Ultrastructural response of potato roots resistant to cyst nematode *Globodera rostochiensis* pathotype Ro 1

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SUMMARY

Histological and ultrastructural changes in the potato cvs Diamant, Anosta and Morag, three days after inoculation with second stage juveniles of *Globodera rostochiensis* pathotype Ro1, were identical. The cells involved in nematode feeding were already transformed into a highly metabolic syncytium. Protein storage in form of crystalline bodies were detected in the plastids contained in the syncytial cells. It is assumed that these crystalline bodies are formed as a consequence of an osmotic imbalance in the cells caused by the nematode invasion of resistant roots. The hypersensitive host reaction was evident seven days after nematode inoculation and was characterized by an extensive necrosis lining the syncytium. Syncytial cells showed an involution of the cytoplasmic content while the nucleus and nucleolus were severely affected, indicating inactivation of the transcription mechanism. The mechanism of resistance to the Ro1 pathotype in the three potato cultivars was determined to be antibiosis, expressed as resistance to the survival and reproduction of the nematode.

RÉSUMÉ

Ultrastructure de la réaction des racines de pommes de terre résistantes au nématode à kyste Globodera rostochiensis pathotype Ro1

Les changements histologiques et ultrastructuraux dans les pommes de terre cvs Diamant, Anosta et Morag, trois jours après la pénétration de larves de second stade de *Globodera rostochiensis* pathotype Ro1, étaient identiques. Les cellules impliquées dans l'action trophique du nématode sont déjà transformées en syncytium doué d'une intense activité métabolique. Les plastides des cellules syncitiales contiennent des corps cristallins représentant des protéines de réserve. Il est supposé que les corps cristallins se constituent en réaction au déséquilibre osmotique des cellules provoqué par la pénétration des nématodes dans les racines du cv. résistant. La réaction d'hypersensibilité de l'hôte, mise en évidence sept jours après l'inoculation des nématodes, est caractérisée par une nécrose étendue entourant le syncytium. Les cellules syncitiales montrent des involutions cytoplasmiques. Le noyau lui-même est lésé et le mécanisme de transcription devient inactif. La réponse des trois cultivars de pommes de terre résistants au pathotype Ro1 est rapportée à un phénomène d' « antibiose », exprimant une résistance à la survie et à la reproduction du nématode.

Studies of the nature of resistance of potatoes to cyst nematodes indicated that potatoes with the H_1 gene from *Solanum tuberosum* subsp. *andigena* were invaded by juveniles, but few of them developed to females because of the failure to maintain the syncytium (Hoopes, Anderson & Mai, 1978; Rice, Leadbeater & Stone, 1985). Evaluation of resistance to cyst nematodes in soybean (Riggs, Kim & Gipson, 1973) and sugarbeet (Yu, 1982) have led to the same conclusion, i.e. that the induction and maintenance of the syncytium and absence of hypersensitive reaction are necessary for the development of the nematode. The initial stages of syncytium formation in resistant and susceptible plants appear to be similar. However, the hypersensitive reaction of the resistant host causes the formation of cytoplasmic granules in the syncytial cells within four days and necrotic lesions within ten days after juvenile penetration (Yu & Steele, 1981).

The results reported here attempt to examine the structural variations that occur in the development of syncytia induced by *G. rostochiensis* pathotype Ro1 on three potato cultivars. The susceptibility of cvs Anosta and Diamant to *G. pallida* pathotype Pa3 are presented elsewhere (Melillo, Bleve-Zacheo & Zacheo, 1990).

Materials and methods

Cysts of *G. rostochiensis* pathotype Ro1 (Dutch population) were placed in 0.6 mM sodium meta-vanadate in water and one week later second-stage juveniles were collected. Potato roots of the cultivars Diamant and Anosta (Italian) and Morag (Scottish) resistant to Ro1 were grown from sprouted potato tuber pieces at 17 °C. They were then transferred into clay pots containing 10 ml of sterilized sand and a suspension of 100 second stage juveniles (J2) of Ro1 pathotype were simultaneously added to each pot. Another ten pots of Morag were inoculated with 100 J2 of Pa3 pathotype, collected as above described. The experiments were conducted in growth chambers at 17 °C. At three and seven days after nematode inoculation, roots were removed and washed.
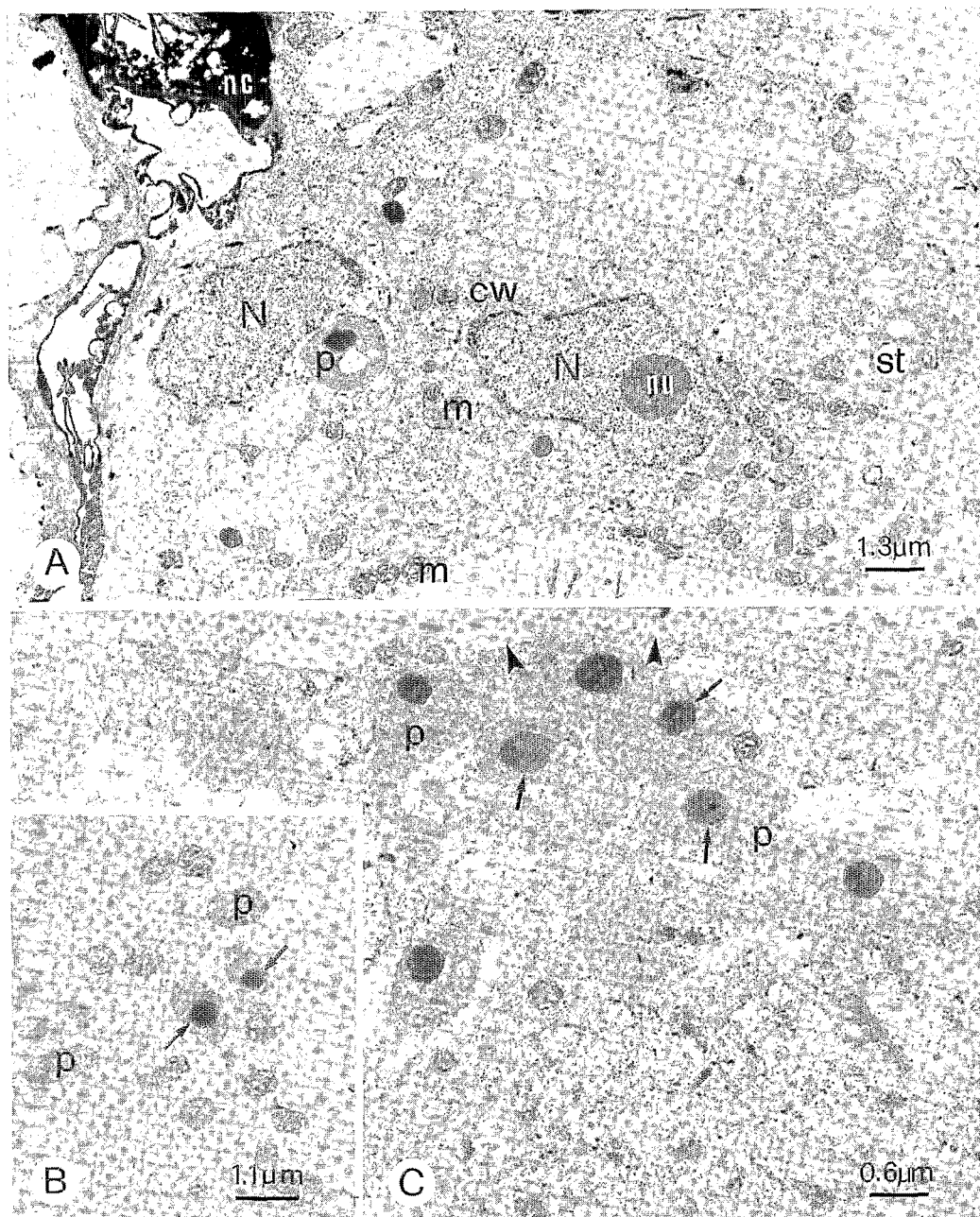
Fig. 1. — A : Micrograph of a transverse section through a syncytium in cv. Diamant root, three days after nematode inoculation. Two layers of cortical cells exhibit a hypersensitive-like response. The modified syncytial cells show the process of wall dissolution with wall stubs in the cytoplasm. The metabolic activity is almost similar to that of unaffected cells apart from starch and protein storage in the plastids. The same features were observed in the syncytia of the other two cultivars. — B : Elongate plastids in unaffected cells in cv. Anosta root. Some contain crystalline inclusions (arrow). — C : Detail of plastids in the syncytial cells in cv. Anosta, three days after nematode inoculation. The shape of the organelles is irregular and crystalline bodies (arrow), assumed to be proteins, are present in their stroma. Vesicular and tubular material are interposed between the cell walls and plasma membranes (head arrow).

List of abbrevations : cw = cell wall; N = nucleus; nu = nucleolus; m = mitochondrion; nc = necrotic cells; ne = nematode; p = plastid; ph = phloem; sy = syncytium; st = starch; v = vacuole; x = xylem.

The root tips (three days) and portions of infested roots (seven days) were fixed in 3 % glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) for 4 h, rinsed in the same buffer, post-fixed in 2 % osmium tetroxide for 4 h at 4 °C, then stained in 0.5 % uranyl acetate, dehydrated in an ascending series to absolute ethanol and embedded in Spurr's medium (1969). Sections 2 µm thick were cut with an LKB ultratome IV, stained with toluidine blue and observed under a light microscope to verify the syncytial location. Ultrathin sections were cut in that region and stained with uranyl acetate and lead citrate and examined under a Jeol 100 B transmission electron microscope at 80 kV.

Results

The three potato cultivars Diamant, Anosta and Morag exhibited an incompatible reaction when inoculated with J2 of *G. rostochiensis* pathotype Ro1. Sections of infested roots, three days after nematode inoculation showed an extensive necrosis in the cortical cells adjacent to the body of the second stage juvenile. Mechanical damage along the pathway of the juveniles through the cortex included more cells than in susceptible cultivars. Degeneration of the cytoplasmic contents to an accumulation of electron dense material coincided with cortical cell death (Fig. 1 A).

The structural features of syncytia in the three potato cultivars were generally similar. In all of the cultivars, the cytoplasm contained numerous ribosomes that occurred either as free particles in the cytoplasm or as polysomes on the surface of endoplasmic reticulum. There were active mitochondria, and many irregularly shaped, membrane-bounded vacuoles were distributed throughout the syncytial cytoplasm. Cell walls between the stimulated cells and the adjacent tissue underwent dissolution (Fig. 1 A). Plastids were numerous, irregularly shaped, bounded by a double membrane envelope and contained crystalline protein bodies and large membranous profiles (Fig. 1 C). Some of them also contained starch grains (Fig. 1 A). In contrast, plastids in unaffected parenchyma cells were of smaller size, regular shape, had a less developed thylakoid system and only a few contained protein bodies (Fig. 1 B). Vesicular and tubular material were localized between the plasma membrane and the cell wall (Fig. 1 C).

Seven days after nematode inoculation the syncytium had greatly expanded. In transverse sections through Diamant roots, syncytial cells were located mostly in the cortical region then developed centripetally surrounding the vascular bundles. The whole syncytial system was contained in a ring of necrotic cells (Fig. 2 A). A longitudinal section demonstrated that a fully developed syncytium extended from the rhizodermis to the outer region of the vascular area; the nematode feeding on this syncytium was located in the area of the syncytium that faced toward the rhizodermis (Fig. 2 B). In either transverse (Fig. 2 A) or longitudinal (Fig. 2 B) sections of the root, the syncytial complex was observed to be bounded by necrotic cells that involved cortical and vascular tissues.

At a later stage more necrotic areas were present both in contact with the nematode and in the cells which would have been incorporated into the syncytium in a susceptible reaction. The cytoplasmic content in the syncytium was weakly stained by toluidine blue and in transverse section most of the cells incorporated into the syncytium had only a thin layer of cytoplasm around the outer walls, especially those near the head of the nematode.

The situation was similar in Anosta roots. In longitudinal section the syncytial components extended deeply into the central portion of the stele. As a consequence the ring of necrotic cells around the periphery of the syncytium extended from one side of the cortical layer to the other. The modified cells were extremely vacuolated (Fig. 2 C).

In Morag roots, both nematode pathotypes, Ro1 and Pa3, established their feeding site in the cortical layer distant from the rhizodermis. The bulk of the syncytium was usually located outside the cambial zone but in contact with the vascular tissue, when it reached its maximum expansion (Fig. 2 D). A large number of cells was involved in syncytium formation. As in the other two cultivars, the cytoplasm became more highly vacuolated. The syncytium was not bounded by necrotic tissue and necrosis was observed only along the pathway of the nematode and around its head. Instead, the syncytium was limited mainly to the cortical region by heavily thickened walls which surrounded it (Fig. 2 D).

Seven days after nematode inoculation, the syncytium in Diamant was sandwiched between xylem and phloem elements and delimited by the altered cells. In these cells the cytoplasm was reduced to a boundary layer of dense material, in which some organelles were still detectable. The syncytial cells had expanded by cell wall dissolution of the incorporated cells. In a compatible combination, the cell walls and wall fragments in the syncytia usually become thickened, but in Diamant syncytia both intact and broken walls were very thin, indicating that a process of dissolution was in progress; in some of the cells the plasma membrane was retracted. In the cytoplasmic matrix of the syncytia membranes, membrane aggregates, few organelles and numerous small vacuoles were randomly distributed. Nuclei were of an amoeboid shape, but almost totally degenerated. The process of karyolysis was evident, with a loss of osmophilia in the nucleus and the dispersion of chromatin to the periphery of the nuclear envelope. The nucleolus appeared to be segregated and all that remained was a mass of closely packed fibrils with occasional amorphous zones (Fig. 3 A). The sequence of events became irreversible, passing through the pycnosis of the nucleus.
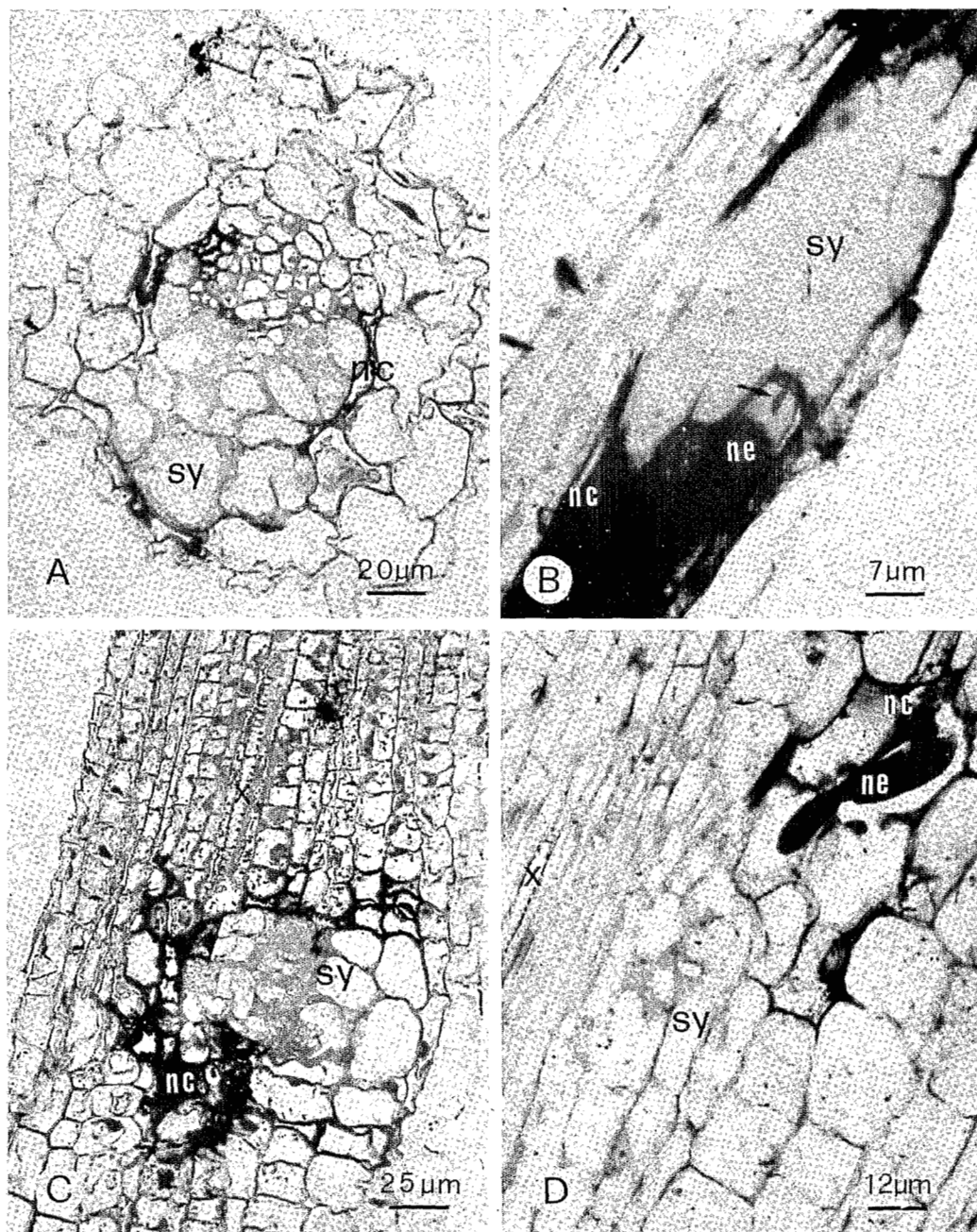
Fig. 2. — A : Light micrograph of a transverse section through Diamant root, seven days after nematode inoculation. The syncytium is greatly enlarged, extending from the outer cortical layer to the cambial tissue. A ring of necrotic cells encloses the syncytial cells, in which there are large vacuoles but scarce cytoplasm. — B : Longitudinal section through a syncytium seven days after nematode inoculation in Diamant root. Extensive necrosis in the cortical cells in the proximity of the nematode juveniles. Cytoplasm in the syncytium is not deeply stained, indicating poor content of cytoplasmic components. Note the head of the nematode with the stylet inserted into the syncytium (arrow). — C : Longitudinal section through syncytial cells in Anosta root, seven days after nematode inoculation. Necrotic cells surround the syncytium. The extension of modified cells is greater than in Diamant. Some of the syncytial cells are completely devoid of cytoplasm. — D : Longitudinal section through a syncytium induced by the pathotype Ro1 in Morag root, seven days after nematode inoculation. Cortical cells are more involved in the formation of the syncytium. There is extensive necrosis in cells where the nematode is positioned. Syncytial cells have interrupted walls and scarce cytoplasm.
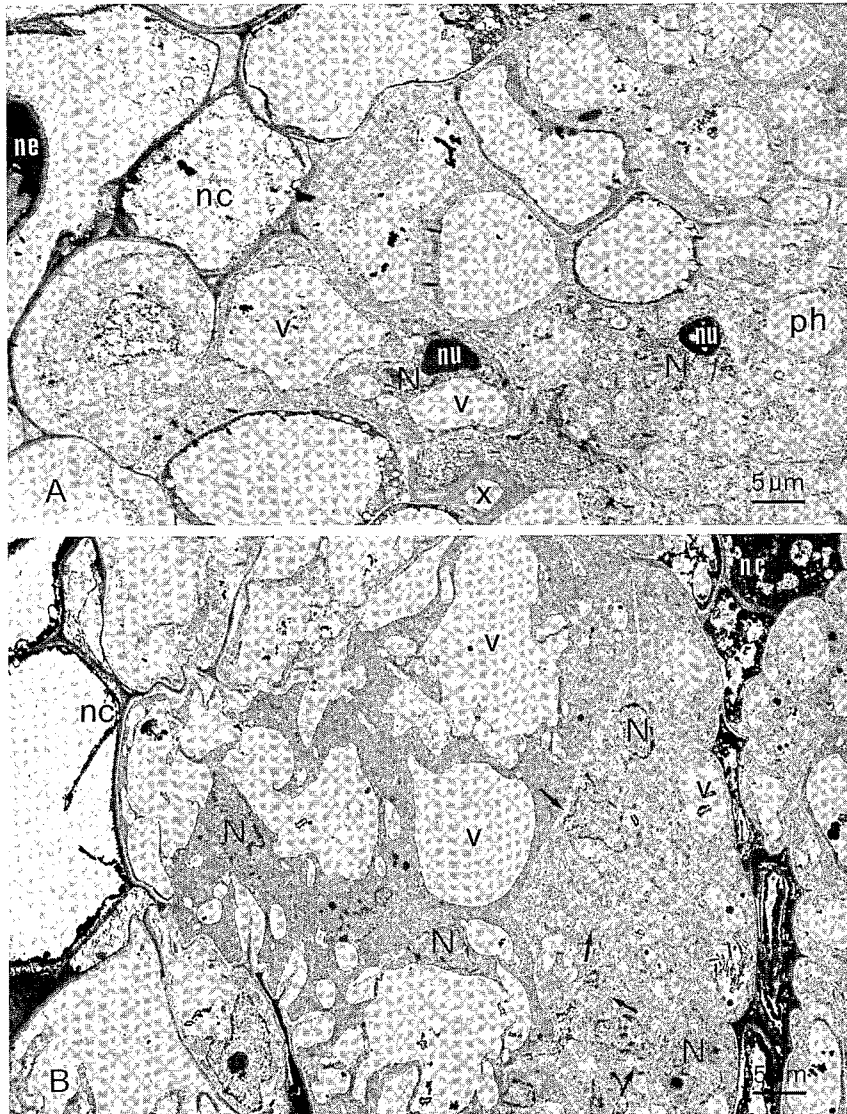
Abbreviations : see Fig. 1.

Fig. 3. — A : Micrograph of a transverse section through part of a syncytium in Diamant root, seven days after nematode inoculation. A ring of necrotic cells has formed around the edge of the syncytium, between the xylem and phloem vessels. The cytoplasm in the syncytial cells is partially degraded, nuclei show margination and clumping of chromatin and segregation of nucleolar components. In cortical cells adjacent to the nematode, the plasma membrane is separated from the cell wall. — B : Transverse section through syncytial cells in Anosta root, seven days after nematode inoculation. The syncytium is delimited by a layer of necrotic cells and appears to consist of two parts : one in which the cell walls are dissolved and the plasma membrane is separated; large vacuoles occupy the cytoplasmic area and nuclei are not extensively damaged as in Diamant. The second part consisting of small cells whose walls are not dissolved but with plasma membrane highly invaginated, indicating secondary wall apposition (arrow).
Abbreviations : see Fig. 1.

In Anosta roots the syncytium was not greatly expanded and was delimited by a ring of necrotic cells. The cell walls and wall remnants of syncytial cells were thin, as in Diamant, and there was extensive separation of the plasma membrane from the wall. Large vacuoles occu-pied the syncytial area. The ground cytoplasm was granular and still rich in organelles, such as mitochondria, ER and ribosomes. Nuclei were amoeboid with large amounts of chromatin in condensed form along the nuclear envelope. Some cells not yet modified as
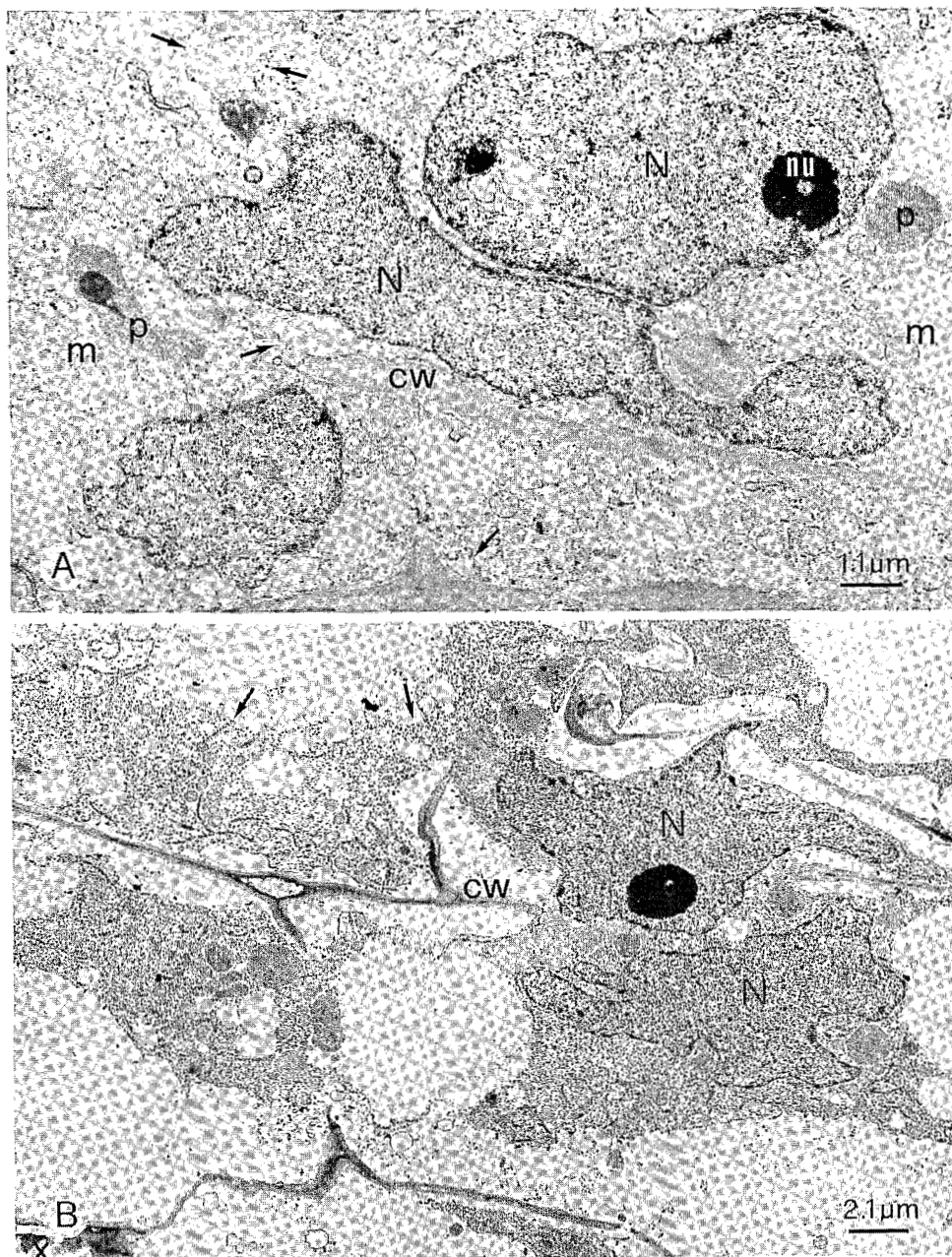
Fig. 4. — A : Micrograph of a portion of syncytial cells in Morag root, seven days after inoculation with second stage juveniles of pathotype Ro1. Cytoplasm is scarce and mitochondria appear to be partially degraded. Karyolysis is evident in the nuclei. A nucleolus, partially segregated is visible. Note the large wall apposition at the thickened and digested walls (arrow). — B : Longitudinal section through a disintegrated syncytium in Morag root, seven days after inoculation with second stage juveniles of pathotype Pa3. The modified cells, adjacent to the xylem vessels, are clearly necrotizing. The cell wall and plasma membrane are dissociated. Cytoplasm and nuclei are in phase of degeneration and the tonoplast has been disrupted (arrow). Note the severity of damage in this syncytium compared with that in Fig. 4 A, in which a hypersensitive response is evident.

Abbreviations : see Fig. 1.

syncytial cells, but delimited by the layer of necrotic cells, exhibited the features of unaffected cells, apart from nuclei that had amoeboid profiles and osmiophilic material (proteins?) in the vacuoles. Their walls were not thickened but wall apposition appeared to progress (Fig. 3 B). In Morag the cell walls and wall fragments of syncytial cells were thicker than in the other two cultivars. Aggregates of vesicular and tubular elements were continuous with the highly invaginated plasmalemma, and also around the wall fragments. The cytoplasm was much reduced and the mitochondria had very few cristae. Nuclei had swollen nuclear envelope and the nucleoplasm showed little osmiophilic reaction (Fig. 4 A). Syncytia induced in Morag by *Globodera pallida* pathotype Pa3, showed a stronger hypersensitive response than in syncytia induced by *G. rostochiensis* pathotype Ro1. Cells surrounding the nematodes and syncytia were severely damaged. The plasma membranes of the syncytial cells were completely disconnected from the partially thickened walls and the advanced state of lysis in the cytoplasm indicated that, after seven days, cellular degeneration was irreversible (Fig. 4 B).

Discussion

The ability of second stage juveniles of *Heterodera* spp. to invade the roots of resistant cultivars and to initiate the development of syncytia has been frequently reported (Riggs, Kim & Gipson, 1973; Yu, 1982; Acedo, Dropkin & Luedders, 1984; Wyss, Stender & Lehmann, 1984; Rice, Leadbeater & Stone, 1985; Rice, Stone & Leadbeater, 1987).

Potato cyst nematodes mainly damage plants by altering host physiology which results in the formation of multinucleate syncytia. Initial stages of syncytium formation appear to be similar in resistant and susceptible plants. However, the hypersensitive reaction of the resistant host early produces unsuitable conditions for the development of the nematode. A conspicuous layer of necrotic cells forms around the periphery of each syncytium and a process of relatively uncontrolled lysis, before on the walls and then in the cytoplasm of the syncytial cells, denies the parasite a suitable energy source for development. The early response of the cells to the presence of the nematode, expressed in the storage of proteins in the plastids and vesicular and membranous structures associated with the plasma membrane, indicates that the plant does not provide adequate nutrients to the parasite. It apparently prevents the syncytium from acting as a food sink and tries to repair or compensate for the (induced) damage. There are instances where ordinary crystalline cell constituents are much more abundant in diseased than in healthy cells. Esau (1975) related the higher number of intraplastid protein crystals found in virus infected spinach cells, as compared with healthy cells, to an effect of the virus on

metabolism and sugar translocation. Uritani (1976) considered that all injuries stimulate, in plant cells, the synthesis of essential components and secondary metabolites, including the hexoses pathway. There is little doubt that the formation of crystalline structures is the consequence of an increased synthesis of proteins induced by pathogens. Accumulation of proteins during the invasion of resistant tomato roots by *Meloidogyne incognita* is reported as an effect of osmotic imbalance due to lytic enzymes produced by the nematode (Bleve-Zacheo *et al.*, 1982). Other authors experimentally obtained the formation of crystalloids by treating the tissues with hypertonic solutions (Perner, 1963) or placing them in a very low concentration of sucrose (Wrischer, 1973). Gunning and Steer (1975) described this feature in plastids which had been subjected to physiological stress. Boundary formations are also reported as an early response in Maris Piper potato infested by *G. rostochiensis* pathotype Ro1 (Rice, Leadbeaten & Stone, 1985) and resistant soybean invaded by *H. glycines* (Riggs, Kim & Gipson, 1973). The boundary formations were associated with abnormal cell wall thickening, similar to those associated with the thickening of cell walls surrounding the necrotic areas in plants infected with virus (Tu & Hiruki, 1971). It is reasonable to argue that they are produced as a response to unfavourable pathological conditions. The retraction of the plasma membrane observed at seven days after nematode inoculation, probably results from a decrease in activity of ion pumps.

This mode of cellular degeneration isolates the injured cell from neighbouring cells. Moreover, nuclear alterations and condensation of the nucleolus, particularly evident in Diamant and Morag, reflect an inactivation of transcription mechanisms (Beaulation & Lockshin, 1982). Failure of the synthetic processes leads to cell death. As syncytia degenerate, the nematodes appear to be immobilized. From our observations the inhibition of nematode development appears to be mainly due to cytoplasmic degeneration prior to the extensive thickening of the wall, as suggested by Webster (1975). In all three cultivars tested pronounced cell wall thickening was not detected.

We suggest that there is a primary hypersensitive-like response, evident as cytoplasm degeneration, in response to the presence of the nematode, followed by wall thickening to repair and/or limit the spread of damage.

The mechanism of resistance to *G. rostochiensis* pathotype Ro1 and to *G. pallida* Pa3 in the cultivar Morag can be classified as antibiosis (Yu, 1982). Antibiosis is expressed as resistance to the survival and reproduction of *Heterodera* spp.

REFERENCES

ACEDO, J. R., DROPKIN, V. H. & LUEDDERS, V. D. (1984). Nematode population attrition and histopathology of *Heterodera glycines*-soybean associations. *J. Nematol.*, 16 : 48'-57.

BEAULATION, J. & LOCKSHIN, R. A. (1982). The relation of programmed cell death to development and reproduction : comparative studies and an attempt at classification. *Int. Rev. Cytol.*, 79 : 215-235.

BLEVE-ZACHEO, T., ZACHEO, G., MELILLO, M. T. & LAMBERTI, F. (1982). Crystal-like structures in plastids of tomato roots, infested by *Meloidogyne incognita. Nematol. medit.*, 10 : 91-99.

ESAU, K. (1975). Crystalline inclusions in thylakoids of spinach chloroplasts. *J. Ultrastruct. Res.*, 53 : 235-243.

GUNNING, E. S. & STEER, M. W. (1975). *Ultrastructure and the Biology of Plant Cells.* London, Edward Arnold, 312 p.

HOOPES, R. W., ANDERSON, R. E. & MAI, W. F. (1978). Internal response of resistant and susceptible potato clones to invasion by potato cyst-nematode *Heterodera rostochiensis. Nematropica,* 8 : 13-21.

MELILLO, M. T., BLEVE-ZACHEO, T. & ZACHEO, G. (1990). Ultrastructural response of potato roots susceptible to cyst nematode *Globodera pallida* pathotype Pa3. *Revue Nématol.*, 13 : 17-28.

PERNER, E. (1963). Kristallisationsercheinungen im Stroma isolierter Spinat-chloroplasten guter Erhaltung. *Naturwiss.*, 50 : 134-135.

RICE, S. L., LEADBEATER, B. S. & STONE, A. R. (1985). Changes in cell structure in roots of resistant potatoes parasitized by potato cyst nematodes. I. Potatoes with resistance gene H derived from *Solanum tuberosum andigena. Physiol. Pl. Pathol.*, 27 : 219-234.

RICE, S. L., STONE, A. R. & LEADBEATER, B. S. (1987). Changes in cell structures in roots of resistant potatoes parasitized by potato cyst nematodes. II. Potatoes with resistance derived from *Solanum vernei. Physiol. Molec. Pl. Pathol.*, 31 : 1-14.

RIGGS, R. D., KIM, K. S. & GIPSON, I. (1973). Ultrastructural changes in Péking soybeans infected with *Heterodera glycines. Phytopathology,* 63 : 76-84.

SPURR, A. R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.*, 26 : 31-43.

TU, J. C. & HIRUKI, C. (1971). Electron microscopy of cell wall thickenings in local lesions of potato virus-M infected Red Kidney bean. *Phytopathology,* 61 : 862-868.

URITANI, I. (1976). Protein metabolism. In : Heitefuss, R. & Williams, P. H., (Eds). *Physiological Plant Pathology,* Berlin, New York, Springer-Verlag : 509-525.

YU, M. H. (1982). Interpretation of mechanism for nematode resistance in sugarbeet. *J. Am. Soc. Sugar Beet Technol.*, 21 : 351-361.

YU, M. H. & STEELE, A. E. (1981). Host-parasite interaction of resistant sugarbeet and *Heterodera schachtii. J. Nematol.*, 13 : 206-212.

WEBSTER, J. M. (1975). Aspects of the host-parasite relationship of plant-parasitic nematodes. *Adv. Parasit.*, 13 : 225-250.

WRISCHER, M. (1973). Protein crystalloids in the stroma of bean plastids. *Protoplasma,* 77 : 141-150.

WYSS, U., STENDER, C. & LEHMANN, H. (1984). Ultrastructure of feeding sites of the cyst nematode *Heterodera schachtii* Schmidt in roots of susceptible and resistant *Raphanus sativus* var. *oleiformis* cultivars. *Physiol. Pl. Pathol.*, 25 : 21-38.