

Istituto di Nematologia Agraria, C.N.R., 70126 Bari, Italy

ULTRASTRUCTURAL ASPECTS
OF THE HYPERSENSITIVE REACTION
IN TOMATO ROOT CELLS RESISTANT TO
MELOIDOGYNE INCOGNITA

by

T. BLEVE-ZACHEO, G. ZACHEO, M. T. MELILLO and F. LAMBERTI (1)

Juveniles of the root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw., enter the roots of resistant or susceptible plants in the same numbers (Reynolds *et al.*, 1970). In the tomato cv. Hawaii cells around the anterior end of root-knot nematodes were killed within 24 hours of invasion of the roots (Riggs and Winstead, 1959).

Rapid death of the cells isolates the nematode and injury is thus confined to a few cells (Rohde, 1972). This incompatible host-parasite interaction (hypersensitive reaction) has been reported as a common type of response in resistant plants for a wide variety of pathogens (Riggs and Winstead, 1959; Rohde, 1965; Klement and Goodman, 1967; Loebenstein, 1972; Brown, 1978; Politis and Goodman, 1978; Heath, 1980 and 1981).

Ultrastructural changes that occur during the development of a hypersensitive reaction (HR) in tomato cv. Nematex, resistant to *M. incognita*, have been described by Paulson and Webster (1972). However, the mechanism by which resistant plants starve parasitic nematodes and prevent their development remains unknown. This study was undertaken to obtain additional data on the ultrastructural changes in response to penetration of *M. incognita* in the roots of tomato cv. Brech, resistant to this nematode. Histochemical tests were also made to investigate the nature of the material occurring

(1) The authors thank Mr. R. Lerario for assistance with photography.

between the cell wall and plasmalemma in cells surrounding the necrosis.

Materials and methods

Tomato cv Brech seedlings were grown in 5 cm diameter plastic pots containing sterilized quartz sand. Each of the pots was inoculated with 100 second stage juveniles of *M. incognita* and placed in a growth chamber (22°C, 65% RH, 3,000 lux). Two days after inoculation the roots were removed and those apices, which appeared to be damaged under a stereomicroscope, were excised, fixed for 4 hours in 3% glutaraldehyde at 4°C, rinsed overnight in 0.05 M cacodylic acid buffered at pH 7.2, stained 12 hours at 4°C in 0.5 M uranyl acetate, and postfixed 6 hours in 2% osmium tetroxide. They were then dehydrated in a graded ethanol series and embedded in Spurr's medium.

Ultrathin and 2 μ m thick sections were made for electron microscopy and histochemical studies respectively. To detect callose, the sections were immersed for 2 hours in a NaOH methanol solution, which was changed repeatedly to eliminate the resin, and stained with an aqueous solution of 0.05% aniline blue in 0.067 M Na₃PO₄ buffer (Peterson *et al.*, 1978).

An examination for autofluorescence was made on sections treated as previously described, but without aniline blue treatment. The sections were examined with a Leitz Dialux 20 EB microscope with fluorescent light and exciter filter KP 490 and barrier filter TK 510/K 515.

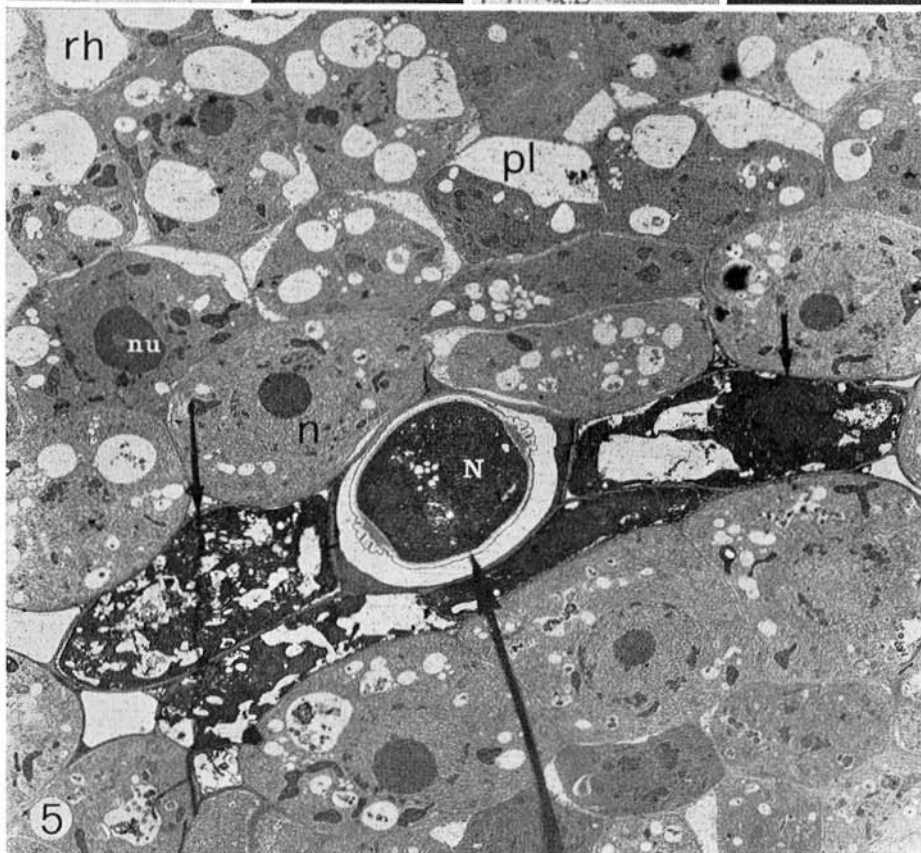
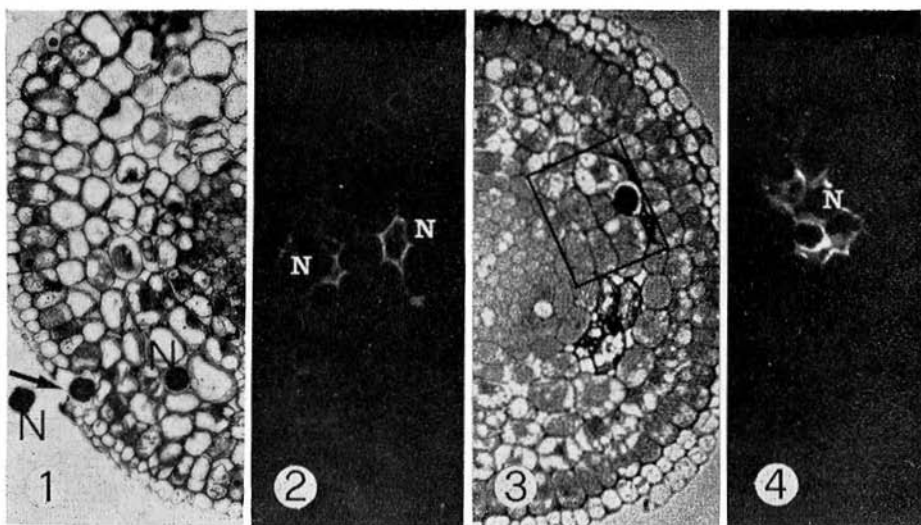
Fig. 1. - Juveniles of *Meloidogyne incognita* in a root section of tomato cv. Brech, resistant to the nematode: nematodes are visible in the third layer of cortical cells, in the cortex (arrow) and near the rhizodermis (x105).

Fig. 2. - Section on the same cells of Fig. 1 stained with aniline blue and observed under fluorescence; fluorescence occurred in the cell walls surrounding two nematodes (x160).

Fig. 3. - Tomato root section with a nematode entrapped by four necrotic cells (arrow) and another nematode in an intercellular space (square) (x100).

Fig. 4. - Detail of a section on the cells of Fig. 3 (square), stained with aniline blue; fluorescence occurred in the wall of the non-necrotic cells close to the nematode (N) (x160).

Fig. 5. - Electron micrograph of part of section in fig. 3. Note a nematode in an intercellular space (arrow) and the necrosis of four cells surrounding the nematode; cells surrounding the necrotic area appear normal while the three cell layers behind the rhizodermis show plasmolysis (false plasmolysis) (x2,300).



Ultrathin sections, cut on an LKB ultratome III, were stained in uranyl acetate and lead citrate (Reynolds, 1963) and examined at 60-80 Kv in a JEM 100 B electron microscope.

Results

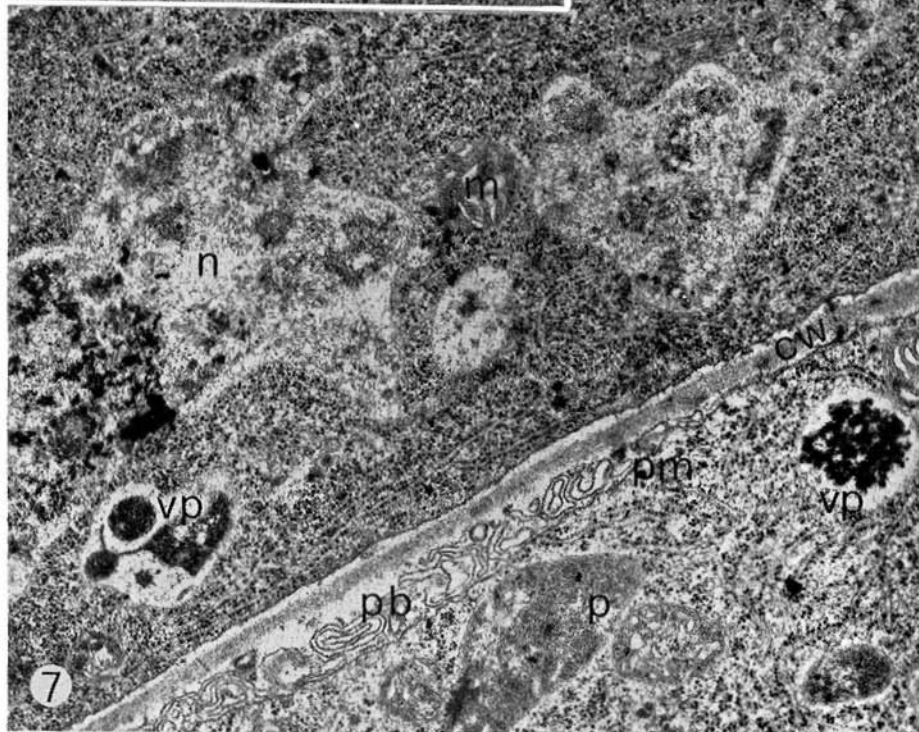
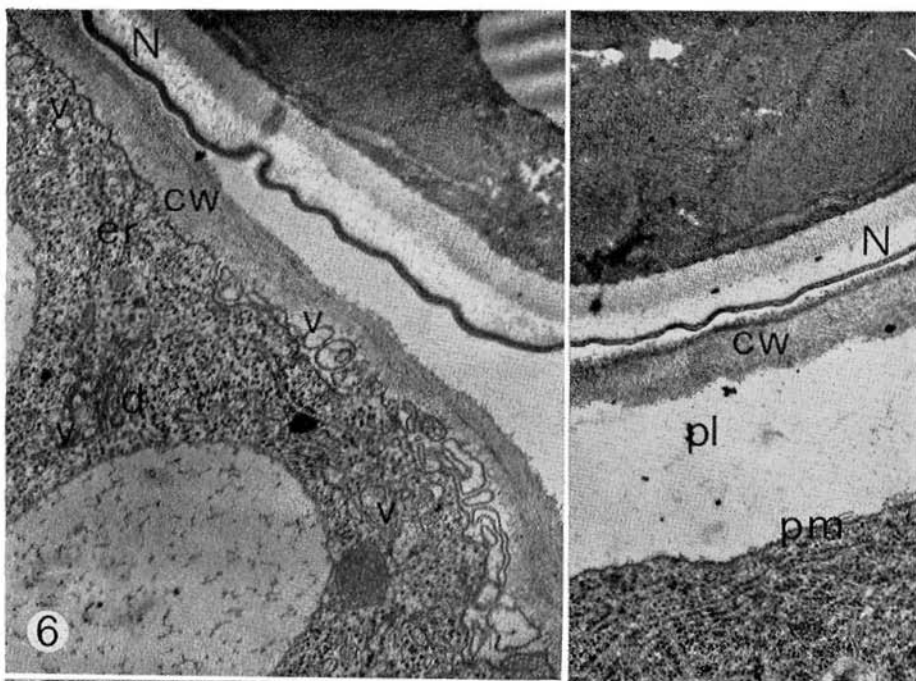
The sections made from the root apices showed that 48 hours after inoculation, penetration of juveniles of *M. incognita* had already occurred at various levels in the cortical cells (Fig. 1). The mechanical and enzymatic effect of penetration caused the breakings of cell walls in the rhizodermis (Fig. 1, arrow). The damage was generally limited, however as root-knot nematodes move through intercellular spaces when invading their host (Dropkin *et al.*, 1969). Once established, the juveniles became sedentary and started their trophic activity. In the resistant roots the cells surrounding the nematode head became disorganised and necrotic (Fig. 3), and the response of giant cell formation did not occur.

Electron microscopy examination of sections of the area of nematode invasion, where cells are differentiating, showed a juvenile in an intercellular space, behind the third layer of cortical cells (Fig. 5). Four necrotic cells, surrounding the nematode head (Fig. 5) seemed to trap it. These cells showed, under the light microscope, high affinity with the stain, while under the electron microscope their cytoplasm appeared as electron dense material in which it was not possible to distinguish the organelles (Fig. 5). Several layers of cells around the necrosis were plasmolysed, which Wheeler (1975) described as false plasmolysis due to the action of pathogens (Figs 5 and 7).

The activity in the cells defining the necrotic area was remarkably intense. Rough endoplasmic reticulum was abundant and associated with numerous ribosomes in the surrounding cytoplasm. Also, parts of the reticulum were enlarged and the Golgi apparatus showed pro-

Fig. 6.- Electron micrograph of a cell adjacent to the nematode showing intense cytoplasmic activity; light vesicles, similar to those present in the cytoplasm are visible between cell wall and plasmalemma; the enlarged endoplasmic reticulum, full of electron light material, and a dictyosome, with electron dense vesicles, are also visible (x 40,000).

Fig. 7. - Electron micrograph with two cells showing intense cytoplasmic activity; note the false plasmolysis in the cell next to the nematode, a large paramural body in the successive cell and vesicles with marked accumulation of protein bodies (x 38,000).



liferation of numerous light vesicles of various sizes. The mitochondria had dense matrices and dilated cristae (Figs 8 and 9). Numerous protein bodies (Fig. 7) were present in the vacuoles (Matile, 1975) and various modifications of the cell wall-plasmalemma interface were observed in these cells. The cell wall was thickened and the plasmalemma disconnected (Fig. 10). Large paramural bodies were located between the cell wall and the plasmalemma (Figs 6 and 7); these consisted of vesicles of various shapes and of a different density to the electrons. Vesicles were sometimes observed similar in appearance to those found in the cytoplasm and to those originating from the Golgi apparatus (Figs 6 and 8). Deposition of translucent material between the cell wall and the plasmalemma extended along the whole surface of the cell wall delimiting the necrotic area (Fig. 10). Staining with aniline blue revealed a marked fluorescence in the walls of these cells. These are thought to be areas of callose deposition which are restricted and closely associated with site of nematode feeding (Figs 2 and 4).

Discussion

The resistant tomato cv. Brech quickly responded to invasion by *M. incognita* juveniles by the necrosis of cells, and the formation of paramural bodies and callose areas in the vicinity of the feeding site of the nematode. This kind of reaction, in which a limited number

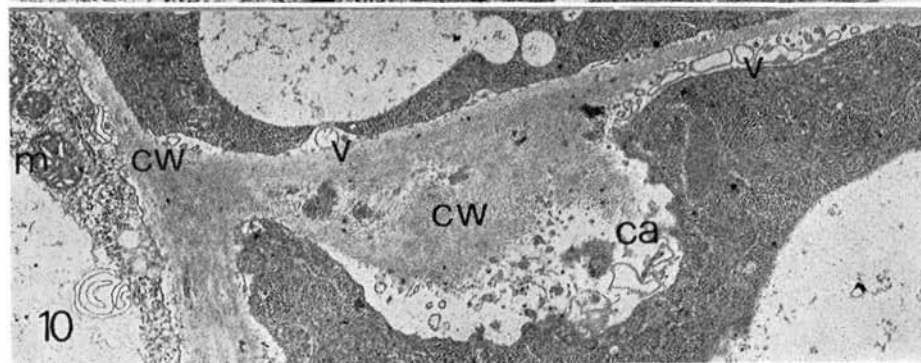
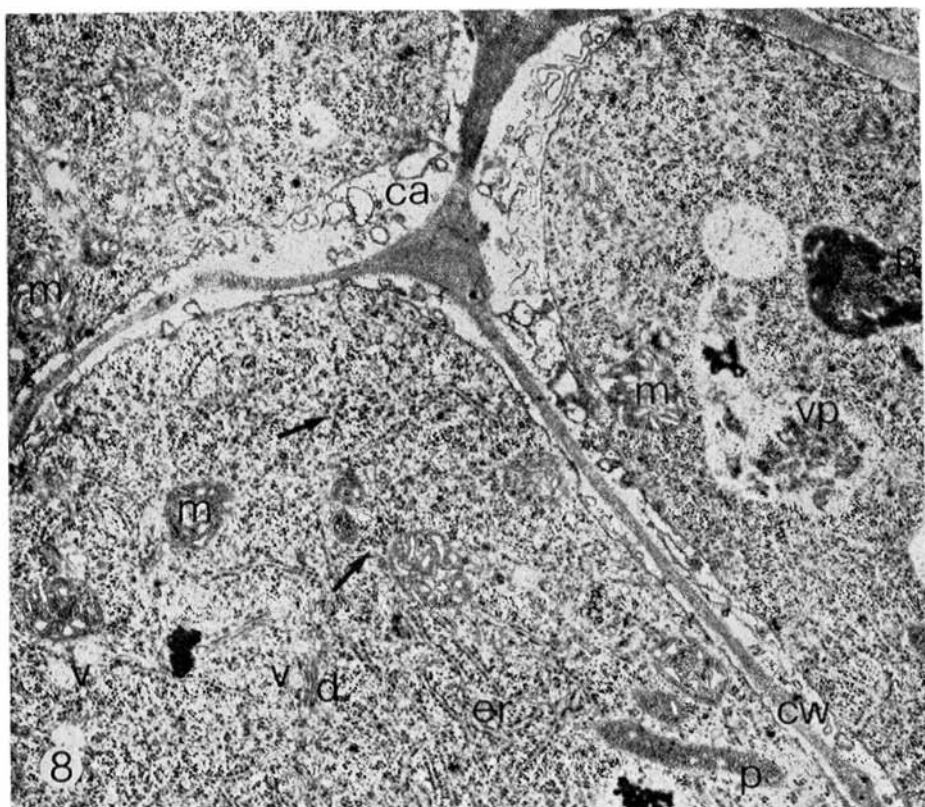
Fig. 8. - Electron micrograph showing callose areas on the whole surface of the cell walls; note the numerous polysomes (arrow), the large quantity of endoplasmic reticulum and several vesicles around the dictyosomes; the mitochondria show enlarged cristae in a dense matrix (x 21,000).

Fig. 9. - Endoplasmic reticulum in active synthesis: accumulation of electron light material is visible between the membranes; note the process of vesicle formation in a portion of the endoplasmic reticulum (x 35,000).

Fig. 10. - Cell wall growths and callose areas: numerous vesicles of various shape are present on both sides of the cell wall (x 16,000).

LIST OF ABBREVIATIONS USED IN THE FIGURES

ca = callose; cw = cell wall; d = dictyosome; er = endoplasmic reticulum; m = mitochondrion; N = nematode; n = nucleus; nu = nucleolus; p = plastid; pb = paramural body; pl = plasmolysis; pm = plasma membrane; rh = rhizodermis; s = starch grain; v = vesicle; vp = vacuolar protein body.



of cells is rapidly killed, is called a hypersensitive reaction (Wallace, 1973; Wheeler, 1975).

It was difficult to establish the exact interval between the penetration of the nematode and the initiation of hypersensitive reaction. However, it seemed evident that this process began when the nematode became sedentary and inserted its stylet into the cells in an attempt to feed. Within 48 hours after nematode inoculation there were completely disorganized cells containing only the remains of the pre-existing structures. These together with degenerative processes, such as development of fluorescence and paramural bodies on the wall of cells surrounding the necrotic area, indicated that the mechanism that leads to the hypersensitivity had already developed. Possibly the hypersensitive reaction takes place quickly and no further cellular changes occur.

A rapid exchange of messages, between host and parasite, takes place at the time the nematode induces mechanical and chemical stimuli in the tissue and, to these messages, the plant responds with drastic changes in those cells which are normally fed on by the nematode.

Paulson and Webster (1972) observed the transfer of material from vacuoles to cytoplasm, resulting in disorder in the cytoplasm. They interpreted this phenomenon as a mechanism to prevent formation of giant cells.

The apposition of callose on the wall of cells surrounding the necrotic areas was considered to be peculiar to the hypersensitive reaction induced by viruses (Moore and Stone, 1972; Pennazio *et al.*, 1978). However, it is known that formation of callose takes place as a quick response of the plant cell after wounding caused by pathogens or by mechanical agents (Lipetz, 1970; Aist, 1976; Pennazio *et al.*, 1981).

Therefore, the presence of callose around the site of penetration of the nematode might be considered as a response of the tissue to stylet piercing or to the movements of the nematode itself, and the restricted extension of the callose areas might be attributed to the short time that elapsed between nematode penetration and examination of the tissues.

It has been suggested that callose accumulation during development of the hypersensitive reaction is a way of physiologically isolating cells from their neighbours to minimize electrolyte losses (Wheeler, 1974). A close relationship has been found between electro-

lyte losses and formation of callose in the cells of leaves of *Gomphrena globosa* after the development of the hypersensitive reaction (Weststeyn, 1978; Appiano *et al.*, 1981).

Callose deposition would thus constitute a barrier against indiscriminate losses of electrolytes from the cells attacked by pathogens. The process of callose deposition as yet is not well understood; nevertheless our results and those of other authors (Matile, 1975; Politis and Goodman, 1978; Bleve-Zacheo *et al.*, unpublished) seem to suggest that the Golgi apparatus and the enlarged endoplasmic reticulum produce numerous vesicles which migrate behind the plasmalemma. It is possible that these vesicles transport the precursor of callose and the enzymes necessary for the process of deposition on to the cell walls.

SUMMARY

The hypersensitive reaction was studied on root apices of tomato roots cv. Brech resistant to *Meloidogyne incognita*. Thin and ultrathin sections taken 48 hours after inoculation of the nematode were observed in a fluorescent light microscope and in an electron microscope. The most relevant ultrastructural changes were restricted to cells close to the nematode. The hypersensitive reaction consisted of the production of a few necrotic cells and in the suppression of giant cells. The cells surrounding the necrotic area showed a marked production of vesicles by the endoplasmic reticulum and the presence of numerous paramural bodies and large areas of electron dense material on the cell walls. Staining with aniline blue revealed fluorescent areas of callose in those cells. Callose deposit are considered to be a quick response to injury caused by nematode penetration.

LITERATURE CITED

- AIST J.R., 1976, Papillae and related wound plugs of plant cells. *Ann. Rev. Phytopathol.*, 14: 145-163.
- BROWN G.E., 1978, Hypersensitive response of orange-colored Robinson tangerines to *Colletotrichum gloeosporioides* after ethylene treatment. *Phytopathology*, 68: 700-706.
- DROPKIN V.H., HELGESON J.P. and UPPER C.D., 1969, The hypersensitive reaction of tomatoes resistant to *Meloidogyne incognita*: reversal of cytokinins. *J. Nematol.*, 1: 55-61.
- HEATH M.C., 1980, Reaction of nonsusceptible to fungal pathogens. *Ann. Rev. Phytopathol.*, 18: 211-236.

- HEATH M.C., 1981, Nonhost resistance. In: Plant Disease Control. Resistance and Susceptibility. (R.C. Staples and G.H. Toenniessen eds) J. Wiley & Sons, N.Y., Chichester, Brisbane, Toronto, pp. 201-217.
- KLEMENT Z. and GOODMAN R.N., 1967, The hypersensitive reaction to infection by bacterial plant pathogens. *Ann. Rev. Phytopathol.*, 5: 17-44.
- LIPETZ J., 1970, Wound-healing in higher plants. *Intern. Rev. Cytol.*, 27: 1-28.
- LOEBENSTEIN G., 1972, Localization and induced resistance in virus-infected plants. *Ann. Rev. Phytopathol.*, 10: 177-206.
- MATILE PH., 1975, The Lytic Compartment of Plant Cells. Springer-Verlag, Wien, N.Y., pp. 183.
- MOORE A.E. and STONE B.A., 1972, Effect of infection with TMV and other viruses on the level of B-1, 3-glucan hydrolase in leaves of *Nicotiana glutinosa*. *Virology*, 50: 791-798.
- PAULSON R.E. and WEBSTER J.M., 1972, Ultrastructure of the hypersensitive reaction in roots of tomato, *Lycopersicon esculentum* L., to infection by the root-knot nematode, *Meloidogyne incognita*. *Physiol. Plant Pathol.*, 2: 227-234.
- PENNAZIO S., D'AGOSTINO G., APPIANO A. and REDOLFI P., 1978, Ultrastructure and histochemistry of the resistant tissue surrounding lesions of tomato bushy stunt virus in *Gomphrena globosa* leaves. *Physiol. Plant. Pathol.*, 13: 165-171.
- PENNAZIO S., REDOLFI P. and SAPETTI C., 1981, Callose formation and permeability changes during the partly localized reaction of *Gomphrena globosa* to potato virus X. *Phytopath.*, Z., 100: 172-181.
- PETERSON R.L., HERSEY R.E. and BRISSON J.D., 1978, Embedding softened herbarium material in Spurr's resin for histological studies. *Stain technol.*, 53: 1-9.
- POLITIS D.J. and GOODMAN R.N., 1978, Localized cell wall appositions: incompatible response of tobacco leaf cells to *Pseudomonas pisi*. *Phytopathology*, 68: 309-316.
- REYNOLDS E.S., 1963, The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.*, 17: 208-212.
- REYNOLDS H.W., CARTER W.W. and O'BANNON J.H., 1970, Symptomless resistance of alfalfa to *Meloidogyne incognita acrita*. *J. Nematol.*, 2: 131-134.
- RIGGS R.D. and WINSTEAD N.N., 1959, Studies on resistance in tomato to root-knot nematodes and on the occurrence of pathogenic biotypes. *Phytopathology*, 49: 716-724.
- ROHDE R.A., 1965, The nature of resistance in plants to nematodes. *Phytopathology*, 55: 1159-1162.
- ROHDE R.A., 1972, Expression of resistance in plants to nematodes. *Ann. Rev. Phytopathol.*, 10: 233-252.
- WALLACE H.R., 1973, Nematode Ecology and Plant Disease. Edward Arnold, London, pp. 228.
- WESTSTEYN E.A., 1978, Permeability changes in the hypersensitive reaction of *Nicotiana tabacum* cv Xanthi nc after infection with tobacco mosaic virus. *Physiol. Plant Pathol.*, 10: 253-258.
- WHEELER H., 1974, Cell wall and plasmalemma modifications in diseased and injured plant tissues. *Can. J. Bot.*, 52: 1005-1009.
- WHEELER H., 1975, Plant Pathogenesis. Springer-Verlag, Berlin, Heidelberg, N.Y., pp. 106.

Accepted for publication on 27 January 1982.