PREPARATION OF NEMATODES FOR EXAMINATION UNDER THE STEREOSCAN ELECTRON MICROSCOPE

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The Cambridge Stereoscan electron microscope facilitates detailed examination of nematode cuticle. Specimens must not be covered and all volatile liquid must be removed. The least distorted specimens were those impregnated with anhydrous glycerol or polyethylene glycol (400) the excess of which was drained from the surface before they were mounted and plated with gold/palladium in a vacuum chamber.

The Cambridge "Stereoscan" electron microscope scans the outer surface of specimens with a focused beam of electrons in an evacuated chamber. A signal due to reflected primary electrons and emitted secondaries is collected and used to modulate the brightness of a cathode ray tube scanned in synchronism with the beam (Oatley, Nixon & Pease, 1965). This gives a picture with great depth of focus. The surface of the sample must be electrically conducting to prevent charge build-up due to the electron beam; resolutions down to 200 A.U. can then be achieved in some cases. The specimen holder used has a 12 mm diameter mounting disc of aluminium alloy. The maximum area of scan was 4 mm² but any part of the mounting area can be examined from almost any angle: this makes it difficult to identify the specimen being examined. As specimens must be exposed to a vacuum of 10⁻⁵ Torr, volatile liquids, mainly water, must be removed during preparation without distorting the cuticle.

Four methods of preparation were tried with different nematodes. (1) The heads and tails of living Meloidogyne females were excised, mounted and air dried. (2) Whole males and females of Heterodera were rapidly frozen in distilled water or sugar solution, by surrounding the tube containing them with a freezing mixture of alcohol and solid carbon dioxide and then drying under vacuum before mounting. (3) Several species of vermiform nematodes were plunged into hot 70% alcohol, slowly transferred by stages to absolute ethyl alcohol and then to butyl acetate. These specimens were mounted and the solvent evaporated at room temperature. (4) The water in several species was replaced by less volatile liquids. After fixing in hot TAF (Courtney, Polley & Miller, 1955), specimens were plunged into hot lactophenol. Distortion was less when, instead of draining and mounting, specimens were first transferred to anhydrous lactic acid to remove all...
traces of water and phenol, for phenol crystallised when the water in specimens evaporated. Alternatively specimens were processed to anhydrous glycerol or polyethylene glycol (400) either by slowly evaporating the water from a 1.5% solution of either compound in water or by the glycerol-ethanol method (Seinhorst, 1959). When polyethylene glycol was used, it replaced the glycerol in the solutions. At 120 × magnification, after air drying, freeze drying or water replacement, the best specimens appeared undistorted and only flattened after alcohol drying e.g. Criconemoides in Fig. 4 *).

The nematodes were mounted on the surface of the aluminium alloy disc with ‘Durofix’ (the recommended adhesive), ‘Glyceel’ or ‘nail varnish’ (nitrocellulose solution in amyl acetate). With practice the nematodes could be mounted quickly leaving the required part exposed before the adhesive dried but too much adhesive or solvent submerged the nematode and obscured the surface. Specimens impregnated with glycerol or lactic acid were flaccid and difficult to attach because the adhesive did not grip the cuticle, but many of these specimens were retained by the surface tension of the fluid draining from them. When specimens were in polyethylene glycol, this left them rigid and dissolved the nail varnish enough to attach the nematodes. After mounting it was necessary to see that the specimens were not covered by adhesive and were correctly orientated. This was difficult on the disc because reflected light had to be used. Later, nematodes were mounted on a cover glass smaller in diameter than the alloy disc, examined with transmitted light and the glass attached to the disc. It was then possible to see and reject distorted or obscured specimens and ensure that stylets or spicules had not retracted during the mounting process.

The surface of the specimens was made electrically conducting by coating in a vacuum chamber with a layer of evaporated metal, gold/palladium mixture. Coating was easy, even when specimens were impregnated with heavy liquid and had wet surfaces. The metal layer was so thin that detail was retained but, as a continuous surface with no ‘shadowing’ is created, it was difficult to detect where dirt or secretions were coated and surface details obscured: e.g. the stoma in Fig. 1.

Cuticular distortion from contraction of the underlying tissues was a common artifact and made interpretation of photographs difficult, but when contraction was slight it emphasised cuticle structure and made features more distinct (Figs. 3, 6 and 8). Too rapid processing of a Heteroderma male to polyethylene glycol caused the head to contract about the cephalic arches (Fig. 3). Fig. 6 shows how slight cuticular distortion and flattening after drying with alcohol emphasised the division of the lateral field and annules of Tylenchorhynchus sp., Heterodera males (Figs. 1 and 9) and the Ecphyadophora sp. (Fig. 5), processed slowly to glycerol, are rounded and little distorted or contracted around underlying structures.

Comparison of the corresponding areas of cuticle in Fig. 9 (glycerol impregnated) and Fig. 12 (freeze dried) shows the distortion from freeze drying. The

*) A list of abbreviations used in the figures can be found at the end of this paper.