ON THE FORMATION AND ULTRASTRUCTURE OF FEEDING TUBES PRODUCED BY TRICHODORID NEMATODES

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Ciné-film analysis, light and electron microscopy showed that the feeding tubes produced by feeding trichodorid nematodes (Trichodorus similis being the species most observed) appear to be initiated within the pharyngeal lumen of the nematode. The region concerned is that part of the cuticular lining that is supported by three 'strengthening rods' embedded in it. At a feeding site, just before the onchiostyle is first protracted for plant cell wall perforation, the anterior end of the strengthened region is closely applied to the wall. As onchiostyle thrusting continues, a secretion from the nematode is released that hardens between the onchiostyle and the lumen lining, forming a tube composed of electron-dense material. With further thrusting, after cell wall perforation, the tube extends in a thin layer through the perforation hole into the cell interior, where it is anchored by a small plug of hardened secretions which forms just inside the cell wall around the perforation.

When as sometimes happens parasitized, dead and empty epidermal cells are punctured again, the onchiostyle is usually thrust deeper and deeper into such cells until it may reach and even perforate the wall of underlying cells. The nematode secretions then harden around the onchiostyle into a growing tube that may extend right into the underlying cells.

Trichodorid nematodes are obligate ectoparasites of plant roots. Generally they attack epidermal cells, but sub-epidermal cells may also be injured, especially when many nematodes feed together. As trichodorids can usually be maintained without difficulty on roots of seedlings in agar culture, several direct observations on their feeding behaviour and mechanisms have been made (cf. recent review by Wyss, 1977). Zuckerman (1961) first noticed that the boundary of the hole made in the cell walls by the onchiostyle of a trichodorid nematode (Paratrichodorus christiei) appeared coated with a refractive substance, 'possibly an exometabolite of the nematode'. Later, Wyss (1971) showed that Trichodorus similis produces a small feeding tube which stays anchored for an indefinite time in the puncture hole in the cell wall. It is now well known that trichodorid nematodes produce feeding tubes which are attached to the cells they attack. Cell response to Trichodorus feeding and the cytology of parasitized cells were described earlier (Wyss, 1974, 1975). We have now studied how the feeding tubes are formed and what their structure is.

MATERIALS AND METHODS

Nematodes (predominantly Trichodorus similis, but occasionally also T. primitivus, T. sparsus and Paratrichodorus pachydermus) were observed feeding on roots of Nicotiana tabacum L. var. 'Samsun' seedlings, using the methods described
earlier (Wyss, 1974, 1975). Information from ciné-film studies on the feeding mechanisms of *T. similis*, made in co-operation with the ‘Institut für den wissenschaftlichen Film’, Göttingen, B.R.D., was also analysed.

For electron microscopy root-tips that were attacked by several nematodes and which showed symptoms of feeding damage were selected. At 2 hourly intervals they were fixed in 3% glutaraldehyde in 0.05 M sodium-cacodylate buffer (pH 6.8), washed in buffer and postfixed in 2% osmium tetroxide in the same buffer, all at room temperature. The root-tips were dehydrated in an acetone series and infiltrated with Spurr’s (1969) low viscosity embedding medium. Ultrathin sections were cut on an LKB Ultratome I with glass knives and mounted on formvar-coated copper grids. The sections were stained for 10 min with 5% aqueous uranyl acetate and then for 15 min with lead citrate. The ultrathin sections were examined in the Elmiskop IA (Siemens) or in the EM 10A (Zeiss).

**RESULTS**

*Formation of the feeding tubes*

Light microscopic observations at high magnification and ciné-film analysis of trichodorid nematodes feeding showed that a specialised region of the pharynx is responsible for moulding the feeding tubes they produce. This is the region where the cuticular lining contains three strengthening rods, described by Raski *et al.* (1969) and Hooper (1975). These rods are clearly visible with the light microscope as refractive structures and indicate the position of that section of the lumen lining at any time. At the beginning of a feeding cycle, when the nematode is exploring possible feeding sites by lip-rubbing along the cell wall, the strengthening rods are still positioned behind the oral aperture (Fig. 1). Having selected a suitable site, the strengthened region is drawn forward so that the tips of the rods appear to be touching the surface of the cell wall to be penetrated. Once contact is made in this way, the onchiosyle is immediately thrust at the cell wall at a rapid rate. Cell wall perforation usually takes less than a minute for *T. similis*. If this species, the one most observed, stopped thrusting after about 20 sec, before perforation appeared completed, it drew back the strengthened cuticle into the stomodeum and left the cell. Then a small tube, 1-2 μm long could be seen adhering to the cell wall at the point of attack. This suggests that the tube is formed by secretions, released by the nematode through the pharyngeal lumen, which harden rapidly around that part of the lumen where the lining is stiffened by the strengthening rods.

If penetration occurs and feeding is continued, it culminates in the ingestion of aggregated cytoplasm after a relatively long period of salivation (which in *T. similis* can last up to approximately 3 min). In these circumstances, a feeding tube is formed that is as long as the strengthening rods and which ends in a small knob-like plug inside the host cell just beneath the perforation in the wall (Fig. 1).

Sometimes a nematode repeatedly tries to feed from an epidermal cell or root hair from which it has previously fed. As the remaining cytoplasm in an attacked