M. W. BRZESKI 1): Technique for mounting vulval cones of Heterodera cysts.

The method of mounting vulval cones that was recently described by Hooper (1970), can be simplified. The cyst is cut in water, the vulval cone is cleaned carefully, and then transferred to 95% ethanol. The clearing can be made in clove oil, but excellent results can be also obtained if cheaper castor oil is used. The cleared cones are transferred to a very thin and evenly spread layer of Canada balsam on the slide, arranged in the desired position and the balsam allowed to dry in an oven. The layer of balsam is thinner than the height of the cone. Then a new drop of balsam is added and covered with a cover slip. Supports may be added, but are not essential providing that the first balsam layer to which the cones were sealed was completely dry.

Using this method there has been no difficulty in mounting a number of cones, all in the preferred position, on one slide.


Of the several methods used to prepare nematodes for examination in the scanning electron microscope, all attempt to remove volatile materials (chiefly water) from the nematode without distorting its shape before coating it with a thin layer of metal. Green (1967) found that specimens fixed in TAF (Courtney, Polley & Miller, 1955), processed to glycerol and coated with gold/palladium were least distorted, but they were flaccid, difficult to handle and difficult to fix to the specimen stub; also some species, for example Heterodera larvae, are easily damaged by the electron beam. Wilson (1969) and Ellenby & Wilson (1969) found that vulval regions of Heterodera cysts and stylets of Heterodera larvae could be examined after being dried in air. De Grisse & Lagasse (1969) used a similar method for specimens of Criconematidae and Mononchidae. Hammond (1969) preferred TAF-fixed, freeze-dried specimens of Acesaris to those fixed in formal-acetic-alcohol or Bouin's fixatives. Slight contraction of the tissues beneath the cuticle of freeze-dried specimens increased the prominence of surface warts. Pasternak (1970) used an elaborate method to prepare specimens of Panagrellus; after fixation in cold acrolein or glutaraldehyde, the nematodes were fixed again in cold osmium tetroxide, and then freeze-dried at liquid nitrogen temperatures before being coated with gold. Although they showed fine cuticular details, the surfaces of the specimens were distorted.

Our method allows specimens fixed by the methods standard for light microscopy to be used, sometimes even after nematodes have been stored in fixative for several years. Larvae of Heterodera spp. killed by heat and fixed in either 4% formalin, TAF or formal-acetic 4 : 1 (Seinhorst, 1966), were transferred to 3 cm diameter glass cavity blocks each containing approximately 0.2 ml distilled water. Dirty specimens were first washed by passing through several changes of distilled water. The blocks were placed in a desiccator containing dry acetone and left at room temperature for at least 24 hours. Water in the blocks was slowly replaced with acetone by vapour exchange, and the volume of liquid in each block increased to several times the original amount, so blocks needed a cavity as large as 3 cm diameter. After acetone had replaced the water the specimens seemed undistorted. Specimen stubs were prepared by coating the aluminium stub surface with a thin layer of adhesive made by washing a 20 cm X 2 cm piece of "Lassotape" in 20 ml ether. The ether evaporates leaving a dry adhesive surface and stubs can be prepared in advance of their use. Nematodes were placed individually on the adhesive surface to which they adhered as they touched it. With care, nematodes could be placed so that their heads or tails projected from the stub surface. The acetone evaporates from the nematodes leaving a dry preparation. The stubs were then coated with gold, 200A thick, on a rotating turntable to give even deposition.

The specimens were examined under a Cambridge “Stereoscan” scanning electron microscope, using 5Kv electron gun potential. At 5Kv the beam caused no obvious damage but at larger potentials image quality deteriorated, probably because Heterodera larvae were penetrated by the electron beam. Useful results were obtained with magnifications up to approximately 25,000 X of

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Fig. 1. A — Larva of Mexican cyst nematode, anterior region of body showing lateral field × 1150; B — Larva of *Heterodera cacti*, anterior end showing infolding of lateral field × 750; C — Larva of *H. sacchari*, lip region × 8400; D — Larva of *H. sacchari*, cuticle in region of lateral field × 6000; E — Male of *H. rostochiensis* (pathotype E), entire × 75; F — Male of *H. rostochiensis* (pathotype E), tail × 1150.