM plates (Percy et al. 1983). The following technique produced slides suitable for photography using Normarski interference-contrast illumination.

The section of cuticle of interest is removed in distilled water or saline. It should be noted that fixatives containing hardening constituents, e.g. alcohol or formalin, should be avoided because hardened tissues cannot be flattened. Next, the piece of cuticle is given a short wash in water and then placed in 10% potassium hydroxide at 60°C for 1 to 3 h to remove soft tissue (Humason, 1967). If the specimen is heavily maculated it may be placed in 10% hydrogen peroxide until sufficiently bleached. After being washed, the cuticle is placed in 45% acetic acid until it is soft and flexible. Usually 1 to 2 h is adequate. The specimen is washed in warm water and placed in a hot water-Glycerin jelly (1:1) mixture ensuring that no air bubbles adhere to it. Finally, the cuticle is mounted in hot Glycerin jelly on a slide and immediately placed on a cold plate or a flat surface in a freezer while strong pressure is applied to the surface of the coverslip to flatten the cuticle. The Glycerin jelly solidifies in several seconds retaining the specimen in this position. The slide can now be observed using Normarski interference-contrast illumination.

This technique works particularly well with larval cuticle allowing discrimination of various surface features (Fig. 1). Since the specimen must be transilluminated, the method works best on cuticle that is not heavily tanned. Unfortunately most adult cuticle is too hard to flatten and too tanned to be transilluminated. While phase contrast or dark field illumination can be used the results are inferior to the more natural appearance produced by Normarski interference-contrast illumination.

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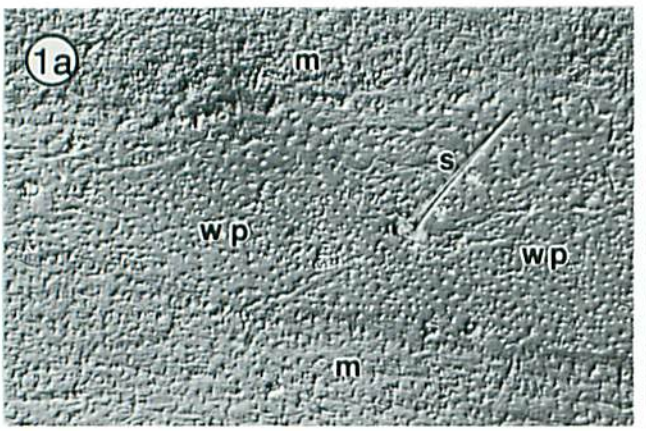

Figure 1a. Normarski interference-contrast micrograph of larval tergal cuticle of the red-backed alder sawfly, *Eriocampa ovata*. wp, wax filament producing surface of a tergal annulet covered with cuticular papillae; m, area of non-wax secreting cuticle covered with muri-cations; s, sensillum tric-hodeum.

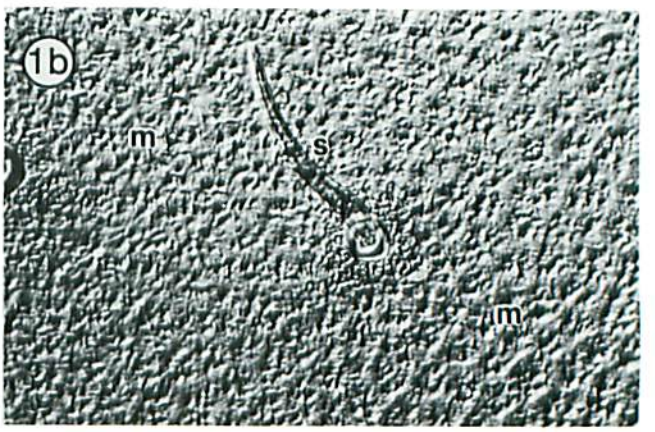

Figure 1b. Normarski interference-contrast micrograph of a portion of tergal abdominal cuticle of the larval silk-worm, *Bombyx mori*. m, muri-cated surface of integu-ment; s, sensillum tricho-deum. Magnification X514.

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