ULTRASTRUCTURE OF INITIAL RESPONSE OF GRAMINACEOUS ROOTS TO INFECTION BY HETERODERA AVENAE

BY

T. BLEVE-ZACHEO1, M. T. MELILLO1, M. ANDRES2, G. ZACHEO1 and M. D. ROMERO2)

1) Istituto di Nematologia Agraria, CNR, Amendola 165/A, 70126 Bari, Italy; 2) Centro de Ciencias Medioambientales, Serrano 115, Madrid, Spain

Ultrastructural changes induced by two pathotypes, Ha22 and Ha71 (Spanish populations), of the cereal cyst nematode *Heterodera avenae* on wheat cv. Anza and on oat cv. Cometa are described from 24 h to 5 days after nematode inoculation. Pathotype Ha22 selected as a feeding site the stele parenchyma cells of wheat roots, which responded with extensive hypertrophy, increase in cellular organelles and formation of elaborate ingrowths of the cell walls adjacent to xylem tracheal elements as seen in a 5 day-old syncytium. On the same host, pathotype Ha71 fed upon xylem parenchyma cells that reacted early (24 h) with callose-like and osmiophilic granule deposits on their walls, leading to the degradation of the induced syncytium within 5 days. Feeding of both pathotypes on oat roots induced a hypersensitive host reaction, characterized by extensive necrosis of the endodermis and pericycle. Pathotype Ha22 induced small syncytia in the stele where cellular response was expressed through involution of the cytoplasmic content, indicating a lack of nutrients from plant to nematode. Pathotype Ha71 did not form syncytia in the stelar tissue and fed only on cortical cells, which were transformed into secretory transfer-like cells.

Keywords: cereal cyst nematode, host-parasite relations, wheat, oat, resistance

In describing the development of syncytia in root tissues of resistant and susceptible wheat infected by *Heterodera avenae*, Grymazsewska & Golinowski (1991) emphasized that they were initiated earlier in susceptible than in resistant tissues. In susceptible roots syncytia commenced their development in the root apices and were mostly composed of parenchymatic cells adjacent to the tracheal elements, whereas in resistant roots they were initiated exclusively in the maturation zone, involving fewer cells with less immediate contact with the vascular tissue. However, the ultrastructural modifications in response to *H. avenae* penetration into the roots of Gramineae and cytological responses to different pathotypes have not yet been described.

During a morphometric study and biochemical analysis of proteins to characterize pathotypes of cereal cyst nematodes from Spanish populations, two pathotypes of *H. avenae*, Ha22 and Ha71, were differentiated; one is close to the pathotypes British Ha11 and Ha12 as well as Swedish Sw1 and the other similar to but clearly separate from British Ha23 (Valdeolivas & Romero, 1990). These observations prompted us to study the initial stage of infection of the two pathotypes and their relation to cytological modifications of host tissues in a cultivar of wheat. Furthermore, we examined the structural changes in oat roots resistant to *H. avenae*, with a view to elucidating the morphological and...
ultrastructural alterations associated with resistance in this host-nematode interaction.

MATERIALS AND METHODS

Cysts of _H. avenae_ pathotypes Ha$_{22}$ and Ha$_{71}$ (Spanish populations) were placed in wheat root diffusate at 10°C and second-stage juveniles (J$_2$) were collected about one month later.

Seeds of wheat cv. Anza and oat cv. Cometa were germinated under sterile conditions. When root initials appeared, each seedling was transplanted into clay pots containing 10 ml of sterilized sand and inoculated with 60 J$_2$ of pathotype Ha$_{22}$ or Ha$_{71}$. The plants were maintained in growth chambers at 17°C with a 14 h photoperiod at 5000 lux and relative humidity of 70%. Twenty-four hours after nematode inoculation, the seedlings were washed to remove J$_2$ that had not penetrated the roots. The pots were filled with fresh sterilized sand and the plants were grown under the same conditions until they were examined.

Segments of infected roots were excised 24 and 48 h and 3 and 5 days after inoculation and fixed in buffered 3% glutaraldehyde (0.05 M sodium cacodylate buffer, pH 7.2) at 4°C for 4 h; rinsed in the same buffer and post-fixed in 2% osmium tetroxide in the same buffer for 4 h at 4°C; dehydrated in an alcohol series and infiltrated with a low viscosity medium (Spurr, 1969). Approximately 10 segments of each sample were sectioned. Sections, 2 μm thick, were cut with a LKB ultratome IV, stained with toluidine blue and observed under a light microscope to verify the syncytial location. Ultrathin sections were cut in that area and stained with 3% aqueous uranyl acetate and then with lead citrate and viewed in a Philips 400 T electron microscope.

RESULTS

Microscopic evidence indicated that one of the first changes in host roots in the _H. avenae_-wheat interaction is the swelling of the root apices 24 h after nematode inoculation. In wheat at 24 h, J$_2$ of both pathotypes, Ha$_{22}$ and Ha$_{71}$, became sedentary and started to induce a syncytium in the meristematic tissue. Root swellings resembled those induced by _Meloidogyne_ spp. in susceptible hosts (Fig. 1A) but they showed only a hypertrophic response and an early cell vacuolization (Fig. 1A) adjacent to cells selected as a feeding site (Fig. 1B). Within 5 days, syncytia induced by both pathotype infections were greatly developed in the vascular cylinder of wheat roots (Fig. 1C, D) and emergence of initials close to the syncytia had occurred (Fig. 1D). The new roots were formed at various sites along the infected root, behind the terminal swelling. Nevertheless, the swollen tip remained functional and further elongation was not impeded.