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MARY T. FRANKLIN [1]): *Preparation of posterior cuticular patterns of* Meloidogyne *spp. for identification.*

Since Chitwood (1949) split '*Heterodera marioni*' into several species, examination of the cuticular pattern in the posterior region of the female in the genus *Meloidogyne* has become the chief means of identifying species. The so-called 'perineal pattern' is characteristic of the species, but it is obvious to anyone examining many patterns that there is great variability within species and it is not always possible to identify an individual specimen with certainty. The features forming the pattern are the vulva, anus, phasmids, lateral fields and the surrounding striae. As shown by Triantaphyllou (1960) the cuticular markings are formed partly by striae and partly by folds.

Because nematodes are easily distorted by fixation the common methods of preparation were examined to make sure that the patterns observed were independent of the process employed. A second object was to find the quickest method of getting good preparations, because many specimens of a root-knot nematode population must be examined to allow for within-species variability and the occurrence of populations of mixed species.

Sasser (1954), Taylor, Dropkin & Martin (1955), Goffart (1957) and Triantaphyllou & Sasser (1960) described methods of preparing posterior cuticular patterns. All their specimens were fixed in formaldehyde — 5% for 24 hours or longer, or 2% for at least 3 days (Triantaphyllou & Sasser). No mention was made of the temperature of fixation, but live nematodes put into cold formaldehyde usually become distorted. After fixation the posterior ends of the females were cut off individually and mounted in glycerine or lactophenol. Most nematodes transferred from water or formalin to cold glycerine or lactophenol collapse rapidly, which might affect the cuticular pattern. Taylor et al. chopped infested roots in a homogeniser, sieved out the larger particles and picked the required pieces of nematode cuticle from the residue collected on a 200-mesh sieve. A disadvantage of this method is that young females cannot be separated from egg-laying ones, and identification is based on egg-laying ones. Preparations in which whole nematodes are simply flattened under a coverslip do not allow the pattern to be seen clearly; the minimum of body contents should remain with the cuticle so that examination can be by transmitted light. Taylor et al. advise that specimens should not become dry before fixation.

To examine the effects on posterior cuticular patterns, tomato roots bearing a population of *M. javanica* or (treatments 4 & 7) *M. incognita* were treated as follows.

Freshly dug galled roots were shaken to remove soil, then: —

1. Processed immediately — a) put in water, females removed and dissected, cuticle mounted in cold cotton-blue lactophenol (CBLPh); or b) put into cold CBLPh, females removed, dissected and mounted in it; or c) put into boiling CBLPh, allowed to cool, dissected and mounted as above.
2. Put in a polythene bag, tightly closed, and kept at room temperature for 1 week, then treated as in a), b) or c) above.
3. As 2 but polythene bag kept in refrigerator for 1 week, then treated as in a), b) or c) above.
4. Dried out over radiator for 1 week, then: — a) put in cold 5% formalin for 24 hours, females removed and dissected in cold CBLPh; or b) put in cold CBLPh for 24 hours, females removed and dissected in the same or c) put in boiling CBLPh, cooled in it, females removed and dissected in fresh CBLPh.
5. Put into cold 5% formaldehyde for 2 weeks, then: — a) females removed and dissected in formalin, cuticle in cold CBLPh; or b) roots put into boiling CBLPh, as 4c.
6. Put into 5% formaldehyde at 60-65° C, cooled and left for 2 weeks, then: — a) as 5a); or b) as 5b.
7. Immersed for 42 hours in one of the following, cold, — a) 70% alcohol; or b) glacial acetic acid; or c) formal acetic 4 : 10.

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Liquids replaced by cold CBLPh, left for several days and females dissected in CBLPh.

In all treatments dissection involved cutting off the required piece of cuticle and cleaning the body tissues from it with a nylon bristle under a microscope at a magnification of × 30. For each treatment ten posterior cuticular patterns were mounted on a slide in lactophenol and examined at a magnification of × 1000 under a × 45 fluorite oil-immersion objective. Observations were made on the ease of preparation of the specimens and the condition of the patterns.

*Ease of preparation*

The most difficult specimens to prepare were those fixed in formalin, whether hot or cold (4a, 5a, 6a). Formal acetic (7c) specimens were nearly as difficult. The cuticle was hard and easily torn and the body contents were difficult to remove, the vulva often becoming torn in the process. When transferred to hot cotton-blue lactophenol (5b, 6b), formalin specimens were softened and became easier to process. Dried specimens put into either cold or hot lactophenol swelled and softened overnight and were easily dissected (4b, 4c). Specimens treated with 70% alcohol or glacial acetic acid followed by several days in cotton-blue lactophenol were readily dissected and cleaned (7a, 7b). Preparations of specimens from lots 1, 2 and 3 were all made relatively easily, no difference being observed between fresh and stored material except that fungi grow on roots stored at higher temperatures. Cotton-blue helps in determining the position of the vulva because the tissues around it take up the stain. A satisfactory slide of ten good specimens from fresh or lactophenol material can be made in less than 1 hour.

*Effect of treatment on posterior cuticular pattern*

In examining the patterns note was taken of the striae and whether phasmids could be seen. No distortion or abnormality could be related to any of the treatments. Formalin-fixed specimens with remains of body contents were darkly stained and the striae difficult to see. Phasmids were seen most easily in specimens not fixed in formalin. Of those tested, no method of preparing cuticular patterns appears to influence the characters used for identification.

*Recommended Method*

It is recommended that, for examination of the posterior cuticular patterns of *Meloidogyne,* galled roots should be plunged into boiling 0.1% cotton blue in lactophenol without previous fixation. The roots may be freshly dug or stored for up to 2-3 weeks at about 4° C in a polythene bag after removal of soil. They are best kept very slightly moist as rotting will be more rapid if there is excess water. After cooling in the lactophenol the roots should be transferred to clear lactophenol to prevent excessive staining. They may be stored indefinitely in this. Females should be removed from the roots and dissected in lactophenol. The required piece of cuticle should be cleaned from body contents and mounted in clear or faintly tinted lactophenol. If roots have become dry or have been fixed in one of the usual fixatives, plunging them into boiling lactophenol will make the nematodes suitable for processing.

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