Free Phenols and Root Necrosis in Nematex Tomato Infected with the Root Knot Nematode

Charles H. Brueske and Victor H. Dropkin

Associate Professor, Department of Biology, Mount Union College, Alliance, Ohio 44601; and Professor, Department of Plant Pathology, University of Missouri, Columbia, Missouri 65201.

Accepted for publication 30 September 1972.

ABSTRACT

Nematex tomato seedlings are known to be resistant to certain root knot nematodes at 27 C, and susceptible at 32 C. At the lower temperature, brown necrotic areas are usually associated with larval penetration of the roots; at the higher temperature, this necrosis is greatly reduced or is absent. Uninfected and infected seedlings, 4 to 7 days old, were analyzed for changes in free phenol content under both temperature regimes. In the resistant state (27 C), free phenol concentrations declined more rapidly in the infected seedlings than in the corresponding uninfected seedlings. In the susceptible state (32 C), the decline in the free phenol concentration appeared to be correlated with temperature rather than with infection, since free phenol concentrations declined rapidly in control and infected seedlings. Changes in phenolase activity in the roots were also measured in the susceptible and resistant states. An increase in phenolase activity was found in resistant-infected roots and in susceptible-infected and uninfected roots. There was no increase in phenolase activity in the resistant-uninfected roots.

Phytopathology 63:329-334

Phenol metabolism and hypersensitivity in plants have been examined as resistance mechanisms to pathogenic organisms (5, 12, 13). Phenol metabolism as a resistance mechanism to parasitic nematodes is not well defined. Phenolics have been reported to accumulate in the roots of strawberry, celery, and apple parasitized by *Pratylenchus penetrans* (17, 21, 22). The unsuitability of *Nicotiana repanda* as a host for root knot nematodes was linked to the oxidation of chlorogenic acid (14). Roots with high phenol

content develop brown lesions when they are invaded by *P. penetrans*, but the brown lesions do not develop in roots with low phenol content (26). The browning of chrysanthemum leaves, parasitized by *Aphelenchoides ritzemabosi*, was linked to the oxidation of isochlorogenic and chlorogenic acid; however, the oxidation of these compounds was not related to the growth and multiplication of the nematode (24, 25). Giebel (8), surveying 21 species of Solanaceae for resistance to *Heterodera rostochiensis*, found a high ratio of mono- to polyphenols in nonhosts and a low ratio of mono- to polyphenols in host species. An increased concentration of phenolic compounds was found in roots and leaves of tolerant citrus cultivars that had been parasitized by *Radopholus similis* (6). The resistance of Nematex tomato seedlings to *Meloidogyne incognita* Chitwd. can be reversed with cytokinins (4) or an increase in temperature (3). This paper will deal with the quantitative changes of free phenols in relation to root necrosis in Nematex tomato infected with *M. incognita*.

MATERIALS AND METHODS.–Seedlings of *Lycopersicon esculentum* Mill. 'Nematex' were germinated on moist blotters enclosed in plastic bags. At 96 hr after germination, the roots were covered with fine sand and inoculated with a drop of water containing 75-100 newly emerged larvae. Control roots received a drop of water only (3). The roots were then incubated at 27 and 32 C. Nematodes that had not penetrated the roots were removed by washing 24 hr after inoculation. Root tissue was analyzed for free phenols at 0, 6, 12, 24, 48, and 72 hr after inoculation. At each sampling time, several roots were processed in Southard's stain, and larvae within the roots were counted to determine the degree of infection (4).

At each time period, the seedlings were washed in running water and the hypocotyls removed. The fresh weights were determined, and the roots immersed in hot 80% ethanol. Fresh weights ranged from 180 to 450 mg/experimental series. Free phenols were extracted and partitioned into ethyl ether without acid and alkali hydroylsis (10). The ether extract was dried in a stream of air, and the residue dissolved in 1 ml of 95% ethanol.

One hundred μliters of this solution were strip loaded on a thin-layer chromatography (TLC) silica gel plate (0.25 mm) and developed in benezene:acetic acid:water (6:7:3, v/v, upper phase). The air-dried plates were divided into 20 sections. Each section was scraped from the plate and eluted overnight in 80% ethanol. Following elution, the samples were centrifuged and the fluorescence emission measured with a Turner Fluorometer (Model 110) using a 7-60 primary filter, a 2A secondary filter, and a 1X setting. The readings were converted into μg of chlorogenic acid equivalents/100 mg fresh wt.

Phenolase activity was measured in extracts from a series of seedlings germinated and inoculated as previously described. Somewhat higher levels of inoculum were used (ca. 150 larvae/root). The root tissue was collected at 0, 6, 12, and 24 hr after inoculation. The roots were ground with sand in chilled mortars and enzyme extracts prepared as cytoplasmic enzyme (F1), ionically cell wall bound enzyme (F2), and covalently cell wall bound (F3) (18).

The reaction mixture consisted of 2.5 ml chlorogenic acid (20 μg/ml in 0.05 M phosphate buffer, pH 6.0) and 0.5 ml of enzyme solution. The solutions were placed in the cuvet and mixed, and the cuvet was placed in a Beckman DB-G spectrophotometer connected to a continuous recorder. The reaction was followed by the disappearance of chlorogenic acid from solution at 322 nm. The rate of reaction is expressed as ΔOD/min per 100 mg fresh wt.

RESULTS.–Chromatographic analysis of free phenols extracted from control and inoculated root tissue is shown in Fig. 1. At the time of inoculation (0 hr), there were moderate to strong peaks on the chromatograms at the R_F values of 0 to 0.2, 0.3 to 0.4, and 0.6 to 1.0. At 6 hr after inoculation, nematodes had not entered the roots, and differences between control and inoculated roots were not significant. At 12 hr, penetration of the roots had begun at both temperature regimes. Extracts from roots infected at 27 C showed no decline in the concentration of free phenolic compounds when compared to the controls. Root tissue incubated at 32 C showed a general decline in phenolic content in both control and inoculated tissue.

At 24 hr, inoculated roots were well infected with the nematode, and cellular necrosis was evident in roots incubated at 27 C. These roots also showed a marked decrease in free phenolic content. This decrease is most evident at the R_F values of 0 to 0.2 and 0.6 to 1.0 (Fig. 1). Both the control and inoculated tissue in the 32-C regime showed a general decline in phenols. At 48 and 72 hr after inoculation, infected tissue continued to have a lower concentration of free phenols than the control tissue at both 27 and 32 C.

Figure 2 shows the total free phenols (A) and the F1 phenolase (B) in control and inoculated tissue grown at 27 C. There was no measurable phenolase in the F2. In all experiments, phenolase activity in the F3 was unaffected by infection or temperature. Uninfected tissue at inoculation time had a concentration of free phenols at 12 μg/100 mg fresh wt (exp. 1 = 12.9 μg/100 mg; exp. 2 = 11.11 μg/100 mg). During the next 24 hr, the free phenol content decreased to 8.7 μg/100 mg fresh wt. At 48 hr, the concentration stabilized at 9.3 μg/100 mg fresh wt. The concentration of free phenolic compounds in root tissue inoculated with larvae declined at 6 and 12 hr at a rate similar to that of the control tissue. However, between the 12th and 24th hr after inoculation, the concentration of free phenols decreased from 9.2 μg/100 mg to 4.2 μg/100 mg fresh wt. This decrease occurred simultaneously with larval penetration of the root tissue.

Phenolase activity in control and inoculated roots grown at 27 C (Fig. 2-B) decreased during the 12 hr of the experiment, but the phenolase activity in the

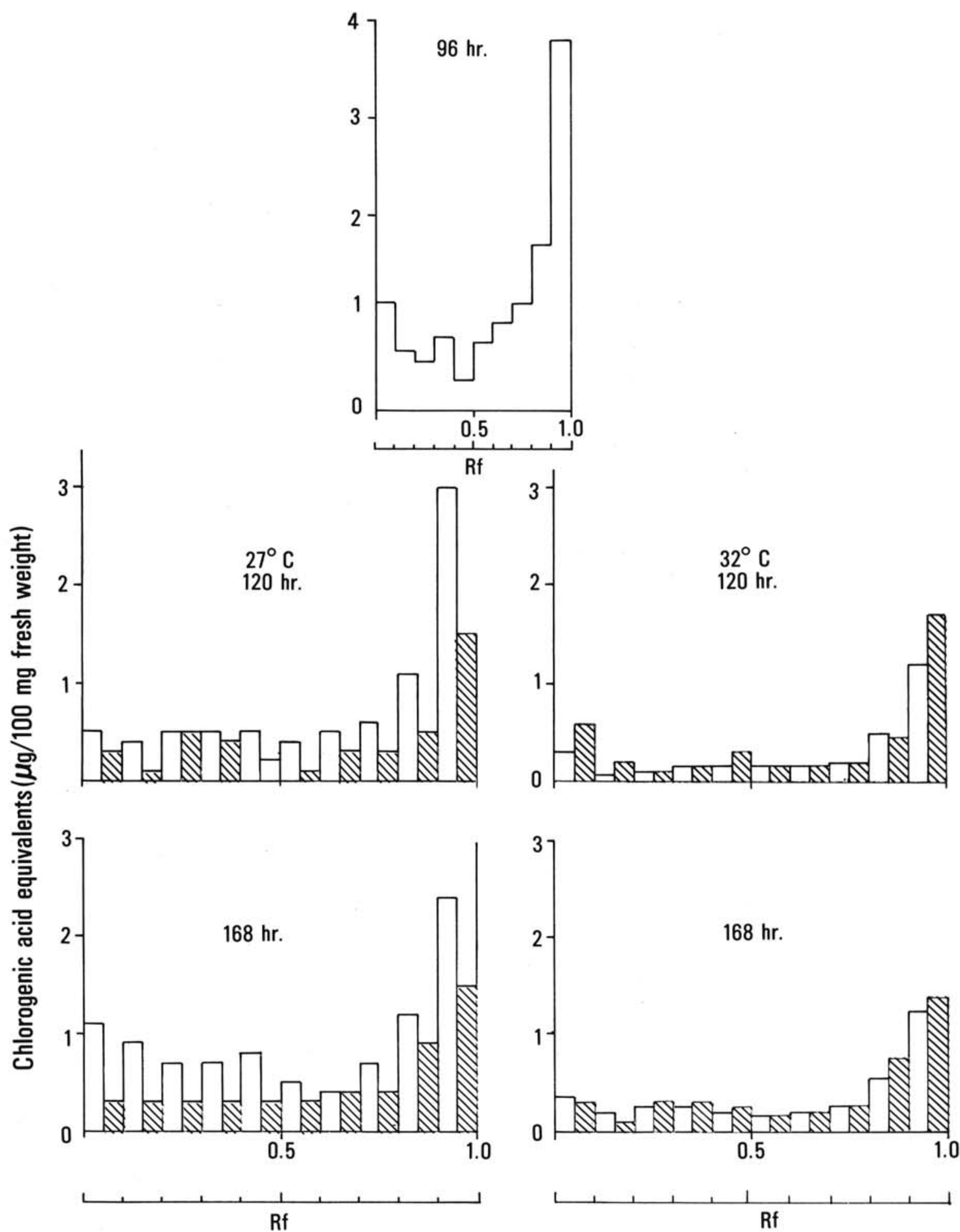
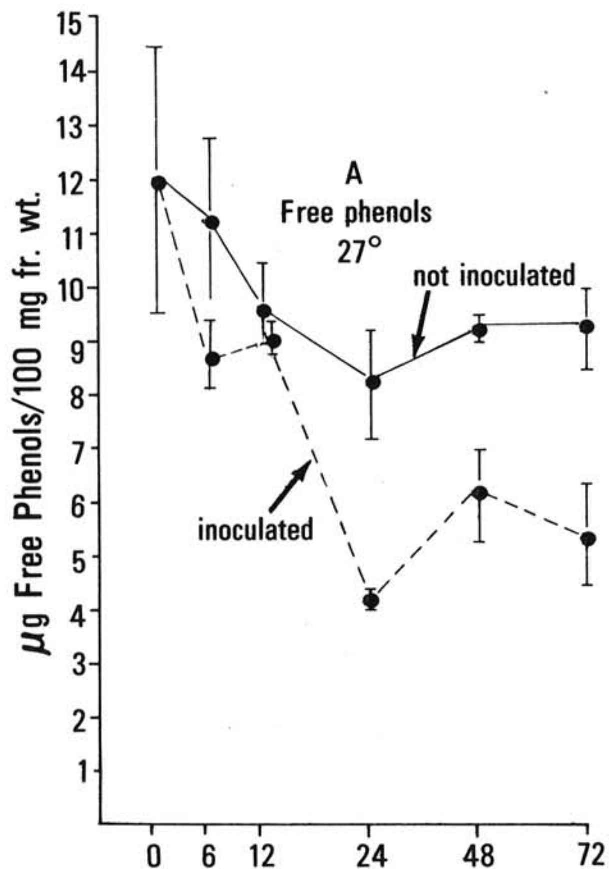
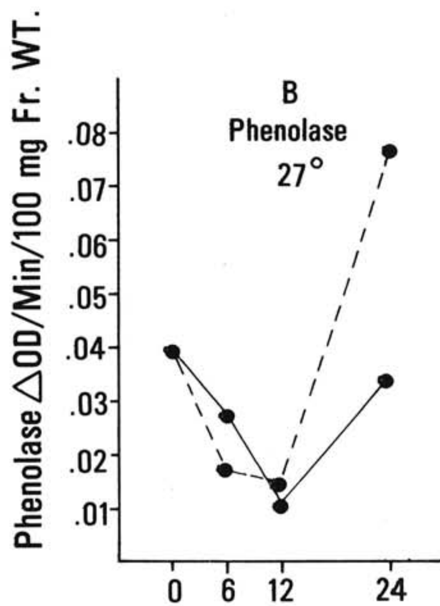


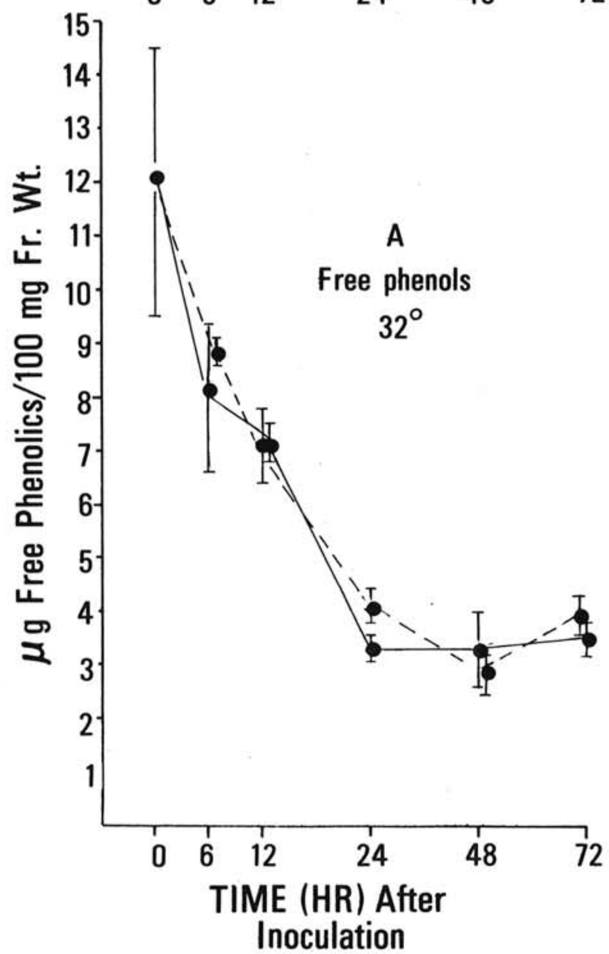
Fig. 1. Chromatographic analysis of phenolic compounds in Nematex tomato roots incubated at 27 and 32 C with and without nematodes; [open box] indicates control; [hatched box] indicates inoculated roots. Time is expressed as elapsed time from the moment of infestation, which occurred at 96 hr after seeding.



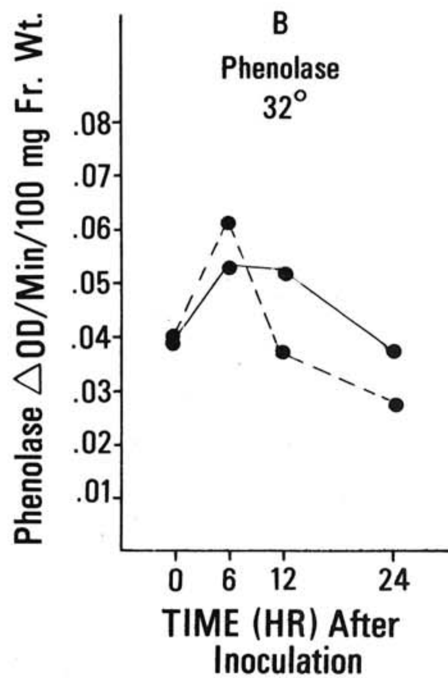
μg Free Phenols/100 mg fr. wt.
A
Free phenols
27°
not inoculated
inoculated
0 6 12 24 48 72



Phenolase △OD/Min/100 mg Fr. WT.
B
Phenolase
27°
0 6 12 24



μg Free Phenolics/100 mg Fr. Wt.
A
Free phenols
32°
0 6 12 24 48 72
TIME (HR) After
Inoculation



Phenolase △OD/Min/100 mg Fr. Wt.
B
Phenolase
32°
0 6 12 24
TIME (HR) After
Inoculation

inoculated roots increased from 0.016 (ΔOD/min per 100 mg fresh wt) at 12 hr to 0.077 at 24 hr. During the same time period, phenolase activity in the control roots increased from 0.01 to 0.036 optical density units.

Total free phenols and F1 phenolase activity in roots grown at 32 C are shown in Fig. 2-C and -D. The concentration of total free phenols declined at a parallel rate in both control and inoculated roots for the first 24 hr, and thereafter remained at a steady state. At 32 C (Fig. 2-D), both the control and inoculated tissue showed an immediate increase in enzyme activity during the first 6 hr of the experiment. There were no larvae present in the tissue at this time. At 12 and 24 hr, the enzyme activity in both tissues had decreased as the concentration of free phenols decreased.

DISCUSSION.–In roots of Nematex tomato seedlings incubated at 27 C (resistant condition), the concentration of free phenols gradually declined as the seedlings aged. In the presence of root knot nematodes, this decline proceeded more rapidly. At 32 C (susceptible condition), the concentration of free phenols in the absence of nematodes declined to lower levels than at 27 C, and the presence of nematodes did not influence the rate of change. There was also a change in relative quantities of free phenol components, particularly in the infected roots incubated at 27 C.

Correlations between phenolase activity and infection occur in other diseases. Van Kammen & Brouwer (23) demonstrated increased phenolase activity in tobacco inoculated with tobacco mosaic virus (TMV). This increase in phenolase activity was found in both inoculated and noninoculated leaves. The induction of tyrosinase activity in TMV-infected tobacco has been demonstrated (11). Specific increases in subcellular phenolase activity occurred in tobacco infected with the tobacco etch virus (15). Hyodo & Uritani (9) reported actinomycin and puromycin suppression of *o*-diphenol oxidase synthesis in sweet potato either injured by slicing or infected with *Phytophthora infestans*. This indicates de novo synthesis of the enzyme.

Plant resistance to parasites may also involve the synthesis of phenolics. The application of phenolic compounds enhanced lesion formation in TMV-infected tobacco (16). The ability of grapefruit seedlings to grow well in the presence of the burrowing nematode was increased with a supply of exogenous phenols (7). Exogenous phenols also increased resistant reactions of potato tissue inoculated with *P. infestans* (19). Ferulic acid and catechin have been reported to increase in cabbage roots infected with *P. penetrans* (1). The high phenol content associated with nematode infections of plants may also reflect high concentrations of endogenous phenols prior to infection. Beckman & Mueller (2) histochemically demonstrated discrete microbodies containing phenolic material in cortical cells of banana roots. Infection of the roots with *Fusarium oxysporum* caused the phenolic compounds to leave the microbodies and mobilize at the site of infection.

Since the combining of phenolase enzyme and substrate and the resulting oxidation of the substrate typically leads to the type of brown discoloration observed at 27 C (20), the absence of this response at 32 C may be related to phenol metabolism. Figures 1 and 2 (C and D) reveal a decrease in the quantity and a change in the composition of free phenols in roots incubated at 32 C. This decrease in phenols occurs in both control and inoculated tissue. The decrease in phenols is also accompanied by an increase in phenolase activity before the entrance of larvae into the root. The decrease in phenolics below a critical level at 32 C is associated with loss of necrosis as a reaction mechanism against the nematode.

LITERATURE CITED

1. ACEDO, J., & R. ROHDE. 1971. Histochemical root pathology of Brassica oleracea capitata L. infected by Pratylenchus penetrans (Cobb) Filipjev and Schuurmans Stekhoven (Nematoda: Tylenchidae). J. Nematol. 3:62-68.
2. BECKMAN, C., & W. MUELLER. 1970. Distribution of phenols in specialized cells of banana roots. Phytopathology 60:79-82.
3. DROPKIN, V. 1969. The necrotic reaction of tomatoes and other hosts resistant to Meloidogyne: reversal by temperature. Phytopathology 59:1632-1637.
4. DROPKIN, V., J. HELGESON, & C. UPPER. 1969. The hypersensitivity reaction of tomatoes resistant to Meloidogyne incognita: Reversal by cytokinins. J. Nematol. 1:55-61.
5. FARKAS, G., & Z. KIRÁLY. 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. Phytopathol. Z. 44:105-150.
6. FELDMAN, A., & R. HANKS. 1968. Phenolic content in the roots and leaves of tolerant and susceptible citrus cultivars attacked by Radopholus similis. Phytochemistry 7:5-12.
7. FELDMAN, A., & R. HANKS. 1971. Attempts to increase tolerance of grapefruit seedlings to the burrowing nematode (Radopholus similis) by application of phenolics. Phytochemistry 10:701-709.
8. GIEBEL, J. 1970. Phenolic content in roots of some Solanaceae and its influence on IAA-oxidase activity as an indicator of resistance to Heterodera rostochiensis. Nematologica 16:22-32.
9. HYODO, H., & I. URITANI. 1966. The inhibitory effect of some antibiotics on increase in o-diphenol oxidase activity during incubation of sliced sweet potato tissue. Agr. Biol. Chem. 30:1083-1086.
10. IBRAHIM, R. K., & G. H. N. TOWERS. 1960. The identification, by chromatography, of plant phenolic acids. Arch. Biochem. Biophys. 87:125-128.

←

Fig. 2. Free phenolic content (A) and phenolase activity (B) in Nematex tomato seedlings inoculated with root knot nematodes and incubated at 27 C. Free phenolic content (C) and phenolase activity (D) in Nematex tomato seedlings inoculated with root knot nematodes and incubated at 32 C; —— indicates control tissue; --- indicates inoculated tissue.

11. JOHN, V., & M. WEINTRAUB. 1967. Phenolase activity in Nicotiana glutinosa infected with tobacco mosaic virus. Phytopathology 57:154-158.
12. KLEMENT, A., & R. GOODMAN. 1967. The hypersensitive reaction to infection by bacterial plant pathogens. Annu. Rev. Phytopathol. 5:17-44.
13. KOSUGE, T. 1969. The role of phenolics in host response to infection. Annu. Rev. Phytopathol. 7:195-222.
14. MILNE, D., D. BOSHOFF, & P. BUCHAN. 1965. The nature of resistance of Nicotiana repanda to the root-knot nematode, Meloidogyne javanica. South Afr. J. Agr. Sci. 8:557-570.
15. NYE, T., & R. HAMPTON. 1966. Biochemical effects of tobacco etch virus infection on tobacco leaf tissue – II. Polyphenol oxidase activity in subcellular fraction. Phytochemistry 5:1187-1189.
16. PARISH, C., M. ZAITLIN, & A. SIEGEL. 1965. A study on necrotic lesion formation by tobacco mosaic virus. Virology 26:413-418.
17. PITCHER, R., Z. PATRICK, & W. MOUNTAIN. 1960. Studies on the host-parasite relations of Pratylenchus penetrans (Cobb) to apple seedlings. Nematologica 5:309-314.
18. RIDGE, I., & D. OSBORNE. 1971. Role of peroxidase when hydroxyproline-rich protein in plant cell walls is increased by ethylene. Nature (New Biology) 229:205-208.
19. SAKUMA, T., & K. TOMIYAMA. 1967. The role of phenolic compounds in the resistance of potato tuber tissue to infection by Phytophthora infestans. Ann. Phytopathol. Soc. Japan 33:48-58.
20. SHIROYA, M., T. SHIROYA, & S. HATTORI. 1955. Studies on the browning and blackening of plant tissues. IV. Chlorogenic acid in the leaves of Nicotiana tabacum. Physiologia Plantarum 8:594-605.
21. TOWNSHEND, J. 1963. The pathogenicity of Pratylenchus penetrans to strawberry. Can. J. Plant Sci. 43:75-78.
22. TOWNSHEND, J. 1963. The pathogenicity of Pratylenchus penetrans to celery. Can. J. Plant Sci. 43:70-74.
23. VAN KAMMEN, A., & D. BROUWER. 1964. Increase of polyphenoloxidase activity by a local virus infection in uninoculated parts of leaves. Virology 22:9-14.
24. WALLACE, H. 1961. Browning of chrysanthemum leaves infested with Aphelenchoides ritzemabosi. Nematologica 6:7-16.
25. WALLACE, H. 1961. The nature of resistance in chrysanthemum varieties to Aphelenchoides ritzemabosi. Nematologica 6:49-58.
26. ZUCKERMAN, B. 1971. Gnotobiology, p. 159-184. *In* B. M. Zuckerman, W. F. Mai, & R. A. Rohde [ed.]. Plant parasitic nematodes, Vol. II. Academic Press, New York.