D. P. TAYLOR & C. NETSCHER [1]) : *An improved technique for preparing perineal patterns of* Meloidogyne *spp.*

Species in the genus *Meloidogyne* Goeldi are identified most commonly on the basis of characteristics of the perineal pattern, as originally proposed by Chitwood (1949). Specialized techniques are required in order to observe the details of the pattern clearly. Sasser (1954), Taylor, Dropkin & Martin (1955), and Triantaphyllou & Sasser (1960) used a rather crude technique in which the posterior third of the female was mounted in lactophenol with no attempt made to remove body tissues. Photographs of perineal patterns in these publications are frequently unclear because of extraneous material beneath or attached to the cuticle. J. B. Goodey (1957) and Whitehead (1968) recommended that inner or body tissues be removed and that the cuticle containing the pattern be trimmed to a small size prior to mounting. Complete removal of these tissues is not easily accomplished, and many of Whitehead's (1968) photographs of perineal patterns are unsatisfactory because diagnostic details are obscured by unsuccessful or incomplete removal of adhering tissues (eg. Whitehead's Figs 75 and 77).

During cytological examination of *Meloidogyne* females, using the technique of Triantaphyllou & Hirschmann (1966), we observed that perineal patterns in these preparations were exceptionally clear and free of body tissues. Laboratory tests showed that immersion of cuticles of *Meloidogyne* females in a 45 % aqueous solution of acetic acid (V/V) permitted the easy removal of all adhering tissues; however, the acetic acid was a difficult medium in which to work. Additional acids were tested, and it was found that 45 % lactic acid produced excellent results without any of the difficulties

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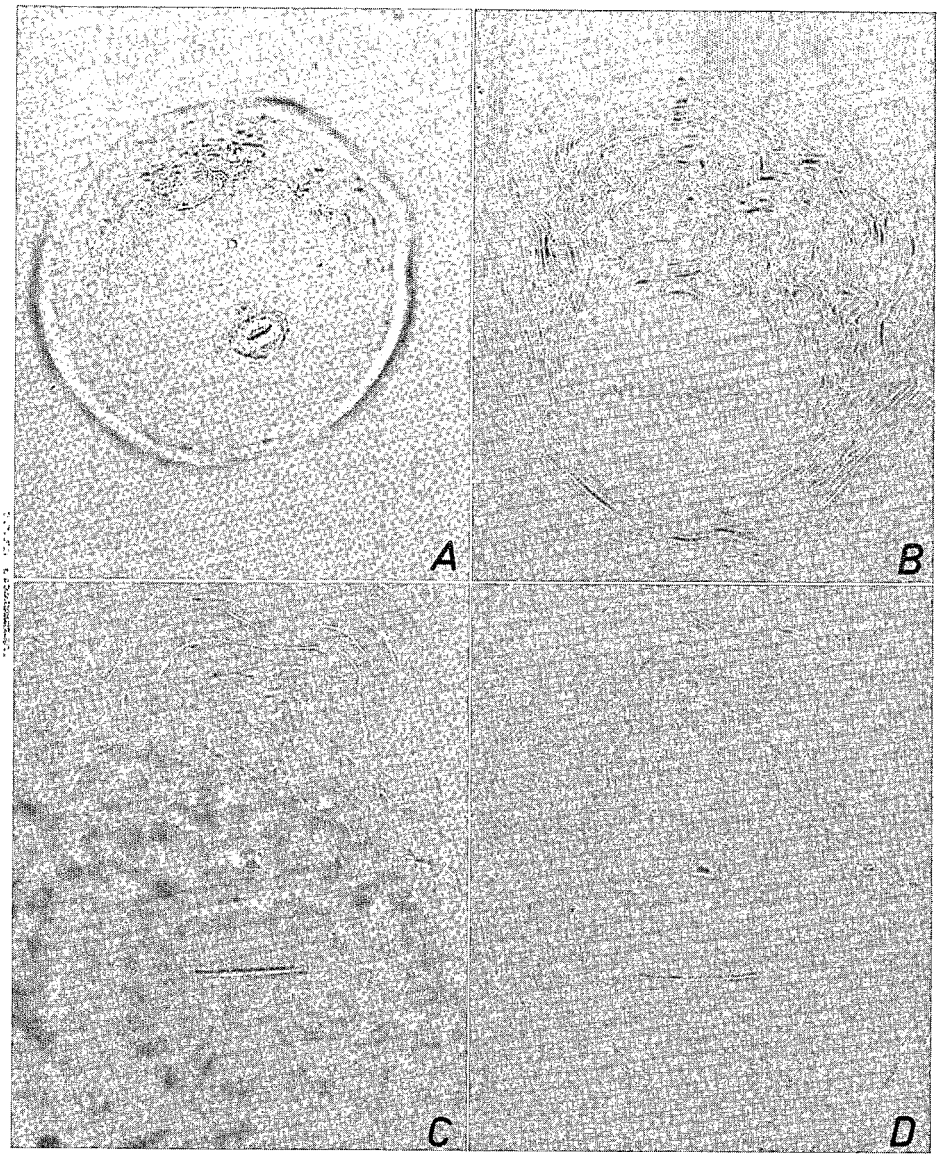

Fig. 1A. Posterior portion of *Meloidogyne* sp. female cuticle after cleaning in lactic acid. — B. Perineal pattern prepared as described in the text — C & D. The same perineal pattern as originally prepared in 1968 and after cleaning in lactic acid in 1974, respectively.

encountered with acetic acid. Fig. 1A illustrates the posterior portion of a *Meloidogyne* female cuticle after cleaning in lactic acid showing the location of the perineal pattern very clearly. Fig. 1B is a photomicrograph of a perineal pattern made using this technique. The clarity of even old perineal patterns can be greatly improved as shown in Figs. 1C-D. Fig. 1C is a photomicrograph (using a 50 × oil immersion objective) of a perineal pattern made in 1968. Fig. 1D is the same specimen cleaned in lactic acid in 1974 and remounted in glycerine.

The entire procedure now used to prepare perineal patterns is as follows :

1. Intact *Meloidogyne* females are placed in 45 % lactic acid on a perspex slide and the posterior end cut off with an optical scalpel.

2. Body tissues are removed by lightly brushing the inner surface of the cuticle with a slightly flexible bristle.

3. When all tissues are removed, the cuticle is transferred to a drop of glycerine where it is carefully trimmed so as to be only slightly larger than the perineal pattern.

4. The piece of cuticle with the perineal pattern is then transferred to a drop of glycerine on a microslide. A coverslip is applied and sealed with glyceel.

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