A TECHNIQUE FOR STUDYING THE MICRODISTRIBUTION OF NEMATODES IN UNDISTURBED SOIL

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The geographical distribution of nematodes within countries and macrodistribution of nematodes within fields and experimental plots has been extensively studied (Barker & Nusbaum, 1971 ; Heath, Brown & Boag, 1976). The microdistribution of nematodes in undisturbed soil has not been directly investigated although Pitcher (1967) was able to observe trichodorid nematodes around roots in an underground laboratory. Indirect evidence obtained from laboratory experiments also indicated that probably small nematodes live in the water film around soil particles and large nematodes between soil aggregates (Jones, Larbey & Parrott, 1969).

The technique described here for identifying the location of nematodes in undisturbed soil is an adaptation of existing techniques for the preparation of thin soil sections (Jones & Griffiths, 1964 ; Fitzpatrick, 1970 ; Cent & Brewer, 1971 ; Jongerious & Heintzberger, 1975). Aluminium boxes 75 × 55 × 45 mm were used as sample containers, and undisturbed soil samples were collected from naturally occurring soil profiles, and were inoculated with *Rotylenchus robustus* or *Longidorus elongatus* in the laboratory. As soon as possible after collection and inoculation, the samples were frozen and stored at —30° in a deep freeze cabinet.

Following freeze-drying in an Edwards Model EF2 freeze-drying unit, the samples, still in their original sample boxes, were placed in trays and impregnated under vacuum (5 torr) with crystic resin, the viscosity of which was decreased by the addition of 30% styrene monomer (Tab. 1A). After this drip-feed impregnation, the samples were left under vacuum overnight and then removed from the impregnation tank and transferred to a fume cupboard. After two to three weeks (dependent on ambient temperature) gelation and curing of the resin was complete.

The now coherent, resin-impregnated soil blocks were removed from their containers and slices 5 mm thick were cut from each block using a 250 mm diameter diamond saw. Each slice was ground flat on a Jones and Shiplan 540VS surface grinding machine fitted with a 90 mm diameter cup wheel, and lapped on 150 mm diameter diamond-impregnated bronze lapping plates (240 and 320 diamond grit size) mounted on a modified Cutrock LMS200 lapping machine (Robertson & Normington, 1976).

Following ultrasonic cleaning, the prepared slices were mounted on glass plates (108 × 83 × 1.5-1.8 mm) using crystic resin as adhesive (Tab. 1). After curing of the mounting resin (24 hours) the bulk of excess material was removed using the diamond saw. The sections were reduced to a final thickness of 20-25 µm using the surface grinder and diamond lapping plates described above, final thickness being determined by visual examination under the polarising microscope.

The completed thin soil sections were stained with toluene blue, or mounted with a coverslip using crystic resin (Tab. 1B) and 61 × 83 mm No. 0 cover slips, after which they were ready for detailed optical examination.

Table 1

The composition of the styrene monomer and crystic resin

A. Resin mixture for impregnation		
BK	17449 crystic resin	3 000 ml
Z	17453 styrene monomer	1 500 ml
Q	17448 accelerator	7.5 ml
Q	17447 catalyst	15 ml
B. Resin mixture for mounting/cover slipping		
BK	17449 crystic resin	25 ml
Q	17448 accelerator	0.5 ml
Q	17447 catalyst	0.5 ml

Note : These materials are vailable from B. & K. Resins Ltd., Unit 2, Ashgrove Estate, Ashgrove Road, Bromley, Kent, BR1 4TH, UK, who will also advise on safe handling procedures.

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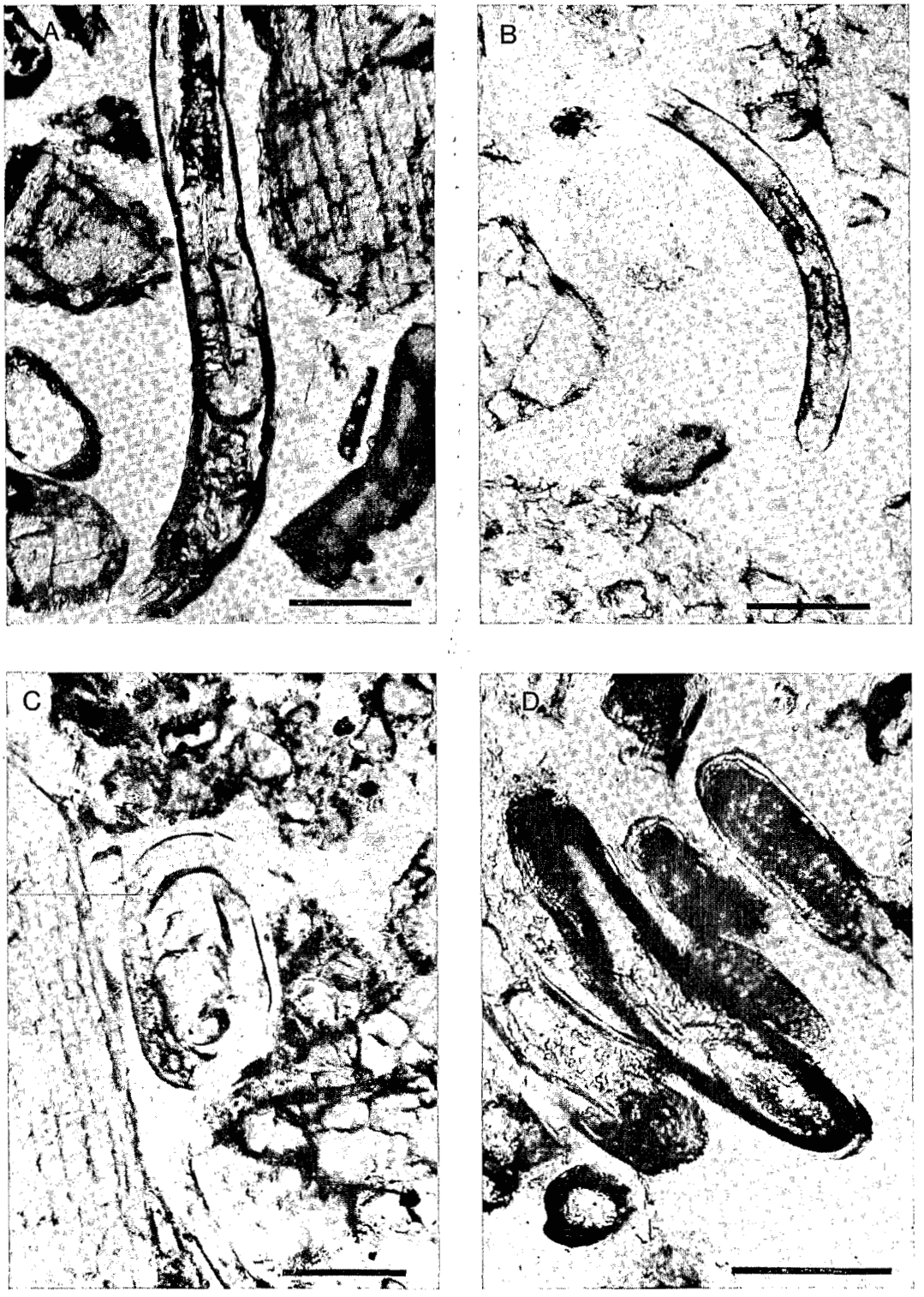

Fig. 1. Sections of soil showing nematodes. A : Oesophageal region of large dorylaimid, Bar represents 100 μm ; B : intestinal region of dorylaimid, Bar represents 10 μm ; C : Coiled *Rotylenchus robustus* alongside root, Bar represents 100 μm ; D : Cross section of *R. robustus*, showing body annules, Bar represents 50 μm.

Nematodes were readily identified when cut obliquely or along their longitudinal axis so that some of their internal organs could be recognised (Fig. 1A). Other nematodes cut in cross section often had their internal organs missing but could still be identified due to the general appearance of the cuticle and its circular form with a diameter corresponding to that of nematodes. In certain situations the width of the nematode annules could be measured and anastomoses observed in the lateral line (Fig. 1D).

This technique could be used to identify the ecological niche occupied by nematodes in different soil types and may help explain why different nematodes species appear to vary in their susceptibility to certain nematicides (Boag, 1979).

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A FURTHER OBSERVATION ON SPERM STRUCTURE IN A *HETERODERA* SP.

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Spermatogenesis and the ultrastructure of sperm in some cyst nematodes has been described by Shepherd, Clark and Kempton (1973). These authors noted differences in the condensation of the nucleus during spermatogenesis between members of the then subgenera *Heterodera (Heterodera)* and *H. (Globodera)*. More recently Behrens (1975) and Mulvey and Stone (1976) proposed that these subgenera *(Globodera* and *Heterodera)* be raised to generic rank and in support cited the differences in sperm development described by Shepherd, Clark and Kempton (1973), and other ultrastructural studies. During their studies of spermatogenesis in cyst nematodes Shepherd *et al.* (unreported) searched

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