HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES IN CEREAL ROOTS CAUSED BY FEEDING OF HELICOXYLENCHUS SPP.

BY

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Parasitism of cereal roots by Helicotylenchus spp. induced two types of lesion, a cortical necrosis in which nematodes physically disrupted cells allowing contamination by secondary micro-organisms, and a small feeding lesion, of up to five cells, around a central food cell in which both the cytoplasm and numbers of cell organelles had increased. Lipid droplets and proteinaceous deposits occurred in food cells. Feeding lesions were usually adjacent to protoxylem cells.

The stylet pierced the food cell and a droplet, assumed to be the dorsal gland exudate, enveloped the stylet tip. The droplet was structureless but whorls of rough endoplasmic reticulum connected the droplet and the cytoplasm.

The functioning of the food cell, and the role of the gland exudate are discussed, and structural changes are compared with those of sedentary feeding plant-parasitic nematodes.

Helicotylenchus spp. have been recorded as causing a superficial bulb-shaped necrosis in the cortex of host roots (Davis & Jenkins, 1960; Taylor, 1961; Stover, 1972; Orbin, 1973), where cell contents were dispersed and walls ruptured. Orbin (1973) observed that soybean roots became lignified. Yeates (1971) attributed the formation of such a necrosis to browsing feeding.

Perry et al (1959) stated that H. digonicus fed preferentially from xylem and phloem cells of lima-bean and Poa pratensis causing cytoplasmic coagulation. Jones (1978) observed that H. dibystera and H. varicaudatus fed as sedentary parasites from one cell and while feeding, females laid many eggs.

The histopathology of cereal roots parasitised by H. digonicus, H. pseudorobustus, and H. varicaudatus and the ultrastructural changes of wheat roots attacked by H. dibystera were studied.

MATERIALS AND METHODS

Pots, containing 100 ml of soil infested with either H. digonicus, H. pseudorobustus or H. varicaudatus were sown with two varieties of barley, wheat or oats. After one month, washed roots were fixed in boiling F.A.A. and segments with nematodes were embedded in paraplast and sectioned at 8 μm (Feder & O'Brien 1968). Sections were stained in Weighert's haematoxylin.

Wheat roots, grown for one month in similar pots containing soil infested with H. dibystera were washed and examined in 3% gluteraldehyde in 0.025% phosphate

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buffer (pH 6.8). Segments with nematodes were transferred to fresh fixative. These roots, post fixed in OsO4, dehydrated in acetone, were embedded in low viscosity resin (Spurr, 1969). Light microscope sections, cut at 0.5 μm were stained in toluidine blue in borax, and sections, cut for examination on a Philips 300 electron microscope, were stained in uranyl acetate and lead citrate.

Root segments of each host with each species of nematode were stained in 0.1% cotton blue in lactophenol, cleared and mounted on slides in lactophenol.

RESULTS

Cotton Blue stained roots

All roots, except the root tip, were invaded by larval and adult nematodes that had either partly or fully entered the roots (Fig. 1a, b). Egg clusters (Fig. 1c) and groups of hatched larvae occurred around female nematodes, slightly swelling the root. A blue-stained lesion surrounded the nematodes. The head of feeding nematodes was adjacent to food cells, which were filled with unstained globules.

Root sections

Nematodes fed from either pericyclic (Fig. 2b, d), endodermal (Fig. 1f) or cortical cells adjacent to endodermal passage cells (Fig. 2a), but non-feeding nematodes and the posterior of feeding nematodes lay coiled in superficial cortical cells, forming a cavity in the cortex.

Some nematodes, having penetrated cell walls, had their bodies constricted by the small wall openings (Fig. 1d). The walls of penetrated cells were lined by a dense deposit and in paraplast sections this deposit resembled a thickening of the cell wall (Fig. 1e). In wheat and barley, the necrosis was usually restricted to superficial cortical cells, but in oat roots, cells around the nematode's head were also necrotic.

The nematode's stylet penetrated the cytoplasm of food cells (Fig. 2c, d) each of which was surrounded by four or five cells with an enlarged cytoplasm. In cortical (Fig. 2a) and endodermal (Fig. 1f) food cells this enlargement was not as pronounced as in pericyclic food cells (Fig. 1e, 2b, c, d) but the feeding lesion associated with the former food cells included adjacent pericyclic and protoxylem cells (Fig. 1f, 2a). The lesion around pericyclic food cells included only adjacent protoxylem cells that contained a dense structureless material. Occasionally an enlarged pericyclic food cell lay between other unaltered pericyclic cells (Fig. 2b).

A knob-like wall thickening occurred around the nematode's stylet (Fig. 2a, c), caused by a localised enlargement of the lignin layer of food cells. In cortical cells (Fig. 3a) lignin was also deposited along the outer tangential cell wall.

The enlarged cytoplasm of food cells of H. dihystera contained numerous mitochondria, plastids, amyloplast-like organelles and rough endoplasmic reticulum (RER) (Fig. 3b, c). The central vacuole of pericyclic cells had collapsed, but numerous small vacuoles occurred.