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TECHNIQUES FOR MANIPULATING SMALL NEMATODES

BY

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Details of construction and operation are given for some apparatus used to observe and manipulate small soil nematodes under the microscope. An observation cell is simply made from rigid P.V.C. Its open sides allow insertion of microtools and depth can be varied in steps. Glass suction cannulae of 20 to 50 μ aperture, connected to syringes, hold nematodes securely. Finer microcannulae (5 to 10 μ aperture) can be inserted through the cuticle for pressure recording. Microdissection knives are made from plate glass slivers fused or glued to glass handles.

To study internal pressures in some nematodes we developed instruments for micromanipulation that may be of general use.

Observation cell for micromanipulation

A cell was needed in which specimens could be manipulated in water while being viewed and filmed through a compound microscope. The cell had to be shallow enough to fit between a 25X objective and a normal condenser, yet provide inside it ample lateral and vertical clearance for microtools. To allow tools to be inserted it had to be open at the sides yet the openings had to be small to keep evaporation to a minimum. Fig. 1 shows a pattern satisfying these requirements. The material used, "Darvic" rigid PVC¹⁾, is easily worked with wood- or metal-cutting tools. It is water-repellent, so that water in the cell is held between upper and lower glass coverslips and does not readily run from the open sides. Steps of different heights at the front and back of the cell provide alternative supporting ledges for the upper coverslip, so that with a shorter slip the cell is shallower. Both upper and lower coverslips can be gummed in place with a tacky paraffin-vaseline mixture (Doncaster, 1962), but we prefer not to gum the upper coverslip, so that when it is touched inadvertently by the microtools, it moves and the tools are not broken. The upper slip can be made narrower to give more clearance for microtools, or wider to limit evaporation. The cell is also suitable for hanging-drop preparations. Our observation cell was milled from solid Darvic sheet 1 cm thick and the central hole was bored and rebated on a lathe. Alternatively, shaped laminae of thinner Darvic can be bonded together with such adhesives as "Surasolve Solvent Cement"²⁾ or "I.S.-12 Cyanoacrylate Adhesive"³⁾.

1) I.C.I. Ltd., Marketed by G. H. Bloore Ltd., 480 Honeypot Lane, Stanmore, Middlesex, England.

2) Durapipe Fittings Ltd., Winnock Rd., West Drayton, Middx., England.

3) Douglas Kane Group Ltd., Welwyn Garden City, Herts., England.

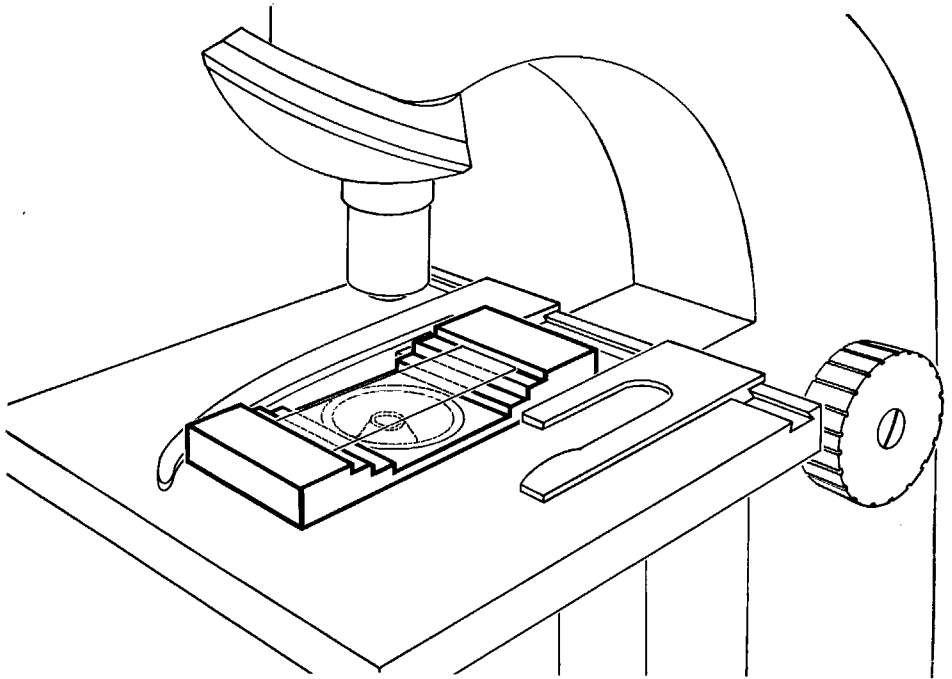


Fig. 1. Microscopical observation cell for micromanipulation. Suggested length, 75 mm; width, 30 mm; lower step height, 3 mm; upper step height, 2 mm.

Microcannulae

Two types of microcannula were used: one to insert into nematodes through the cuticle or stoma in attempts to record internal hydrostatic pressure, the other to hold nematodes securely by suction for observation and experimental procedures.

A piece of "Pyrex" tubing about 15 cm long, 3 mm o.d. and 2 mm bore was heated to dull red over a small gas burner and drawn out by hand to 0.5 mm o.d. The two resulting cannulae were broken apart and the tip of each gently drawn out with forceps while passing it steadily through the flame of a very small gas burner (e.g. a large-bore hypodermic needle clamped upright). The tip was broken off and flamed to produce a holding cannula with an aperture of 20-50 μ (Fig. 2, A & B, right). A holding cannula filled with observation medium (ringer, tap or distilled water) was mounted in a micromanipulator and connected by thin-walled rubber tubing to a fixed, small-bore, smooth-acting hypodermic syringe. Under the microscope the tip of the microcannula was brought close to the subject and suction applied by the syringe, drawing the nematode into or onto the end of the cannula. The rubber tube flattened and exerted a slight, steady suction, which held nematodes placed across the cannula tip in place for minutes or hours. When a nematode had been drawn *into* a cannula, further suction was unnecessary and small syringe adjustments moved the nematode further in or out as required. The

M. K. SEYMOUR & C. C. DONCASTER: *Manipulating small nematodes.*

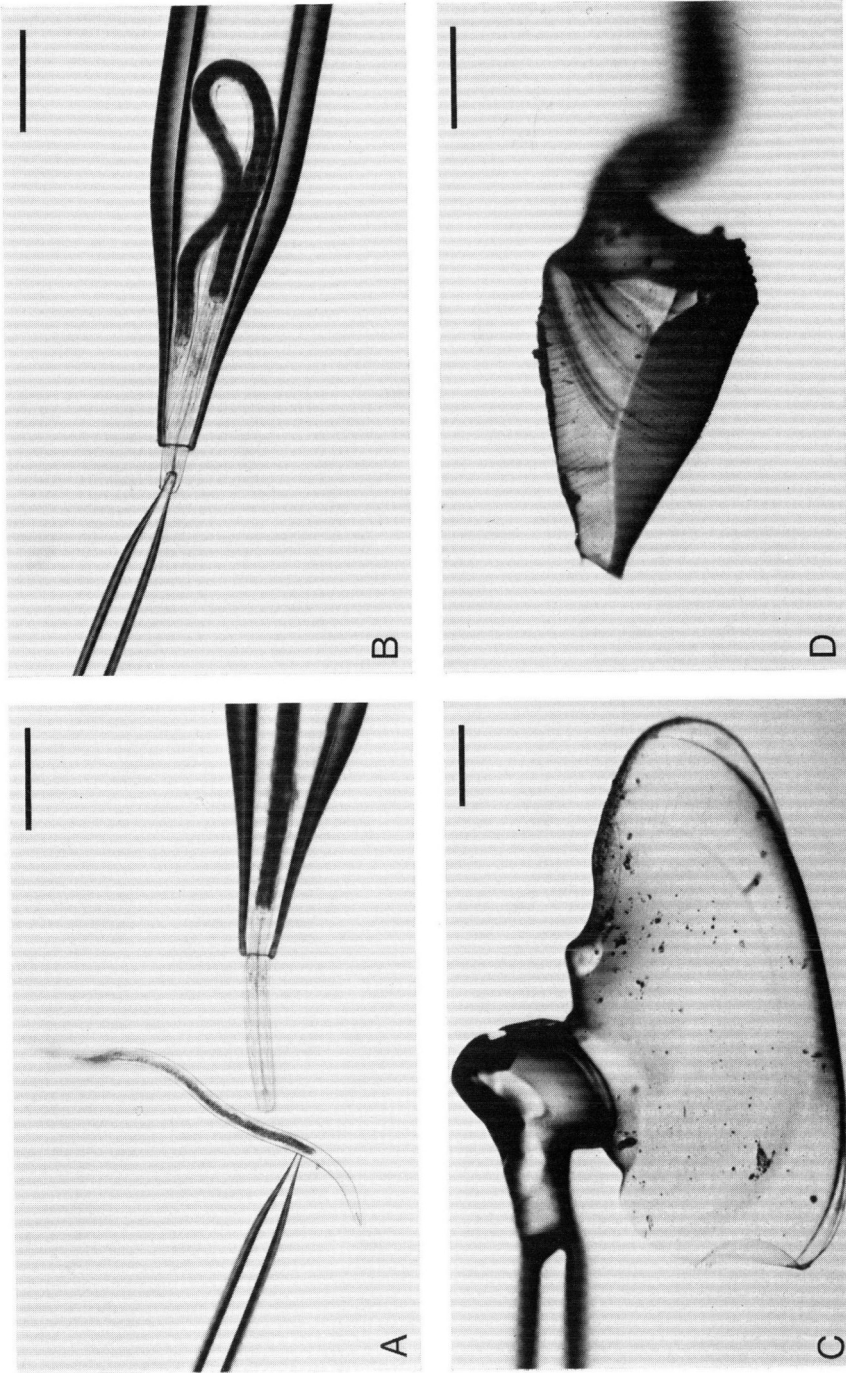


Fig. 2 Nematode holding cannulae arranged for feeding studies. A soil-dwelling mononchid drawn into a large bore cannula, and a tylenchid held across a smaller cannula by suction; B — A mononchid held in a large bore microcannula with a small cannula inserted into the stoma; C & D — Microknives made from slivers of plate glass fused onto glass capillaries. Scale bars represent 0.2 mm.

cannula aperture could be enlarged by breaking off the tip and repolishing in the flame; nematodes of different sizes could thus be accommodated. Fig. 2A shows an arrangement for feeding a mononchid, with the mononch in a wide cannula and a tylenchid, the potential prey, held across a finer one.

Once drawn into a holding cannula, mononchs alternated locomotory lashing with quiescent periods during which they did not respond to touch stimuli. While being held, one swallowed a small stylet-bearing nematode within two min; another attempted to eat a second, larger, mononch.

To construct penetrant cannulae and holders much smaller than 20 μ diameter, the preliminary pulls were made in the same way as for the larger holding cannulae. A further controlled pull, with strong local heating, was then required. For this operation some kind of microforge had to be used (e.g. the De Fonbrune⁴) or the homemade unit described by Thaysen & Morris (1947). After the first two pulls, the drawn-out end was made into a hook for attaching a weight or spring. A light pull (about 5 gm wt) was applied and the bright red heating filament was brought near the glass, which thinned and parted; the filament was then rapidly withdrawn. This left a short, shouldered point tapering to less than 1 μ diameter. The tip was stubbed under water beneath the microscope to enlarge it to a sharp-edged hole of the required diameter (usually 5-10 μ). The abrupt taper minimised capillarity and viscosity effects and ensured maximum strength. Mounted on a micromanipulator and connected by rigid polyethylene, nylon⁵) or thick-walled rubber tube to a micrometer syringe, short penetrant microcannulae could easily be inserted through a nematode's body wall into the pseudocoelom, gut or elsewhere. Microcannulae of this pattern but with heat-polished tips serve as suction holders (Fig. 2A, left) and when placed in a nematode stoma (Fig. 2B) induce a swallowing reaction.

Glass microknives

To make microdissection knives a cleanly cut edge of plate glass was lightly tapped or gripped with pliers and thin slivers broken off onto black paper. Under a dissecting microscope, conchoidal or pointed sharp-edged fragments were selected. A microcannula, as described above (unfinished or broken) served as a handle, to which a sliver was attached in the desired orientation. Blades may be fused to the handles with a microforge or stuck with epoxy resin glue (e.g. "Araldite"). Two large examples are illustrated in Fig. 2, C & D.

ZUSAMMENFASSUNG

Methoden zur Handhabung kleiner Nematoden

Es werden Einzelheiten von Aufbau und Wirkungsweise einiger Apparate mitgeteilt, die für die Beobachtung und Handhabung kleiner Nematoden unter dem Mikroskop benutzt werden. Eine Beobachtungskammer ist einfach aus starrem PVC hergestellt. Ihre offenen Seiten erlauben das

⁴) Agents in Britain: Scientific Techniques Ltd., Reliant Works, Brockham, Betchworth, Surrey, England.

⁵) Portex Plastics Ltd., Hythe, Kent, England.

Anbringen von Mikrowerkzeugen, und ihre Tiefe kann stufenweise verändert werden. Mit Ansaugkanülen aus Glas (20-50 μ Öffnungsweite), die mit Injektionsspritzen verbunden sind, lassen sich Nematoden sicher halten. Feinere Mikrokanülen mit Öffnungen von 5-10 μ können für Druckmessungen durch die Cuticula geführt werden. Mikropräpariermesser werden aus Glassplittern gemacht, die an Glasstäbe angeschmolzen oder angeklebt werden.

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Accepted for publication: February 22, 1972.