Phenol Levels in Leaves of Tomato Cultivars Infected with Pratylenchus penetrans[1]

PAUL FRIEDMAN and R. A. ROHDE[2]

The accumulation of phenols in lesions has been shown to accompany injury by *Pratylenchus penetrans* (Cobb) Chitwood & Oteifa in roots of a number of plant species (1, 4, 6). The presence of these compounds is thought to influence the activity of nearby cells in the root cortex and endodermis as well as to inhibit nematode activity (6).

Investigations into the physiology of nematode-infected plants have been directed almost exclusively toward the metabolic changes occurring in the infected root system. So far, little attention has been given to the metabolic changes occurring above the crown when nematodes are infecting roots. In this study, the possibility that altered phenol metabolism occurred in the leaves of tomato plants infected with *P. penetrans* was investigated.

Tomato (*Lycopersicon esculentum* Mill.) culitvars used in this project were 'Manalucie', 'Rutgers' (suitable hosts of *P. penetrans*), and 'Valiant' (poor host of *P. penetrans*). *Pratylenchus penetrans* was cultured aseptically in alfalfa callus tissue grown in nutrient agar containing 2, 4-dichlorophenoxyacetic acid (2, 4-D) (5). Greenhouse-grown plants of each tomato cultivar were inoculated, after the appearance of the second set of true leaves, by pipetting a suspension of nematodes into the root zone. Noninoculated plants served as controls.

Four grams fresh weight of leaf tissue were added to 200 ml of distilled water, boiled for 20 min, and the suspension filtered through Whatman No. 41 filter paper. Free phenols were extracted with 100 ml of ethyl acetate (reagent) by continuous shaking for 1 h. The water layer was then removed, acidified to pH 2.0 with 1 N HCl, and placed in a boiling water bath for 1 h. Bound phenols were then extracted from the acidified water layer with 100 ml of ethyl acetate. The ethyl acetate extracts were concentrated to 5 ml in a rotary evaporator at 70-72 C.

Samples (50 μl) of these crude extracts were chromatographed on cellulose thin-layer chromatography (TLC) plates (EM Laboratories Inc., Elmsford, N.Y.) with butanol:acetic acid:water 6:1:2 (V:V:V) in the first direction and 7% acetic acid: 0.03% sodium acetate in the second. After being dried at room temperature and exposed to NH_3, the chromatograms were observed and photographed under ultraviolet light (254 and 360 nm). The following chromogenic reagents were also used for the detection of phenolic compounds on thin-layer plates: reagent I—a mixture containing 6 ml of a 10% solution of sodium tungstate, 6 ml of a 5% solution of trichloroacetic acid, and 3 ml of 0.5 N hydrochloric acid to which 6 ml of freshly prepared 5% sodium nitrate solution were added; reagent II—0.5 N sodium hydroxide solution (2).

Ethyl acetate extracts of 1 ml were evaporated to dryness and reconstituted in 20 ml of 90% methanol. Total bound and free phenols were determined from methanol fractions by a modification of the Folin-Ciocalteu method (3). Results are reported as μg equivalents of chlorogenic acid/gram fresh weight of tissue.

The bound phenol content in leaves of nematode-infected plants 28 and 42 days after inoculation increased in all cultivars (Table 1). Although there appeared to be small differences in the free phenol content of nematode-infected plants, these differences were not significant at the 5% level.

When examined on TLC (Fig. 1), extracts from healthy and diseased plants contained the same bound phenols. On the basis of chromatographic behavior and reaction to chromogenic reagents, the major fluorescing spots were tentatively identified as chlorogenic acid and its isomers (isochlorogenic and neochlorogenic acid).

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[2]Graduate Research Assistant and Professor, respectively. Department of Plant Pathology, University of Massachusetts, Amherst, MA 01002. Present address of senior author, Department of Nematology, University of California, Riverside 92521. The authors thank Dr. N. T. Keen for his suggestions in the preparation of this manuscript.

TABLE 1. Total bound and free phenols in leaves of healthy and *Pratylenchus penetrans*-infected tomato plants.

Cultivar	(μg/gm fresh weight)[a] Bound phenols[b, c] Healthy	Diseased	D/H[d]	Free phenols[b, c] Healthy	Diseased	D/H[d]
Experiment 1[e]						
Manalucie	245.3	256.0*	104	19.5	17.8	91
Rutgers	91.6	108.5**	118	22.8	18.0	80
Valiant	218.6	265.1**	121	27.0	25.0	92
Experiment 2[f]						
Manalucie	232.5	263.6**	113	4.0	2.7	67
Rutgers	195.0	254.3**	130	6.5	7.0	108

[a]Values reported as chlorogenic acid equivalents.
[b]Total phenols were determined from reconstituted methanol fractions.
[c]Each value represents the average of 6 replications of one sample. Leaves from 5 plants were combined to form each sample. Asterisks * and ** indicate difference from control at $P=0.05$ and 0.01, respectively.
[d]Percent of phenol levels of diseased plants with respect to phenol levels of healthy controls.
[e]Phenol levels determined 28 days after inoculation. 'Manalucie' and 'Rutgers' were inoculated with 10,000 *P. penetrans*. 'Valiant' was inoculated with 4,500 *P. penetrans*.
[f]Phenol levels determined 42 days after inoculation. 'Manalucie' and 'Rutgers' were inoculated with 2,200 *P. penetrans*.

Other phenolic compounds were detected; however, their specific identity was not determined. When TLC plates were observed visually, some of the fluorescing spots in leaf extracts from healthy and nematode-infected plants were of similar intensity while others were more intense in the latter.

Although the root and shoot weights and appearance of infected plants were similar to healthy plants, higher levels of bound phenols were detected in leaves of nematode-infected plants. This foliar accumulation of bound phenols may be due to translocation from the roots or to increased production in the leaves as a result of a general alteration of plant metabolism following root infection. The concentration of bound phenols at a considerable distance from the infection site may represent a systemic response to nematode infection.

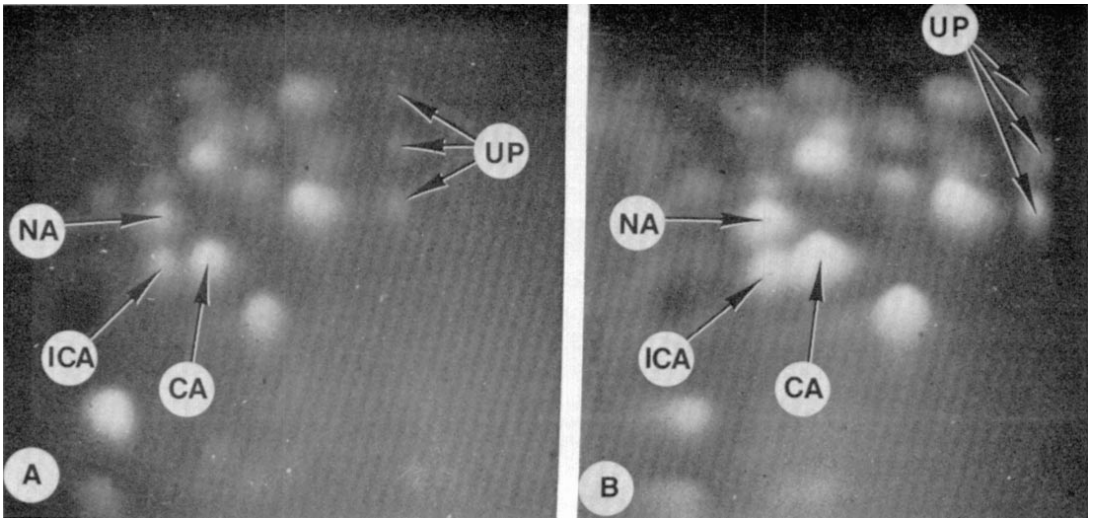

FIG. 1-(A-B). Chromatograms of bound phenols of 'Valiant' tomato 28 days after inoculation with 4,500 *Pratylenchus penetrans*. *A)* Healthy. *B)* Diseased. Chlorogenic acid (CA), neochlorogenic acid (NA), isochlorogenic acid (ICA), and the unidentified phenols (UP) are more intense in the diseased extracts.

The small differences in phenol levels seems to indicate that this response is not a major factor in the disease syndrome.

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