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INFLUENCE OF DIFFERENT TEMPERATURES ON RESISTANCE OF TOMATO PLANTS TO *MELOIDOGYNE INCOGNITA* [1]

by
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Temperature is one of the most important factors influencing the expression of resistance in plant attacked by nematodes. Many reports indicate that plants, when grown at higher temperatures, become more susceptible to nematode infection.

High temperatures reduced the resistance of alfalfa to the stem nematode *Ditylenchus dipsaci* (Grundbacker and Stanford, 1962) and the penetration and development of *Meloidogyne incognita* in resistant tomato roots increased at temperatures above 30 °C (Holtzmann, 1965). Resistance to *M. incognita* decreased progressively in Nematex tomato roots as the temperature was increased from 28 to 33 °C (Dropkin, 1969), and was partially repressed at 35 °C in cotton plants (Carter, 1982). High temperatures also modify the hypersensitive necrotic response of resistant tomato roots to *M. incognita.* At 28 °C host cell necroses developed within a few hours after nematodes had entered the roots; in contrast, no necroses occurred in plants infested at 33 °C (Dropkin, 1969).

The breakdown of resistance and subsequent susceptibility of heat treated plants has been explained in terms of accumulation of heat units (Dropkin, 1969; Carter, 1982), but no systematic studies have been made on the effect of temperature on cellular and biochemical diseases in plants attacked by nematodes. The present study was initiated to identify cytological and biochemical changes that

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occurred in VFN 8 tomato seedlings, infested by *M. incognita* (Kofoid *et* White) Chitw. following the loss of resistance at high temperatures.

Materials and Methods

Seedlings of tomato cultivar VFN 8, resistant to *M. incognita*, were grown at 28 °C and transplanted at about 3-4 cm height into 5 cm diameter clay pots containing quartz sterilized sand. The pots were placed in a growth chamber at 28, 31, 34 or 37 °C, 65% RH 5000 lux and watered with Hogland's solution. After a week, the seedlings were inoculated with 200 *M. incognita* larvae and one week later some of the plants grown at 28 and 34 °C were removed. Infested or galled roots were fixed in 3% glutaraldehyde in 0.05 M cacodylate buffer pH 7.2 for 6 hours at 4 °C, rinsed several times in the same buffer and post-fixed in 2% osmium tetroxide for 4 hours at 4 °C, then prestained overnight in 0.5% aqueous uranyl acetate at room temperature. The galls were dehydrated in an ethanol series and embedded in Spurr's medium. Two microns thick sections were examined with a light microscope and ultrathin sections were observed with a Philips 400 T electron microscope.

Twenty grams of roots from plants grown at 28 and 34 °C were homogenized (Zacheo *et al.*, 1983) and the homogenate centrifuged at 600 g x 10 min, the pellet and the supernatant then being collected separately. Mitochondria were precipitated at 7000 g x 20 min and washed twice in a washing medium. The supernatant remaining after the removal of the mitochondria was centrifuged at 30,000 g x 2 hours. Two fractions were obtained: microsomes and a soluble fraction. The pellet collected after centrifugation at 600 g, containing cell debris and nuclei, was washed in 1% Triton x 100 (x2) and water.

The cell wall pellet was then washed three times with 1M NaCl, these washings then constituting the cell wall fraction. Peroxidases were assayed by measuring absorbance at 470 nm using guaiacol and hydrogen peroxide.

Twenty days after inoculation with *M. incognita*, 20 plants from each temperature regime were removed, stained in boiling acid fuchsin-lactophenol and galls and nematodes from each root system were counted. Roots that had been examined were then dehydrated at 65 °C and weighed.

Results

Effect of temperature on plant growth. Dry root weights of VFN 8 seedlings, measured 20 days after inoculation with *M. incognita* larvae, increased from 28 to a maximus of 34 °C, thereafter declining (Fig. 1).

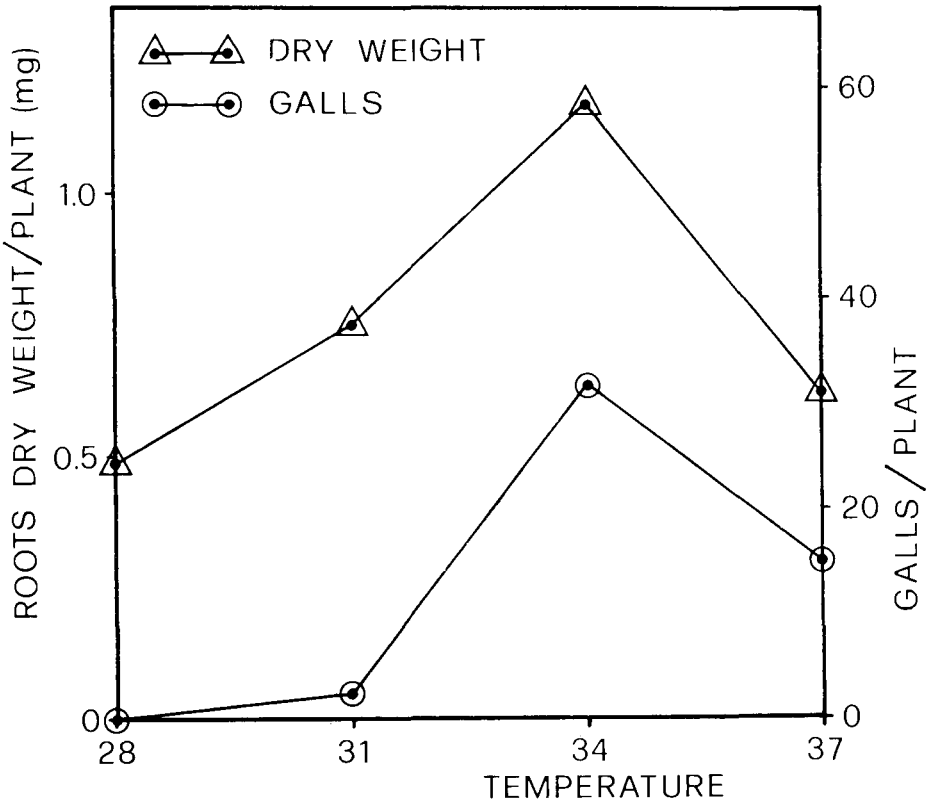

Fig. 1 - Effects of temperature on root growth and root galling. Each point represents the average of twenty plants 20 days after inoculation with M. *incognita*. Temperature varied within ± 0.5 °C of the indicated temperature.

Galling and nematode development. Seedlings were completely resistant to larvae when grown at 28 °C with no galling on the roots. At 31 °C, the presence of few galls on the roots indicated the breakdown of resistance. At 34 °C the largest number of galls was recorded; gall production decreased sharply (about 50%) at temperatures above the optimum (Fig. 1).

The total nematodes (larvae + adults) recovered from roots increased as temperature increased; the number of larvae in the root tissues was greatest at 37 °C. Temperature had considerable effect on the proportion of larvae that developed after penetration. At 28 °C only larvae were recovered from the roots, 20 days after inoculation; at 31 °C, 5% of the larvae that had entered continued development; at 34 °C about 50% of the larvae became females; at 37 °C about 40% had developed to females (Fig. 2). At 34 °C the number of females was not only significantly greater than at other temperatures, but 5% completed their life cycle and produced egg masses.

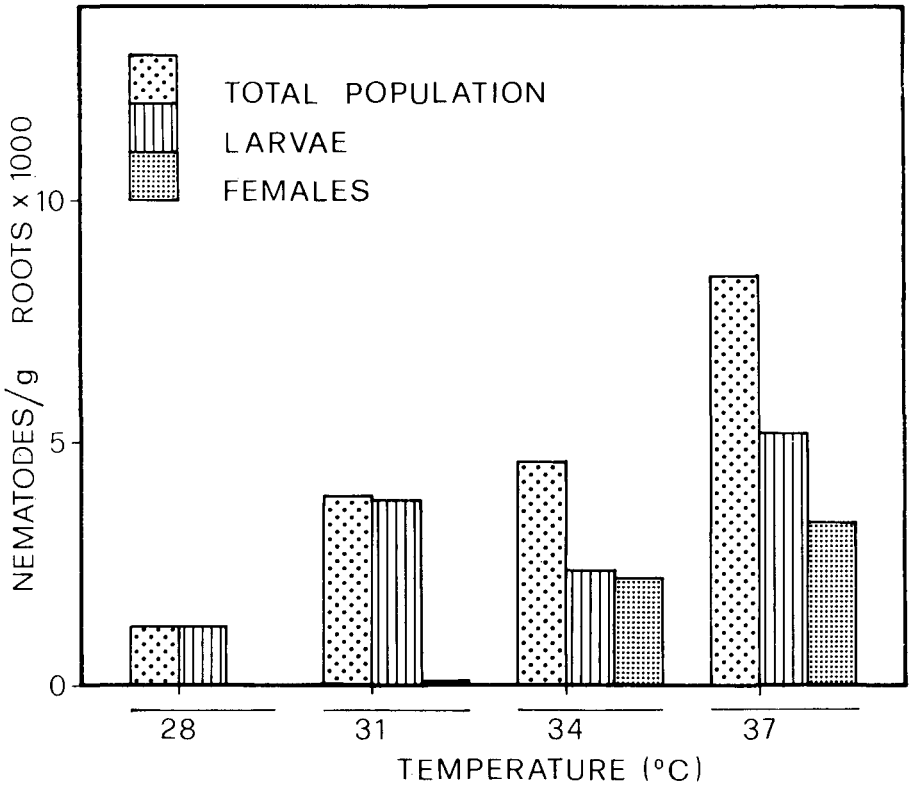

Fig. 2 - Means of total number (larvae + females) of *M. incognita* per g of tomato roots as affected by temperature. Average of twenty plants at each temperature.

Induction of host multinucleate giant cells at 34 °C. Light microscopy studies of galls from infested roots, grown at 34 °C, showed multinucleated giant cells within parenchyma of the vascular tissue (Fig. 3 a, d). This type of cell is very similar to the multinucleate cell induced by *M. incognita* on susceptible tomato roots. However, in the resistant roots, grown under warmer conditions, a reduced number of multinucleate cells (3-4) were found associated with necrotic tissue (Fig. 3 b, c).

In infested cells observed with an electron microscope, differences between reactions in roots grown at 28 and 34 °C were detectable one week after nematode inoculation. In seedlings infested at 28 °C, the hypersensitive reaction had reached a stage where numerous alterations were apparent in the fine structure of the host cells. The cytoplasm of infested cells became electron dense and cellular components could not be distinguished. Hypersensitivity involved only the cells immediately surrounding the nematode; adjacent cells were apparently unaffected. At 34 °C multinucleate cells were present in the tissue surrounding the nematodes. The fine structure of these cells differed little from those described from susceptible roots; cell wall ingrowths were formed next to the vascular elements. Numerous nuclei, mitochondria, profiles of endoplasmic reticulum and ribosomes occurred in the cytoplasm, where the central vacuole was transformed into many small vacuoles. Some adjacent parenchyma cell walls were completely broken down, resulting in a large cavity containing cell debris and evidence of a hypersensitive response.

Peroxidase activity. Following the observations that the resistant cultivar VFN 8 increased peroxidase activities during infestation with *M. incognita* (Zacheo *et al.*, 1982), the activity of these enzymes was tested in healthy and infested plants grown at 28 and 34 °C. At 28 °C a remarkable increase was observed in the peroxidase activity of all the cellular components examined in roots of VFN 8, attacked by nematodes, one week after infestation, compared with the same plants not inoculated. Nematode attack caused about 7-fold increase in the activity of mitochondrial peroxidases, 3-fold in microsomes, 4-fold in cytoplasmic soluble fraction and 2-fold in cell wall fraction salt-extracted. At 34 °C temperature influenced the peroxidases, increasing their activity in healthy plants; no significant changes in enzyme activity of infested plants were detected (Table I).

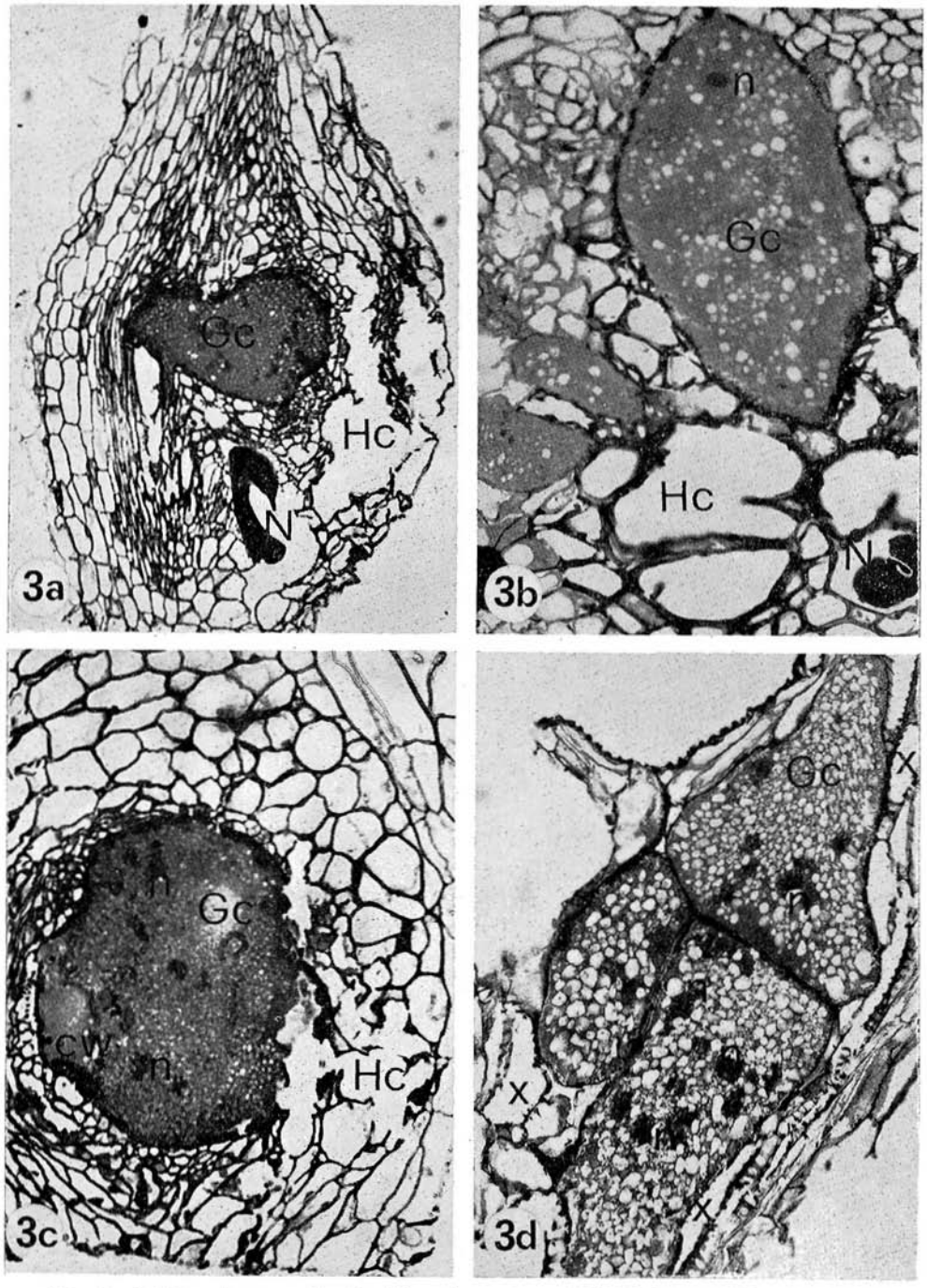

Fig. 3 - Light micrograph of gall-sections from VFN 8 infested by *M. incognita* at 34 °C, one week after infestation. a) cellular responses to infection; nematode is associated with a multinucleate cell and necrotized parenchyma cells (x 400); b) three multinucleate cells next to the vascular tissue and a nematode are evident (x 800); c) well developed giant cell, showing cell wall ingrowths and numerous nuclei, is associated to abnormal xylem tissue (x 800); d) three giant cells delimited by vascular elements (x 800). N = nematode, Gc = giant cell, n = nucleus, Hc = hypersensitive cell, cw = cell wall ingrowth, x= xylem.

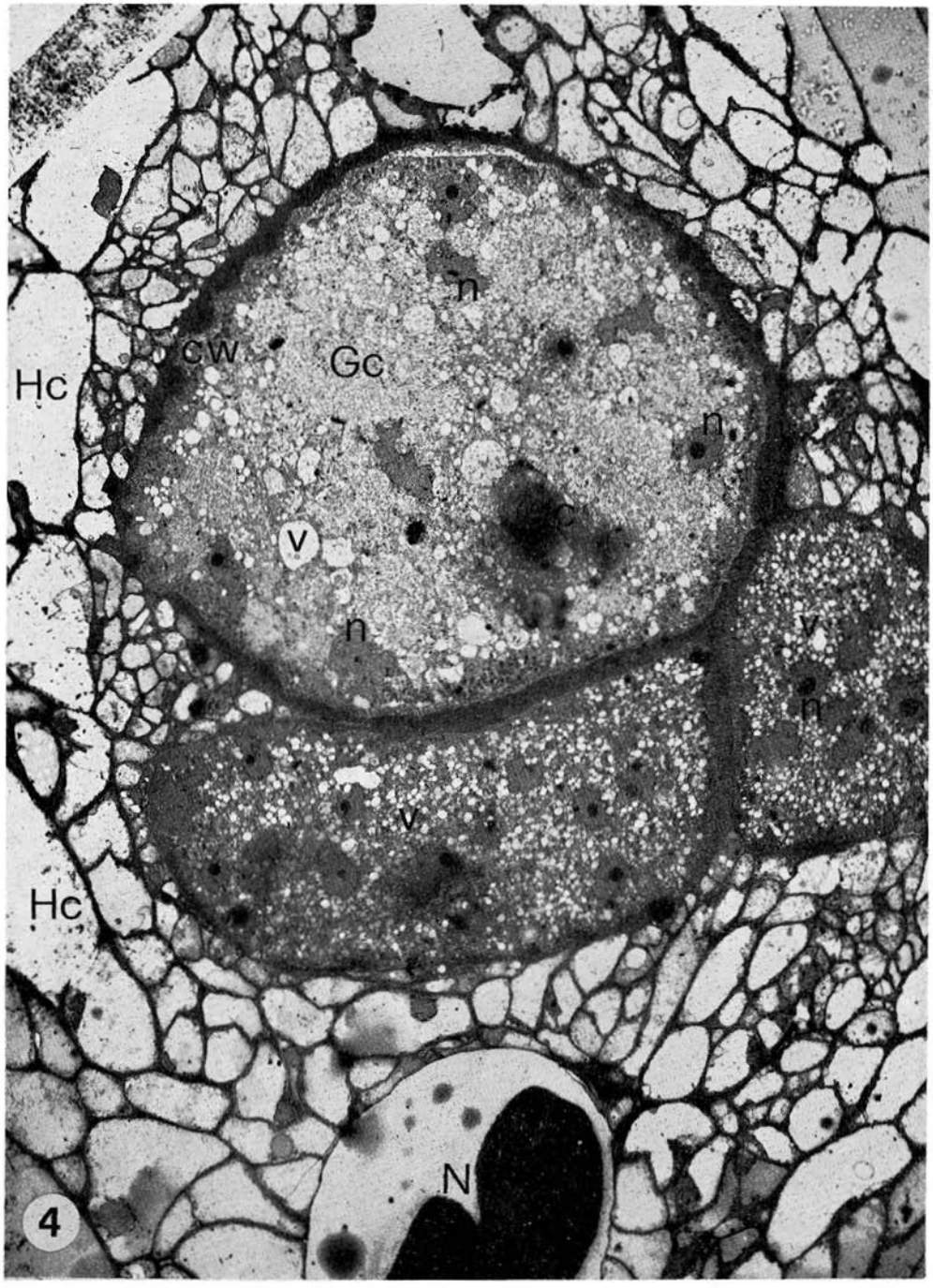

Fig. 4 - Electron micrograph of heat treated root resistant to *M. incognita*, a week after inoculation. Note the presence of hypersensitive reaction (Hc) in the cells adjacent to the giant cells (Gc) which cytoplasm is rich of many small vacuoles (v) x 1600.

Table I - *Effect of temperature on cytoplasmatic and cell wall peroxidase activities in resistant tomato seedlings, seven days after* M. incognita *inoculation. Each value represents the average of four experiments.*

Treatments		Peroxidase as Δ OD/min/mg proteins			
		Mitochondria	Microsomes	Soluble Fraction	Cell Wall Fraction
Roots healthy	28 °C	3.63	3.78	0.48	1.82
Roots infested	28 °C	24.33	13.90	2.18	4.37
Roots healthy	34 °C	5.02	8.89	1.13	5.88
Roots infested	34 °C	6.03	6.63	1.16	7.17

Discussion

The results indicate that the rise of temperature from 28 to 37 °C can induce the breakdown of resistance VFN 8 tomato plants, which become increasingly susceptible to *M. incognita.* The effects of induced susceptibility by temperature elevation in VFN 8 is in agreement with earlier reports (Grundbacker and Stanford, 1962; Dropkin, 1969).

The susceptibility did not appear to be associated with a loss of vigour in the seedlings because, in our experiment, elevated temperature led to an increase in the dry weight of the roots. As temperature increased so did root galling.

This indicates that genetic resistance mechanism are partially repressed at 31 °C and significantly reduced at 34 and 37 °C.

The presence of galls and subsequent formation of multinucleate cells in the invaded tissues support the maturation of nematodes to a significant level. In our studies the highest final population of *M. incognita* occurred at 37 °C but the ratio of penetrated larvae to developed females was highest at 34 °C.

It is suggested that the more favourable conditions for VFN 8 growth and nematode maturation is around 34 °C. The lower ratio larvae-females, obtained at 37 °C, may have been due to the poor condition of the root tissue, at this temperature.

The reduction of host resistance at high temperature was accompanied by a low incidence of necrosis and induction of multinucleate cells in invaded tissues. The reduction in necrosis associated with

nematode infestation, at elevated temperatures, suggests that heat treatment affects the hypersensitive reaction, activating or repressing enzyme systems. Our data show that the loss of resistance is related to the reduced capability of the plants to react to the nematode attack by developing high peroxidase activity (Zacheo *et al.*, 1982).

The heat treatment induced increase in peroxidase activity in healthy plants: we assume that heat induced peroxidases may differ from the peroxidases induced by pathogens. Sanden and Moore (1978) demonstrated four electrophoretic bands of distinct peroxidases in heat treated tobacco plants infected and not. These bands were not present in nonheated extracts.

Because production of free radicals was demonstrated in tomato roots during nematode infestation (Zacheo *et al.*, 1983; Zacheo and Bleve-Zacheo, 1984) and, if the role of peroxidases is to generate oxygen free radicals (Piatt *et al.*, 1977; Klebanoff and Clark, 1978), the decreased activity of peroxidases in heat treated plants may block the production of superoxides. Recent data (Doke, 1983) indicated that superoxide generating system may be activated in potato tissue during the incompatible interaction induced by invading fungi or fungal wall components also that the generation of free radicals may be involved during hypersensitive cell death as a trigger of the sequence of resistance reactions.

SUMMARY

Seedlings of resistant tomato cultivars VFN 8 were grown at 28, 31, 34 and 37 °C and infested with *Meloidogyne incognita*. Resistance of tomato seedlings fell progressively as temperature rose. The total number of nematodes increased as temperature increased; most galling occurred at 34 °C and 50% of the larvae that had invaded the roots completed their development. Necrosis of root tissue was observed only at 28 °C and one week after infestation; at 34 °C gall sections showed multinucleate cell formation, associated with necrotic cells. The loss of resistance in heat treated plants of tomato influenced the behaviour of the peroxidases. At 28 °C infested plants increased enzyme activity, in all cellular components examined, at 34 °C healthy heat treated plants showed increase of peroxidases, but no changes occurred after nematode infestation.

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