volatile nematicides were applied to plants at 4 monthly intervals; good results were obtained with phenamiphos at 2 to 3 g i.a., carbofuran at 2.5 g i.a. oxamyl at 4 g i.a. and 6 g i.a. but decreased to 3 g i.a. In general granular nematicides were as good as or better than DBCP in improving yields.

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INTERNAL RESPONSE OF RESISTANT AND SUSCEPTIBLE POTATO CLONES TO INVASION BY POTATO CYST NEMATODE HETERODERA ROSTOCHIENSIS [REACCION INTERNA DE CLONES RESISTENTES Y SUSCEPTIBLES A LA INVASION DEL NEMATODO DEL QUISTE DE LA PAPA]

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ABSTRACT

A histological study was made to observe the early response of four resistant and four susceptible potato clones to invasion by race A of *Heterodera rostochiensis* Wollenweber. The resistant clones were hybrids between *Solanum tuberosum* ssp. *tuberosum* and four different sources of resistance: *S. tuberosum* ssp. *andigena, S. spegazzinii, S. vernei,* and *S. multidissectum.* The response of each resistant clone was compared to that of a related, but susceptible, clone. The response to invasion in all of the susceptible clones was the formation, through cell-wall dissolution, of a multinucleate syncytium, characterized by dense, granular cytoplasm. In all of the resistant clones, larvae were able to penetrate the roots and initiate the syncytium, but the amount of tissue included and the maintenance of the dense cytoplasm were limited by the resistant reaction. The mechanisms of resistance involved vacuolation of the syncytial cytoplasm, necrosis, and enclosure of the syncytium, but were not exactly the same for all of the resistant clones.

INTRODUCTION

Although the potato cyst nematode has been in the United States for at least 35 years, only race A of *H. rostochiensis* is known to be represented in the soil population.

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The Cornell University potato breeding program has developed good resistance against this population, using the H1 gene derived from the *Solanum tuberosum* ssp. *andigena* clone CPC 1673, first reported by Ellenby in 1952 (1). To prepare for the introduction or development of new races against which the H1 gene may not be effective, the breeding program is now incorporating a diverse set of genes from several species into commercially acceptable tetraploid potatoes. The objectives of this study were to learn whether these genes from different sources conditioned the same or different internal resistance reactions in the invaded roots and also whether the resistance response could easily be recognized in the early stages of infections.

Several studies of host-parasite interactions between *Heterodera* ssp. and crop plants have led to the conclusion that the syncytium, a specialized feeding site induced in the roots of the host, is the key to successful parasitism by these cyst-forming nematodes. The syncytium in potato roots infected by cyst nematode larvae was reported by O'Brien and Prentice in 1930 (6). Its role as a specialized feeding site for the sedentary nematode was postulated. An ultrastructural study by Jones and Northcote (4) led to a more detailed description of the syncytium and to the discovery of cell-wall protuberances which may serve to facilitate the movement of solutes from the vascular system of the roots to the feeding nematode by increasing the surface area of the syncytium.

Studies of the nature of resistance in potatoes by Williams (9) indicated that potatoes with the H1 gene from Andigena were still invaded by the larvae, but that few females developed to maturity and that there were few 'giant cells' associated with feeding larvae. Piegat and Wilski (7) described a reaction in roots with the same resistance, in which the cells near the nematode's head became highly vacuolated and necrotic instead of forming the dense, granular cytoplasm characteristic of the susceptible plant. Studies of the nature of resistance to cyst nematodes in clover (5) and soybean (2) have led to the same conclusion: that the induction and maintenance of a suitable syncytium are necessary for the development of mature females.

MATERIALS AND METHODS

The plants examined for their internal reaction to cyst nematode infection represented four known resistant and four known susceptible clones: two commercial varieties, 'Katahdin' (susceptible) and M-1783-28 (resistant); and two diploid clones, also full sibs, 72-21-86 (susceptible) and 72-21-1 (resistant). Each resistant clone derived its resistance from a different species: 'Peconic,' from *S. tuberosum* ssp. *andigena;* M-1783-28, from *S. spegazzinii;* 73-92-10, from *S. vernei;* and 72-21-1, from *S. multidissectum.* Each was known to be highly resistant, having in each of several tests permitted the development of less than five mature females per plant in heavily inoculated soil.

Plants to be inoculated were started from sprouted tuber pieces in potting soil in 175-ml styrofoam cups. Eight to ten holes 0.7 cm in diameter were punched in the bottom of each cup. The cups were placed in glass petri dishes which were filled with fine-textured vermiculite. Within 10-12 days, the roots were protruding through some of the holes into the vermiculite. They were then gently removed from the vermiculite and a solution containing about 1000 larvae was pipetted over the roots of each plant. The plants were again placed in the vermiculite-filled petri dishes. After 48 hrs, the roots were again removed from the dishes, washed in tap water to remove larvae which had not penetrated, and replaced in clean vermiculite.

Root samples were collected at 2-3 day intervals for the first 12 days after inoculation, and some collections were made at later stages up to 25 days after inoculation.

The samples were cleaned, fixed for 24 hrs. in Craf III fixative, dehydrated by the tertiary butyl alcohol method (8), infiltrated and embedded in Peel-a-way paraffin, sectioned at 12-15 um on a rotary microtome, and stained with safranin and fast green for observation.

RESULTS

The responses of the four susceptible clones were essentially the same. Following invasion of the roots, second-stage larvae migrated through the cortex and assumed a feeding position in the cortical cells, oriented parallel to the vascular cylinder. Mechanical damage to cells and some necrosis could be seen along the paths of movement, but there was no clearly visible reaction in cells ahead of the larvae at the very early stages. Six days after invasion, some cell-wall breakdown had occurred in the cells near the nematode head, as cells began to fuse into the feeding site, the syncytium. The cytoplasm of these cells was dense and granular in appearance and lacked vacuolation. Ten days after inoculation, there was considerable cell-wall breakdown in the cells between the larval head and the stele and cell-wall fragments were visible in many sections (Figure 1). The cytoplasm still appeared dense and granular. The cell wall

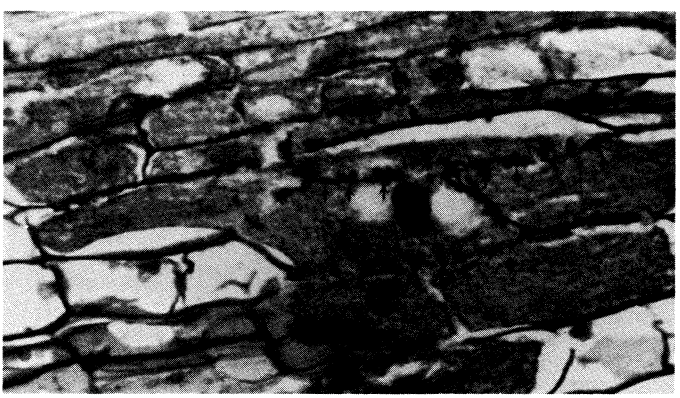


Figure 1. Syncytium in 'Katahdin' root, with dense cytoplasm, lack of vacuolation, and cell wall fragments (indicated by arrows).

pierced by the stylet was considerably thicker than normal, but remained intact. Within 12 days, the syncytium had expanded along the vascular system and after 14 days, the posterior ends of some females protruded toward the root surface. The granular, sometimes reticulated nature of the cytoplasm was becoming more pronounced. Many large nuclei were sometimes grouped together as the walls between cells were broken down and cytoplasm fused (Figure 2). Sections taken 23 days after inoculation (Figure 3) showed swollen females whose posteriors had burst through the root surface and protruded out from it. Egg development was beginning. Extensive syncytial tissue was present along the vascular system. Some syncytial cytoplasm had a striated pattern, giving the appearance of an oriented 'flow' of materials toward the nematode (Figure 4). The edges of the syncytium were not bounded by necrotic tissue, but joined smoothly with the non-affected cells more distant from the nematode.

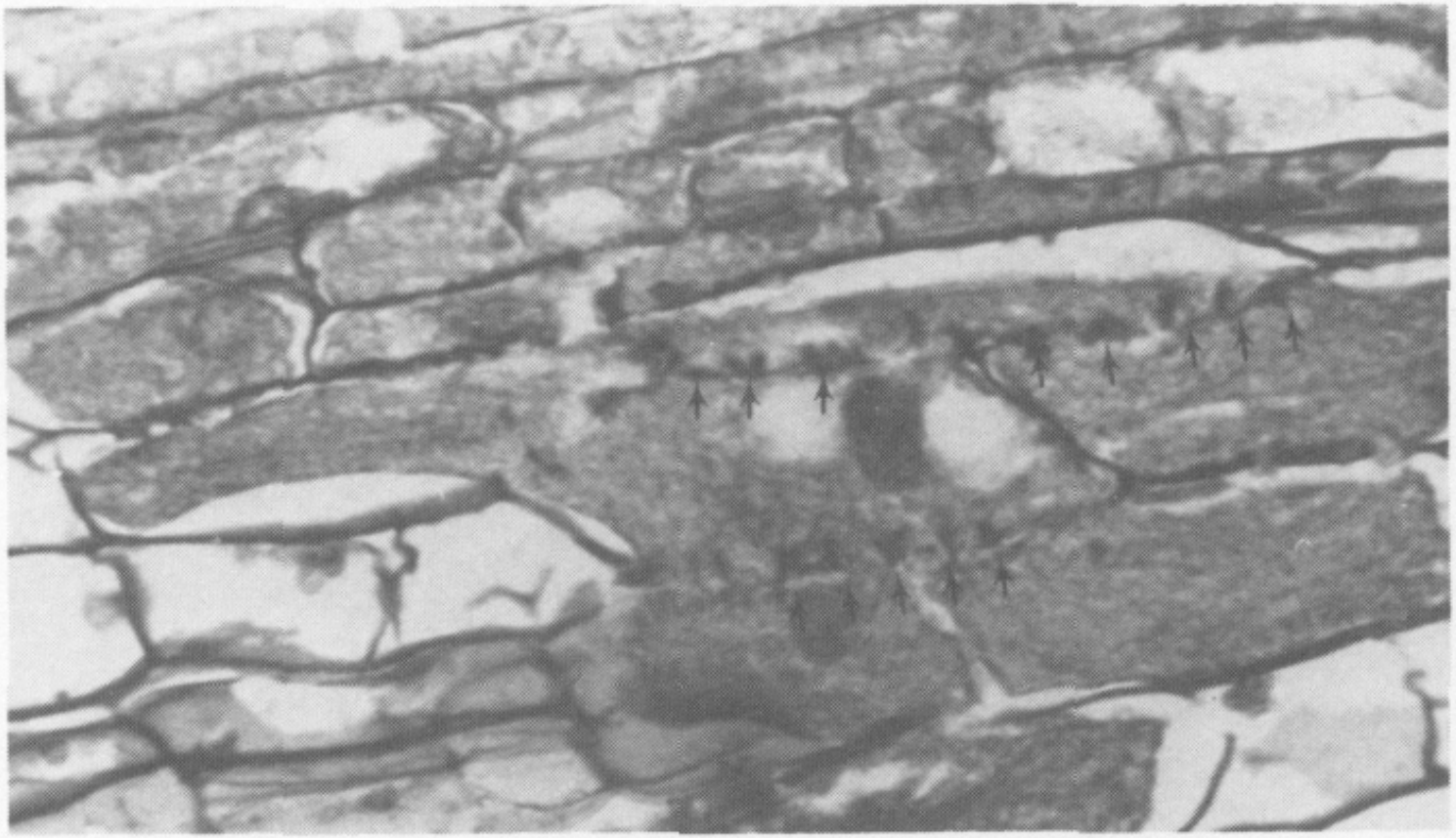


Figure 1. Syncytium in 'Katahdin' root, with dense cytoplasm, lack of vacuolation, and cell wall fragments (indicated by arrows).

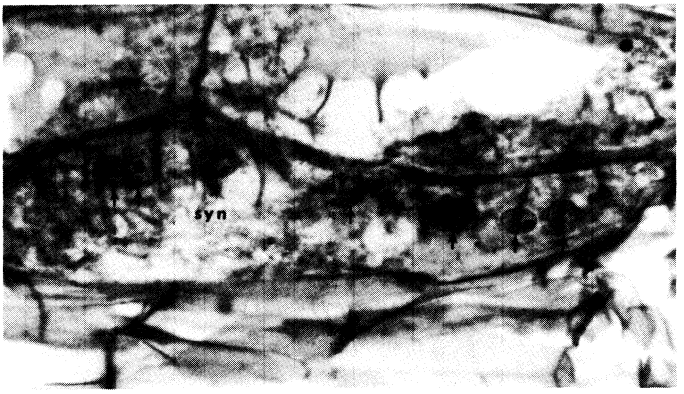


Figure 2. Syncytium (syn) in susceptible 73-92-1. Eight nuclei (arrows) with prominent nucleoli are present.

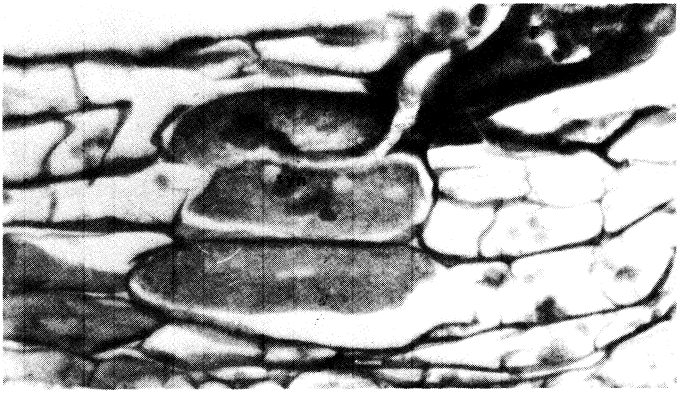
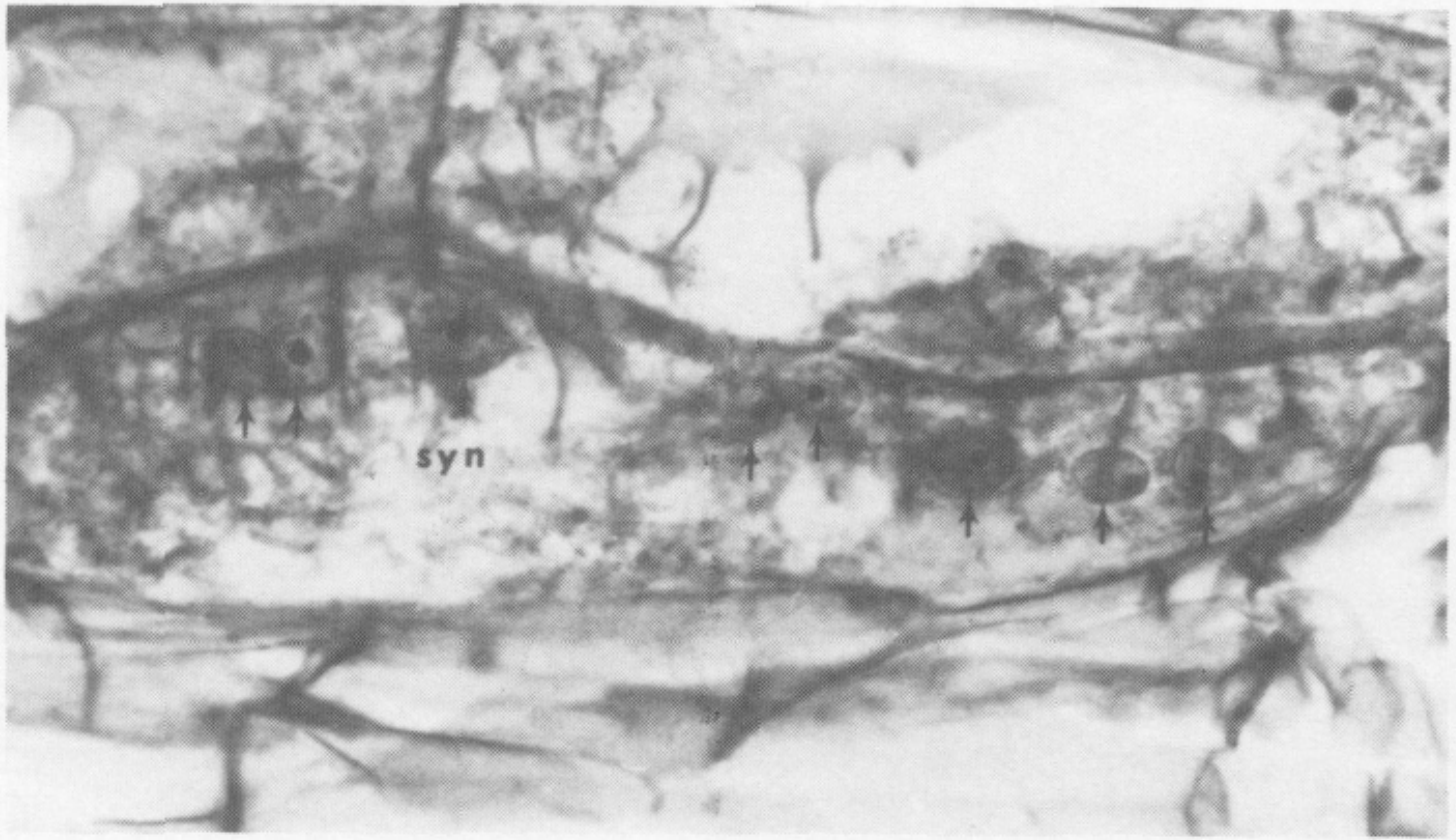


Figure 3. Developing female (F) protruding from 'Katahdin' root, with supporting syncytium (syn).

In all of the resistant clones, larvae readily entered the roots and moved through the cortex to a feeding site. There were differences, however, in the internal reactions which prevented larvae from developing into mature females which could reproduce.

The resistance reaction of 'Peconic,' based on the H1 gene of the Andigena clone CPC 1673, permitted very little larval development. In early stages of infection, a small amount of tissue with the granular cytoplasm typical of the syncytium was formed, but it was surrounded by red-staining and probably necrotic cells. In later stages, 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. There was considerable vacuolation of cells near the nematode's head. Although developing males were observed, the collapse of the dense cytoplasm and the necrosis of the syncytial tissue precluded the development of mature females (Figure 5).



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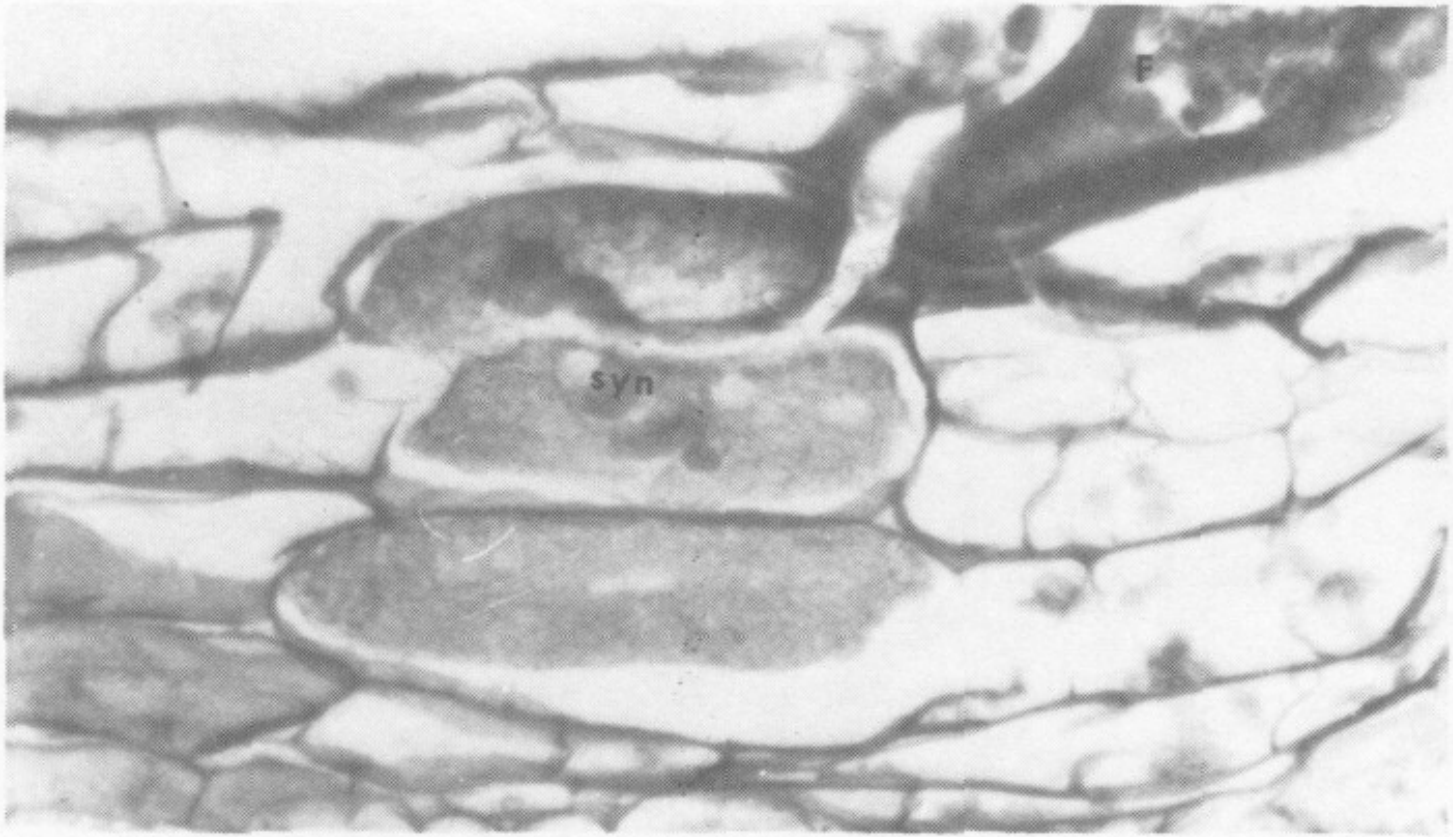

Figure 3. Developing female (F) protruding from 'Katahdin' root, with supporting syncytium (syn).

Figure 4. Syncytium in 'Katahdin' root. Cytoplasm contains striations (indicated by arrows) indicative of an oriented flow of materials toward the nematode (n).

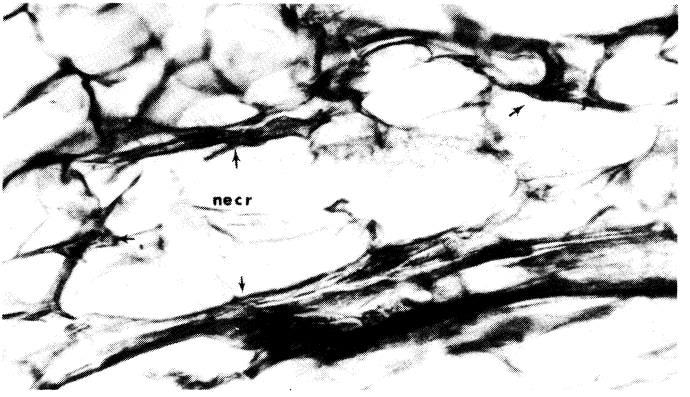

Figure 5. Section of 'Peconic' root, with necrotic and collapsed cells (necr) surrounded by darkly stained tissue (arrows).

Clone M-1783-28, deriving its resistance from *S. spegazzinii*, was also readily invaded by larvae. As in 'Peconic,' there was an initial induction of the syncytium with some cell-wall dissolution and dense, granular cytoplasm. But it failed to develop fully enough to support the development of mature females. In contrast to 'Peconic,' there was no red-staining, necrotic tissue surrounding the incipient syncytium; rather, the cytoplasm became highly vacuolated and the syncytium appeared to collapse from within (Figure 6).

The resistance of clone 73-92-10 was derived from *S. vernei*. Again, larvae readily in-

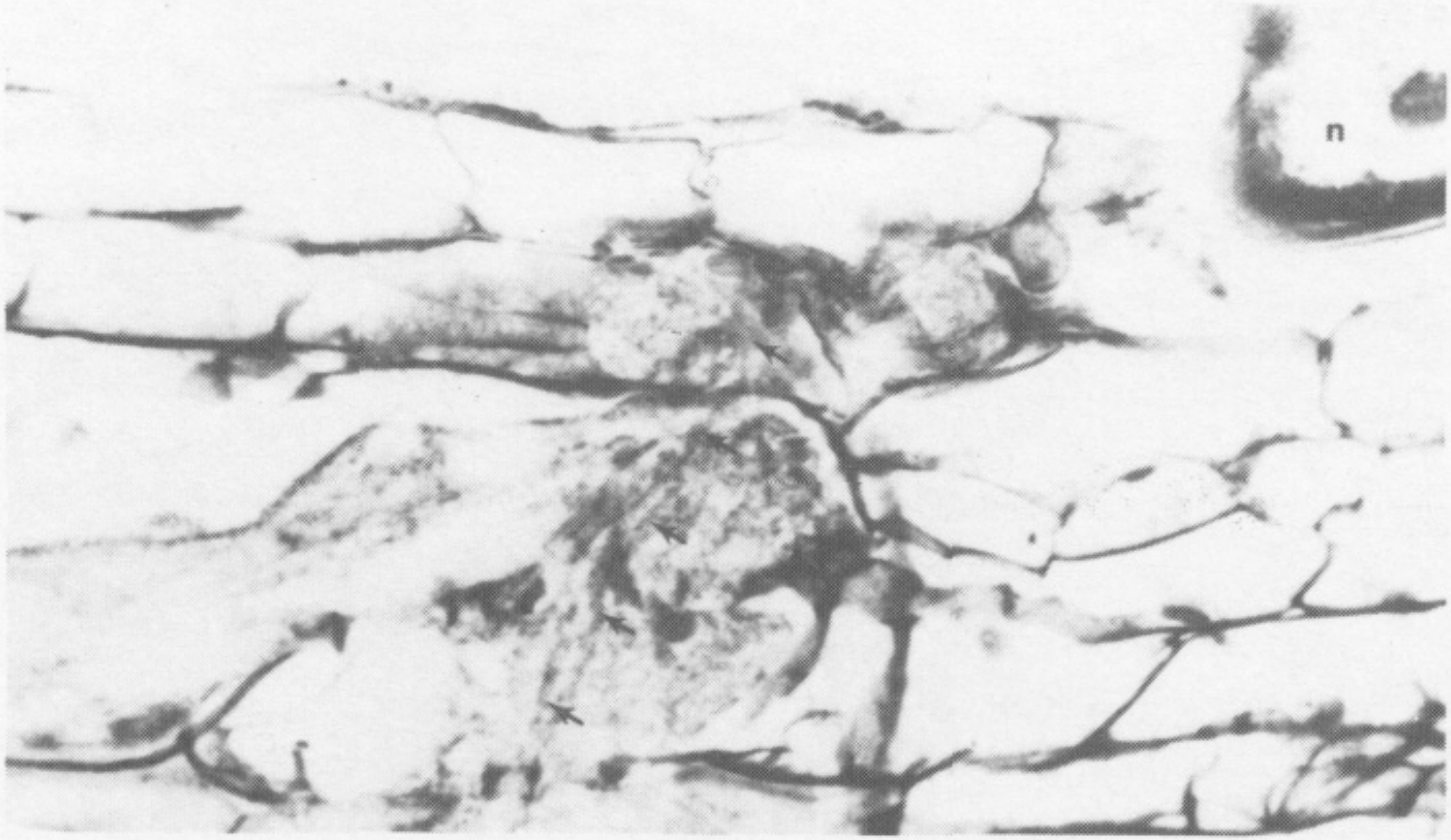

Figure 4. Syncytium in 'Katahdin' root. Cytoplasm contains striations (indicated by arrows) indicative of an oriented flow of materials toward the nematode (n).

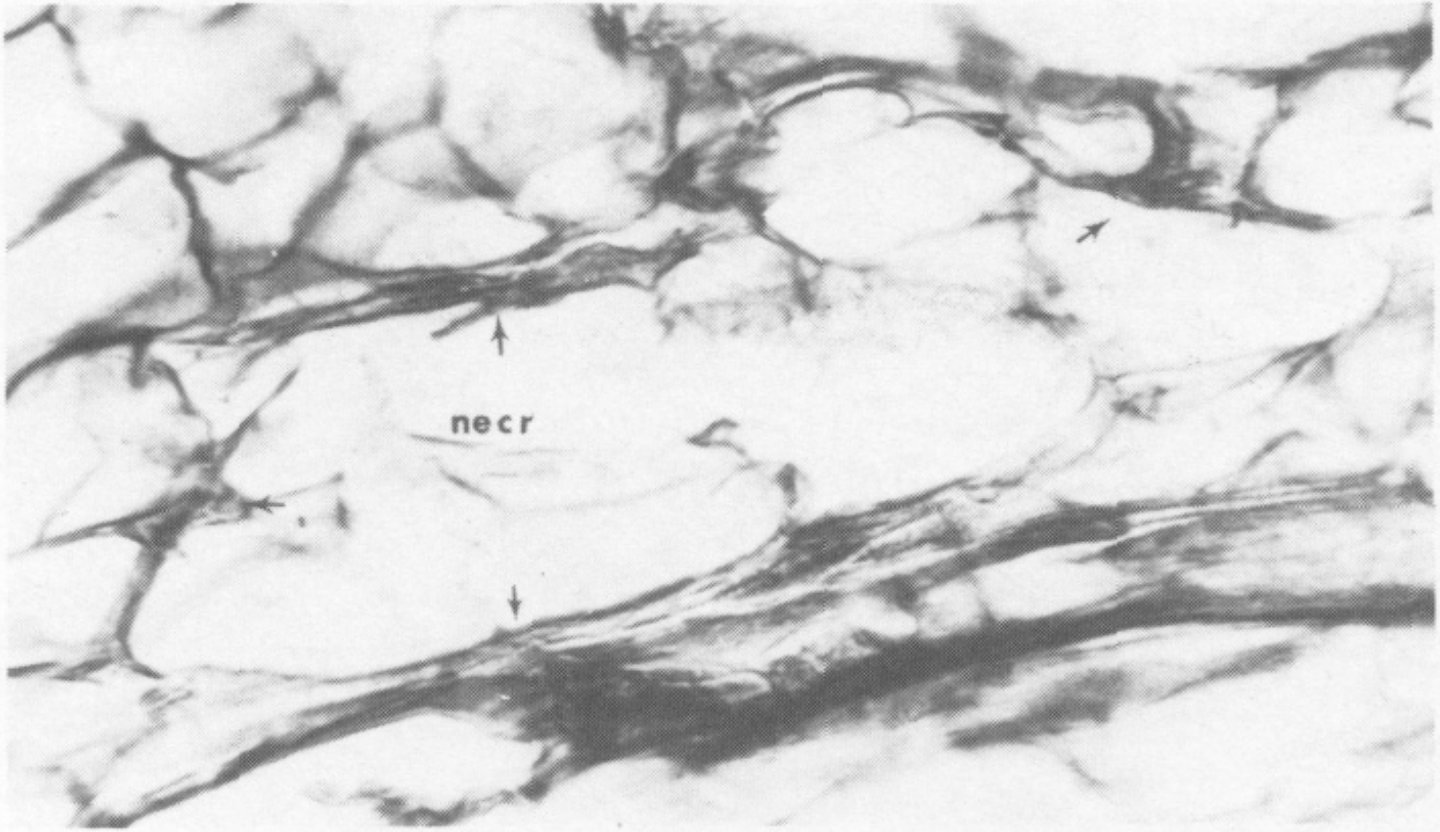

Figure 5. Section of 'Peconic' root, with necrotic and collapsed cells (necr) surrounded by darkly stained tissue (arrows).

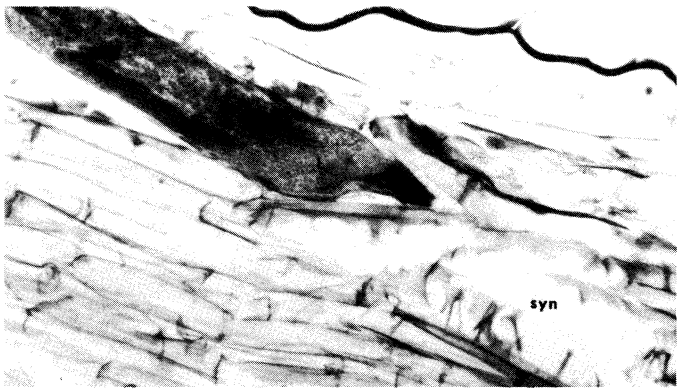

Figure 6. Larva and collapsed syncytium (syn) in *S. spegazzinii*-resistant M-1783-28.

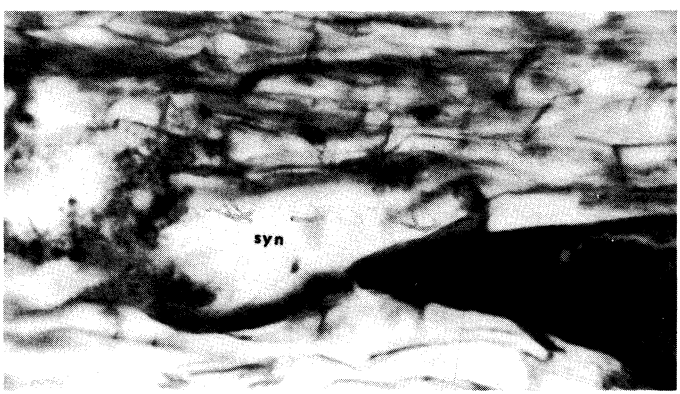

Figure 7. Young female larva (L) in *S. vernei*-resistant 73-92-10, with vacuolated cytoplasm next to head.

vaded the roots and initiated some cell-wall dissolution and dense, granular cytoplasm typical of the syncytium. But in later stages of infection, the syncytial cytoplasm became more and more highly vacuolated and surrounded by a slightly thickened wall. Some necrosis also occurred in the cells that were bordering on the nematode and the region of the syncytium. (Figure 7).

In the clone 72-21-1, deriving resistance from *S. multidissectum,* larvae initiated the formation of dense, granular cytoplasm characteristic of the syncytium. In contrast to the other three resistant clones, the cytoplasm did not become more highly vacuolated. Instead, the syncytium was limited in its spread mainly to the cortical region by the formation of a heavily thickened wall surrounding it (Figure 8).

Table 1 summarizes the resistance mechanisms observed in these four clones.

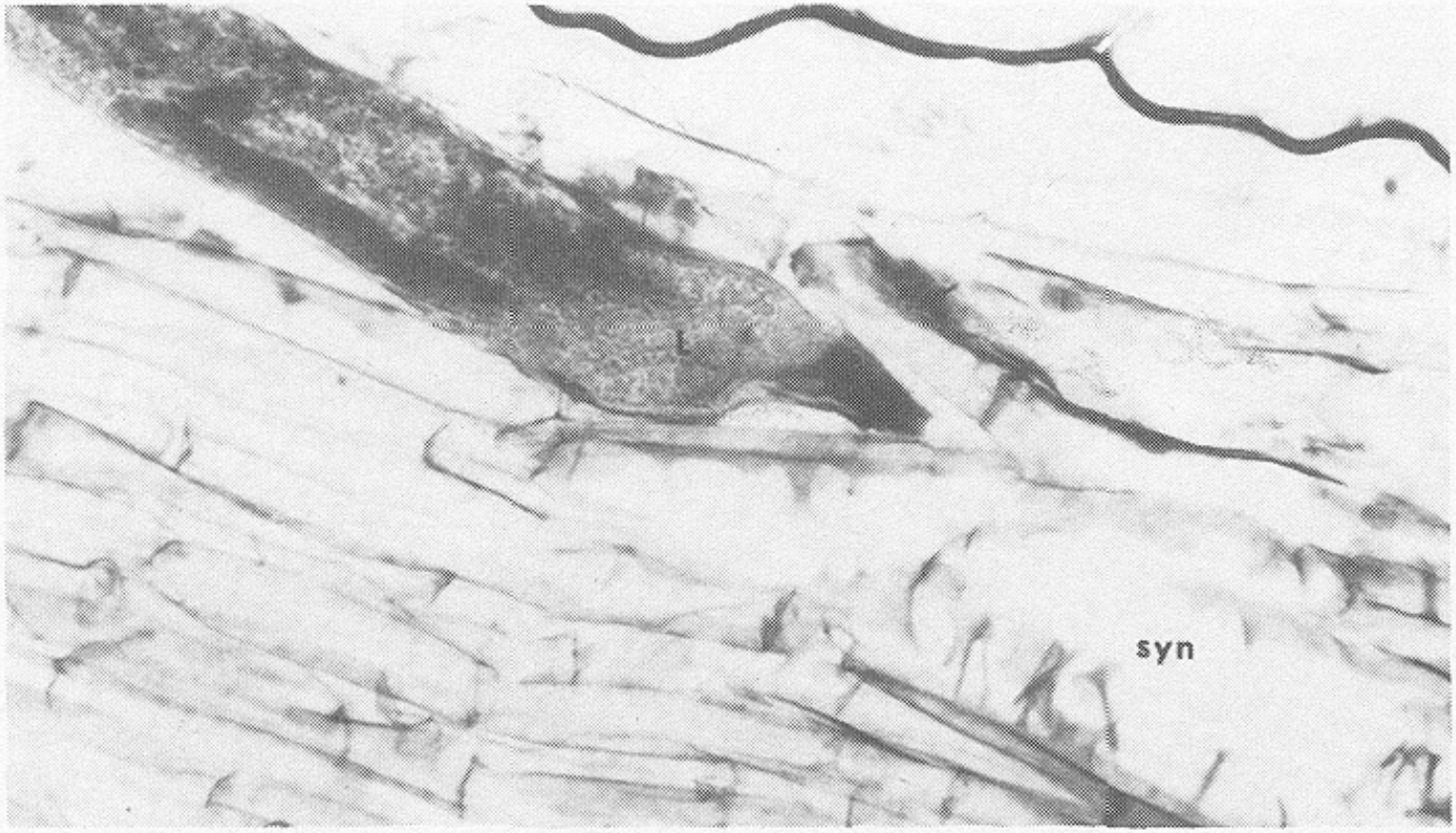

Figure 6. Larva and collapsed syncytium (syn) in *S. spegazzinii*-resistant M-1783-28.

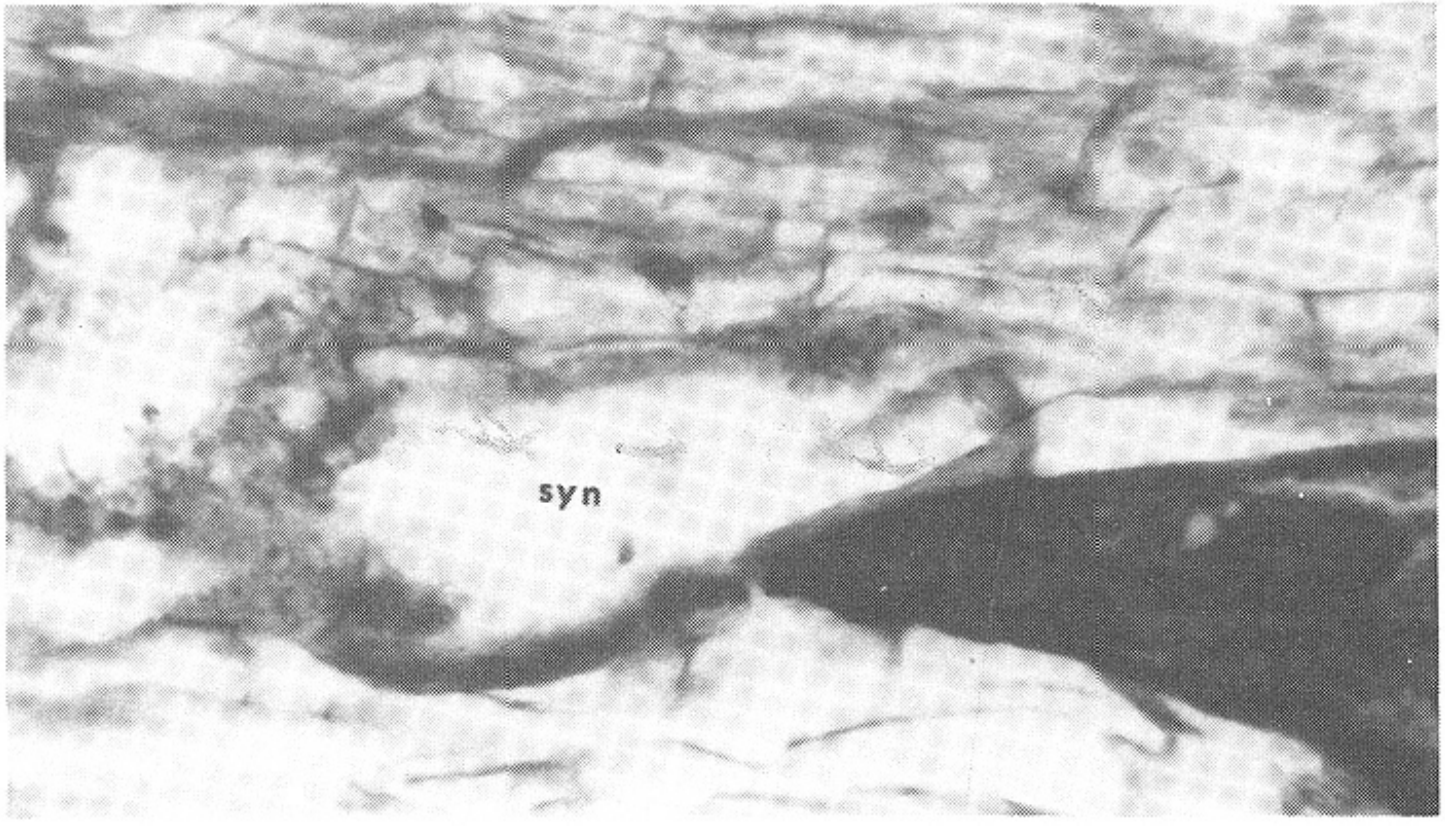

Figure 7. Young female larva (L) in *S. vernei*-resistant 73-92-10, with vacuolated cytoplasm next to head.

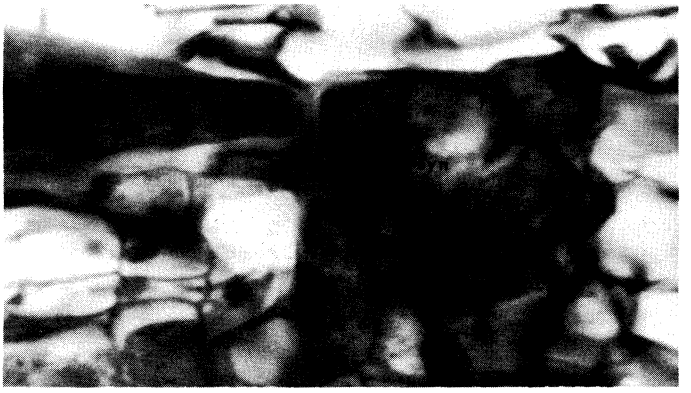

Figure 8. Larva (L) in *S. multidissectum*-resistant 72-21-1, with syncytium (syn) limited by thickening of walls on its boundary, as indicated by arrows.

Table 1. SUMMARY OF THE EXPRESSIONS OF RESISTANCE IN THE CLONES TESTED.

	EXPRESSION OF RESISTANCE			
Source of Resistance	Penetration by Larvae	Initiation of Syncytium	Maximum Larval Development	Mechanism of Limiting Syncytium
S. tuberosum ssp. *andigena* ('Peconic')	yes	yes	3rd stage	vacuolation of cytoplasm; walling off and collapse of cells; necrosis
S. spegazzinii (M-1783-28)	yes	yes	3rd stage	vacuolation of cytoplasm; collapse of cells
S. vernei (73-92-10)	yes	yes	4th stage	vacuolation of cytoplasm; walling off; necrosis
S. multi-dissectum (72-21-1)	yes	yes	3rd stage	walling off

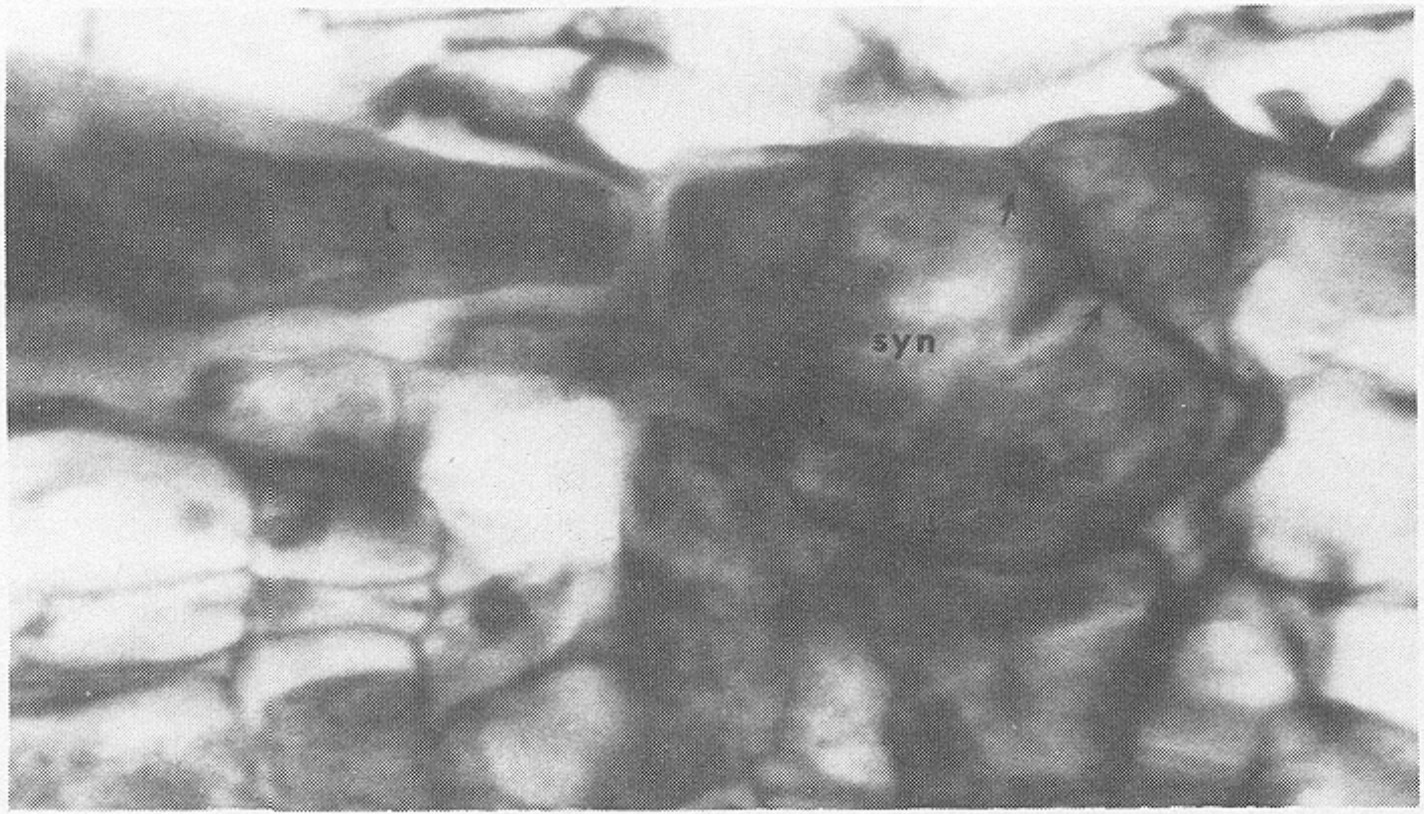

Figure 8. Larva (L) in *S. multidissectum*-resistant 72-21-1, with syncytium (syn) limited by thickening of walls on its boundary, as indicated by arrows.

DISCUSSION

The findings of other workers with respect to the formation of the syncytium in the susceptible plant and the failure of the syncytium to be formed or to be maintained in the resistant plant were confirmed in this study.

Jones (3) suggested that the system by which the nematode induces the syncytium and the system by which the resistant plant 'recognizes' the nematode as incompatible are separate. According to this suggestion, the nematode could induce a suitable syncytium in the resistant plant if it were not for a separate, unrelated, resistance reaction that prevents it. It can be concluded from these results that even the highly resistant clones have a certain degree of susceptibility—that is, they all respond to some extent to the stimulus to form a syncytium on which a nematode can feed and develop. In three of the four clones, the resistant reaction was probably a separate phenomenon from the susceptible reaction, with the plant actively limiting the nematode's ability to induce a syncytium by walling it off in some manner. In the spegazzinii-resistant clone, there was no visible enclosure of the syncytium, and its resistance could have been the result of a failing to respond adequately to the stimulus of the nematode.

The fact that the resistance reactions of these four clones were not all alike tends to make the rapid recognition of the resistant plant more difficult, but if several genes for resistance, each mediating a different reaction, could be incorporated into a single variety, the probability of resistance being overcome by small changes in the nematode population may be greatly reduced.

RESUMEN

Se hizo un estudio histológico para observar la reacción a la invasión de la raza A de *Heterodera rostochiensis* de cuatro clones de papa resistentes y cuatro susceptibles. Los clones resistentes fueron híbridos entre *Solanum tuberosum* spp *tuberosum* y cuatro fuentes de resistencia diferentes: *S. turberosum* spp. *andigena, S. spegazzinni, S. vernei,* y *S. multidissectum.* La reacción de cada clón resistente se comparó con la de uno susceptible y conexo. En los clones susceptibles la reacción a la invasión consistió en la formación, por medio de la disolución del tabique celular, de un síncito polinuclear caracterizado por un citoplasma granuloso y denso. En todos los clones resistentes, las larvas pudieron penetrar las raíces e iniciar el síncito, pero la cantidad de tejido incluido y el mantenimiento del citoplasma denso fueron limitados por la reacción de resistencia. El mecanismo de la resistencia incluyó vacuolación del citoplasma sincítico, necrosis y encerramiento del síncito, pero no fué identico en todos los clones resistentes.

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