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INFLUENCE OF THE LESION NEMATODE, *PRATYLENCHUS PENETRANS* (COBB) ON THE PHYSIOLOGY OF LEAVES OF THREE TOMATO CULTIVARS AND THE EFFECT OF EXOGENOUS PHENOL APPLICATIONS ON PENETRATION

A Thesis Presented

By

PAUL ALAN FRIEDMAN

Submitted to the Graduate School of the University of Massachusetts in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

September 1975

Plant Pathology

INFLUENCE OF THE LESION NEMATODE, *PRATYLENCHUS PENETRANS* (COBB) ON THE PHYSIOLOGY OF LEAVES OF THREE TOMATO CULTIVARS AND THE EFFECT OF EXOGENOUS PHENOL APPLICATIONS ON PENETRATION

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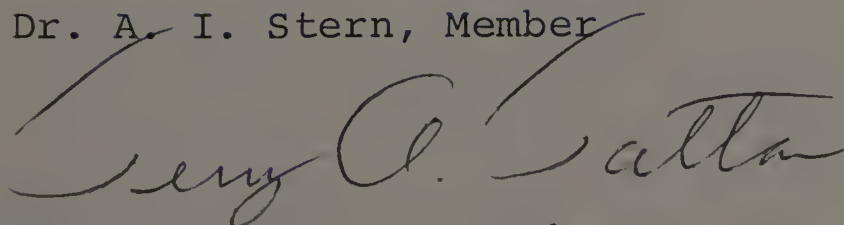

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TABLE OF CONTENTS

	Page
ABSTRACT	1
INTRODUCTION	2
MATERIALS AND METHODS	
Growth of hosts and pathogen	5
Determination of total soluble phenols	5
Chromatography and quantitative determination of free and bound phenols	6
Determination of total chlorophyll and carotenoid	7
Determination of photosynthetic competence-photoreduction	7
Exogenous application of phenolic compounds	8
RESULTS	
Total soluble phenols	10
Chromatography and total free and bound phenols	10
Total chlorophyll and carotenoid and photosynthetic competence	12
Exogenous application of phenolic compounds	13
DISCUSSION	14
LITERATURE CITED	18
TABLES	21
FIGURES	27

ABSTRACT

Manalucie, Rutgers, and Valiant tomatoes were inoculated with axenically cultured *Pratylenchus penetrans*. Chromatographic analysis (TLC) and total phenol determinations (Folin-Ciocalteu method) were conducted on leaf extracts of both healthy and *P. penetrans* infected tomato cultivars. No significant differences were observed in total methanol soluble phenols. In contrast, *P. penetrans* infection resulted in significant increases of 4-30 percent in amounts of bound phenols. Significant decreases in free phenol content from 11 to 2 percent of total (free and bound) phenols however were found to be only a function of increasing plant age. *P. penetrans* infection did not influence the photosynthetic process as measured by chlorophyll and carotenoid content and photosynthetic competence, of the three tomato cultivars examined. Exogenous application of phenols, tyrosine at rates of 10 and 100 ppm and chlorogenic acid at a rate of 100 ppm, increased the susceptibility of Valiant and Rutgers tomato seedlings to *P. penetrans* infection. Phenol applications did not influence the size of lesions or the average number of nematodes per lesion.

INTRODUCTION

The accumulation of phenolic compounds and their relation to the infection process has been observed and outlined in a number of nematode-host-plant interactions. The lesion nematode, *Pratylenchus penetrans*, has been shown to induce phenol accumulation resulting in the discoloration and necrosis of the epidermis, cortex, and endodermis of cabbage, alfalfa, peach, apple, carrot, celery and strawberry (1,5,16,17,19,22,23). Higher amounts of phenols were found in the endodermis for each of these host-parasite interactions and this tissue appeared to act as a barrier to nematode entry into the plant's vascular system during early stages of infection. Another migratory endoparasite, *Radopholus similis*, has been observed to induce resistant reactions and accumulation of phenols in certain host plants. *R. similis* infection of resistant citrus cultivars resulted in a 27-300 percent increase in the bound phenolics in the roots, while in the roots of susceptible cultivars the bound phenolics were reduced 16-34 percent (9).

Investigations into the physiology of nematode infected plants have been directed almost exclusively to

those physiological changes occurring in the infected root system. So far, little attention has been given to the physiological changes occurring in the aboveground plant parts when nematodes are infecting roots. Hanks and Feldman (8,11,12) have observed quantitative changes in the amounts of growth substances, amino acids, and organic acids in leaves, thus suggesting a systemic response in citrus cultivars whose roots were infected with the burrowing nematode, *R. similis*. Both healthy resistant and healthy susceptible citrus cultivars maintained essentially the same kinds and amounts of phenolic compounds in the leaves. However, higher amounts of free phenols were found in the leaves of resistant citrus cultivars infected with *R. similis* than in infected susceptible cultivars. Thus, there appeared to be an accumulation of free phenols in the resistant hosts at a site considerably distant from the infection site. This increase in phenols formed post-infectionally is postulated by Feldman and Hanks to be one of the factors involved in the observed resistance to *R. similis* (9).

In a subsequent study, attempts were made to increase the resistance of *R. similis*-susceptible citrus cultivars through exogenous application of phenolic compounds. Treatments with specific phenolic compounds, e.g., vanillic

acid, were found to increase the individual and total phenolic content in the roots of susceptible citrus cultivars infected with _R. similis_. Phenolic treatments tended to reduce the total phenolic content of healthy seedlings. Although the pathogen was not entirely eliminated from infected root systems there did appear to be some suppression of _R. similis_ activity and essentially normal plant development was maintained (10).

Although injury to root systems, i.e., necrosis and lesion formation, caused by _P. penetrans_ and _R. similis_ are similar, no attention has been given to the metabolism of leaves when _P. penetrans_ is the infecting nematode. This study attempted to ascertain: (i) whether a systemic response for phenol accumulation occurs in the leaves of tomato cultivars when infected with _P. penetrans_; (ii) if _P. penetrans_ infection influenced photosynthetic processes, as measured by chlorophyll and carotenoid content and photosynthetic competence (photoreduction) of tomato cultivars, and (iii) if phenolic compounds added exogenously to tomato cultivars influenced root penetration by _P. penetrans_.

MATERIALS AND METHODS

Growth of hosts and pathogen

The tomato (*Lycopersicon esculentum* Mill.) cultivars used in this study were 'Manalucie' (resistant to *Meloidogyne incognita*), 'Rutgers' (suitable host of *Pratylenchus penetrans*), and 'Valiant' (poor host of *P. penetrans*). *P. penetrans* (Cobb) was cultured asceptically in alfalfa callus tissue grown on nutrient agar containing 2,4-D (15). Greenhouse grown plants from 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 of each plant. Uninoculated plants from each cultivar served as controls.

Determination of total soluble phenols

Methanol extracts were prepared from 0.25 g fresh weight of leaf tissue by grinding each sample in hot (50-55 C) 90 percent methanol for 150 sec at medium speed in a Virtis Homogenizer. Extracts were filtered through Whatman No. 1 filter paper and brought to a final volume of 10 ml. Total soluble phenols were determined on a 0.5 ml sample by a modification of the Folin-Ciocalteu method (13). Results are reported as mg equivalents of chlorogenic acid per gram fresh weight of tissue.

Chromatography and quantitative determination of free and bound phenols

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

Samples (50 uliters) of these extracts were chromatographed on cellulose thin-layer chromatography (TLC) plates with butanol:acetic acid:water 6:1:2 in the first direction and 7% acetic acid:0.03% sodium acetate in the second. After drying at room temperature the chromatograms were observed and photographed under ultraviolet light after exposure to NH_3 vapor. The following chromogenic reagent was 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, and to it added 6 ml of freshly prepared 5% sodium nitrate solution. Reagent II - 0.5 N sodium hydroxide solution (4).

Presence of phenols were detected with chromogenic reagent, however specific phenolic compounds were not identified.

Samples (1 ml) of ethyl acetate extracts 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 (13). Results are reported as ug equivalents of chlorogenic acid per gram fresh weight of tissue.

Determination of total chlorophyll and carotenoid

Acetone extracts were prepared from 0.25 g fresh weight of leaf tissue by grinding each sample in cold 100 percent acetone plus 10 mg $MgCO_3$ for 180 sec at medium speed in a Virtis Homogenizer. The liquid phase was adjusted with distilled water to a final concentration of 80 percent acetone (V/V). Total chlorophyll was determined spectrophotometrically by the method of Arnon (2). Total carotenoid was determined by employing an average extinction coefficient of 2500 $cm^2 \times 10^2 g^{-1}$ at 475 nm (21).

Determination of photosynthetic competence-photoreduction

Chloroplasts were prepared by homogenizing 5 g fresh weight of leaf tissue at 0 to 4 C in a Waring Blender for 30 sec at top speed in a medium containing 80 ml STN (0.2 M sucrose, 0.05 M Tris, and 0.1 M NaCl) adjusted to pH 8.0 with HCl. The homogenate was filtered through a double

layer of cheesecloth and subjected to differential centrifugation. The filtrate was centrifuged at 0 to 2 C for 90 sec at 600 xg. The resulting supernatant was centrifuged for 7 min at 1000 xg and the pellet was resuspended in STN. The suspension was centrifuged for 7 min at 1000 xg and the chloroplast pellet resuspended in 3 ml of STN.

The light reactions were performed using procedures described by Howes and Stern (14). The standard reaction mixture contained in micromoles: Tris, (pH 8.0) 45; KCl, 40; $MgCl_2$, 8; Na, K Phosphate (pH 8.0), 24; ADP (pH 8.0), 4; $K_3Fe(CN)_6$, 15; chloroplasts containing 35 to 80 ug chlorophyll, and distilled water to bring the final volume to 3 ml. Photoreduction of ferricyanide was measured by the colorimetric determination of Avron and Shavit (3). The rate of ferricyanide reduction is expressed as net photoreduction, i.e., light minus dark controls.

<u>Exogenous application of phenolic compounds</u>

Seeds of two tomato cultivars 'Rutgers' and 'Valiant', were heat treated in a hot (52 C) water bath for 10 min, surface sterilized for 15 min in a solution (1:1) 5.25% sodium hypochlorite and distilled water, and rinsed in sterile distilled water. The sterilized seeds were germinated on nutrient agar plates supplemented at the rates of 10 and 100 ppm with tyrosine and chlorogenic acid.

P. penetrans were axenically cultured on alfalfa callus. The nematodes were aseptically extracted from infected callus, placed 24 hr on a 1.5 cm plug of non-absorbent cotton in contact with 10 ml of distilled water in a previously autoclaved, foil capped, 15 ml centrifuge tube (1). Nematode suspensions were pipetted over the roots of 4 day old tomato seedlings. Inoculated seedlings and inoculated controls (without phenolic supplement) were maintained at 24 C.

Roots were fixed and stained 24-36 hr after inoculation by the following procedure: (i) fixation and staining for 2 hr in equal parts of glacial acetic acid and 95% ethanol containing 0.0175 mg/ml acid fuchsin; (ii) clearing for 12 to 24 hr in saturated chloral hydrate; (iii) final preservation and examination in clear lactophenol solution (7).

RESULTS

Total soluble phenols

Leaves of nematode-infected Manalucie, Rutgers, and Valiant tomato plants did not exhibit any significant differences in amounts of total soluble phenols when compared to control plants (Figs. 1,2,3,4 and Table 1). This finding was consistent with all cultivars regardless of inoculum level (1400-10,000 *P. penetrans*) or length of the infection period (14-42 days).

Chromatography and total free and bound phenols

Thin layer chromatograms of free phenols revealed ultraviolet-fluorescing spots at the same locations for all three cultivars. Within each cultivar the fluorescing spots were observed at the same locations regardless of whether the plants were healthy or diseased. The major fluorescing spots were identified as phenolic compounds after TLC plates were reacted with the chromogenic reagent. Chromatograms of free phenols revealed different and fewer fluorescing spots than did chromatograms of bound phenols, and free phenols made up a small percentage of the total (free and bound) phenol content.

Chromatograms of bound phenols also revealed fluorescing spots at the same locations for all three cultivars. Within each cultivar the spots were observed at the same locations regardless of whether the plants were healthy or diseased. The major fluorescing spots were identified as phenolic compounds after TLC plates were reacted with the chromogenic reagent. Several spots on chromatograms of bound phenols of all cultivars were observed to have increased in size and intensity of fluorescence as a consequence of *P. penetrans* infection (Figs. 5-7). Chromatograms of free phenols did not exhibit similar changes.

Free phenol content 28 days after inoculation of healthy and diseased leaves averaged 13 and 9 percent respectively of the total (free and bound) phenolic content for all tomato cultivars. Free phenol content 42 days after inoculation of healthy and diseased leaves averaged 2 percent of the total (free and bound) phenolic content for all tomato cultivars (Tables 2 and 3). The total bound phenols in the leaves of Manalucie 28 and 42 days after inoculation were increased 4 and 13 percent respectively over controls, whereas the total free phenols of healthy and diseased leaves remained relatively unchanged. The total bound phenols in the leaves of Rutgers 28 and 42 days after inoculation were increased 18 and 30 percent respectively over controls. The total free phenol levels of

healthy and diseased leaves of Rutgers 28 and 42 days after inoculation remained relatively unchanged. Leaves of Valiant 28 days after inoculation exhibited an increase of 21 percent in total bound phenols while total free phenols of diseased leaves were not significantly changed when compared with control levels.

Total chlorophyll and carotenoid and photosynthetic competence

Leaves of *P. penetrans* infected Manalucie, Rutgers, and Valiant tomato plants did not exhibit any significant differences in total chlorophyll and carotenoid 28 days after inoculation when compared with control plants (Table 4). The chlorophyll:carotenoid molar ratios for all cultivars averaged 3.21 and 3.14 for healthy and diseased plants respectively. These values were consistent with values previously reported for other plants (18).

The photosynthetic competence, i.e., the photoreduction of ferricyanide, of healthy and nematode-infected plants was essentially the same for all cultivars (Table 5). Determinations of Hill activity in healthy plants yielded an average rate of 182.3 umoles ferricyanide reduced/mg chlorophyll·hr, with Manalucie exhibiting a rate of 145.2 umoles ferricyanide reduced/mg chlorophyll·hr. An average rate of 193.0 umoles ferricyanide reduced/mg chlorophyll·hr was determined for nematode infected plants of the three

cultivars. These values were within the range, 20-430 umoles ferricyanide reduced/mg chlorophyll·hr, reported in a study on Hill activity of 15 genera of broadleaf spermatophytes (20).

Exogenous application of phenolic compounds

Valiant and Rutgers tomato seedlings exhibited increased susceptibility to *P. penetrans* infection when treated with tyrosine at rates of 10 and 100 ppm and chlorogenic acid at a rate of 100 ppm (Table 6). Chlorogenic acid (10 ppm) treated Valiant and Rutgers tomato seedlings exhibited comparable susceptibility to control seedlings. Size of lesions and average number of nematodes per lesion were comparable for all treatments. Lesions averaged in length 337 and 332 microns for Valiant and Rutgers tomato seedlings respectively. Valiant and Rutgers tomato seedlings averaged 3.3 and 2.4 nematodes per lesion respectively.

DISCUSSION

Leaves of nematode-infected Manalucie, Rutgers, and Valiant tomato plants did not exhibit any significant reductions or accumulations in amounts of total methanol soluble phenols. In contrast, chromatographic analysis and total phenol determinations of ethyl acetate extracts revealed significant increases in the amounts of bound phenols present in the leaves of nematode-infected plants. Comparable increases were observed for all three cultivars but did not appear to be a function of inoculum level or length of the infection period. These observations suggest that the increases in bound phenols were masked in the total methanol soluble phenol determinations. Chromatographic analysis and total phenol determinations of ethyl acetate extracts of all cultivars did not reveal significant differences in the amounts of free phenols present in the leaves of healthy and nematode-infected plants. The significant decreases observed in free phenol content of healthy and nematode-infected plants 42 days after inoculation were not a result of nematode infection, but appeared to be a function of increasing plant age.

Separation of leaf extracts into free and bound phenols has provided a method for the more accurate measurement of changes occurring in phenol content.

Feldman and Hanks (9) have observed increases in amounts of free phenols in leaves of resistant citrus cultivars infected with R. similis. This mobilization and accumulation of free phenols at a site considerably distant from the infection site appeared to be characteristic of this disease and suggested a systemic response to infection. Phenols have been observed to accumulate at the infection site of host plants when P. penetrans is the infecting nematode (1,5,16,17,19,22,23). The observed increase of bound phenols in the leaves of tomato cultivars also suggests a systemic response to P. penetrans infection of roots.

The Manalucie treatment suggests an enhancement in photosynthetic competence as a consequence of P. penetrans infection. However, when all treatments were compared the photosynthetic competence of Manalucie healthy plants was lower than the photosynthetic competence of healthy and nematode-infected plants of the other cultivars. This apparent difference in the Manalucie treatment was not considered to be a result of nematode infection. Thus the photosynthetic competence of healthy and nematode-infected plants was essentially the same for all cultivars.

The absence of any significant differences in photosynthetic competence suggests that the structural integrity of the chloroplasts and pigments was maintained during the infection period. The absence of any significant differences in amounts of total chlorophyll and carotenoid further supports this observation. It is apparent that *P. penetrans* infection did not influence the photosynthetic processes investigated.

Size of lesions and numbers of nematodes per lesion were not influenced by cultivar or a particular phenolic treatment. Valiant and Rutgers tomato seedlings were found to be equally susceptible to *P. penetrans* infection. However, the susceptibility of both cultivars to *P. penetrans* infection was greatly increased as a result of tyrosine and chlorogenic acid treatments.

Increased infectivity may be a result of injury to the host. Preliminary studies showed that phenol supplements at a rate of 1000 ppm retarded seedling development. Phenol supplements at the rates of 10 and 100 ppm may have sufficiently weakened the development of these tomato seedlings and increased their susceptibility to *P. penetrans* infection. These results agree with Dolliver (6) who reported higher numbers of *P. penetrans* in the root systems of Wando pea plants that had been physiologically weakened.

Feldman and Hanks (10) reported that long term applications of phenols to susceptible citrus cultivars could increase their resistance by suppression of *R. similis* activity (i.e., reproduction and survivability). Because of the relatively short duration of these experiments, the effects of phenol supplements on *P. penetrans* survival and reproduction in tomato seedlings was not determined. Further investigations are required to determine the influence of phenol supplements on these processes.

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TABLE 1. Total soluble phenols in leaves of healthy and nematode-infected tomato cultivars 28 days after inoculation. Nematode-infected plants of 'Manalucie' and 'Rutgers' were inoculated with 10,000 *P. penetrans*. Nematode-infected plants of 'Valiant' were inoculated with 4,500 *P. penetrans*.

	(mg/g fresh weight)[a]	
Cultivar	Healthy[b]	Diseased[b]
Manalucie	3.92±.83	4.00±.80
Rutgers	3.09±.42	2.70±.18
Valiant	3.88±.67	4.02±.90

[a]Values reported in chlorogenic acid equivalents.

[b]Mean of 5 samples. The 95% confidence limits are reported for each sample mean.

TABLE 2. Total bound and free phenols in leaves of healthy and nematode-infected tomato cultivars 28 days after inoculation. Nematode-infected plants of 'Manalucie' and 'Rutgers' were inoculated with 10,000 P. *penetrans*. Nematode-infected plants of 'Valiant' were inoculated with 4,500 *P. penetrans*.

	(ug/g fresh weight)[a]			
	Bound phenols[b,c]		Free phenols[b,c]	
Cultivar	Healthy	Diseased	Healthy	Diseased
Manalucie	245.3± 4.8	256.0±10.0	19.5± 6.0	17.8± 3.6
Rutgers	91.6± 6.0	108.5± 3.2	22.8± 9.2	18.0± 4.4
Valiant	218.6±11.6	265.1±10.0	27.0±10.7	25.0±10.0

[a]Values reported in chlorogenic acid equivalents.

[b]Total phenols were determined from reconstituted methanol fractions.

[c]Each value represents the mean of 6 replications of one sample. Leaves from 5 plants were combined to form each sample. The 95% confidence limits are reported for each sample mean.

TABLE 3. Total bound and free phenols in leaves of healthy and nematode-infected tomato cultivars 42 days after inoculation. Nematode-infected plants of 'Manalucie' and 'Rutgers' were inoculated with 2,200 *P. penetrans*.

	(ug/g fresh weight)[a]			
	Bound phenols[b,c]		Free phenols[b,c]	
Cultivar	Healthy	Diseased	Healthy	Diseased
Manalucie	232.5±12.7	263.6±11.1	4.0±4.8	2.7+4.0
Rutgers	195.0± 1.5	254.3±10.7	6.5±6.0	7.0±7.6

[a]Values reported in chlorogenic acid equivalents.

[b]Total phenols were determined from reconstituted methanol fractions.

[c]Each value represents the mean of 6 replications of one sample. Leaves from 5 plants were combined to form each sample. The 95% confidence limits are reported for each sample mean.

TABLE 4. Total chlorophyll and carotenoid and chlorophyll:carotenoid ratios in leaves of healthy and nematode-infected tomato cultivars 28 days after inoculation. Nematode-infected plants of 'Manalucie' and 'Rutgers' were inoculated with 10,000 *P. penetrans*. Nematode-infected plants of 'Valiant' were inoculated with 4,500 *P. penetrans*.

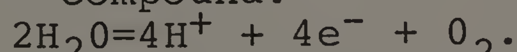
	(mg/g fresh weight)[a]					
	Chlorophyll		Carotenoid		Chlorophyll:Carotenoid (M/M)	
Cultivar	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Manalucie	1.16±.26	1.37±.56	.218±.05	.271±.11	3.17±.14	3.01±.19
Rutgers	1.45±.15	1.52±.23	.271±.03	.280±.05	3.18±.21	3.24±.11
Valiant	1.49±.28	1.24±.28	.271±.14	.233±.05	3.27±.14	3.17±.17

[a]Mean of 5 samples. The 95% confidence limits are reported for each sample mean.

TABLE 5. Photosynthetic competence of leaves of healthy and nematode-infected tomato cultivars 28 days after inoculation. Nematode-infected plants of 'Manalucie' and 'Rutgers' were inoculated with 10,000 *P. penetrans*. Nematode-infected plants of 'Valiant' were inoculated with 4,500 *P. penetrans*.

	(umoles/mg chl/hr)			
	Ferricyanide reduced[a]		Oxygen evolved[a,b]	
Cultivar	Healthy	Diseased	Healthy	Diseased
Manalucie	145.2	213.3	36.3	53.3
Rutgers	179.7[c]	178.6	44.9	44.7
Valiant	221.9	187.0	55.5	46.8
Average	182.3	193.0	45.6	48.3

[a]Each value represents a combined sampling of 5 plants.

[b]Values determined as follows:
Ferricyanide ($FeCN_6^{\equiv}$) is a one electron acceptor compound.

$2H_2O = 4H^+ + 4e^- + O_2$.
Dividing values of ferricyanide reduced by 4 = equivalents O_2 evolved.

[c]Determined from methanol control employed in experiments with DCMU, data not shown.

TABLE 6. Influence of treatments with phenols on infection of tomato seedlings with *P. penetrans*.

Cultivar	Treatment	Rate	% Infection	Total number of lesions	Average lesion length (u)	Average number of nematodes per lesion
Valiant	Tyrosine	10	41.6	7	261	1.6
	Tyrosine	100	53.8	11	283	2.8
	Chlorogenic acid	10	16.6	2	330	2.5
	Chlorogenic acid	100	36.4	4	418	5.3
	Control		15.0	4	395	4.5
Rutgers	Tyrosine	10	33.3	6	307	2.5
	Tyrosine	100	30.8	4	315	1
	Chlorogenic acid	10	10.0	1	300	1
	Chlorogenic acid	100	81.8	16	339	5.3
	Control		8.3	2	350	2

Fig. 1. Total soluble phenols in leaves of healthy and nematode-infected 'Manalucie' tomato. Nematode-infected plants were inoculated with 1,400 *P. penetrans*. Each point represents the mean of 5 samples. The 95% confidence limits are indicated for each mean.

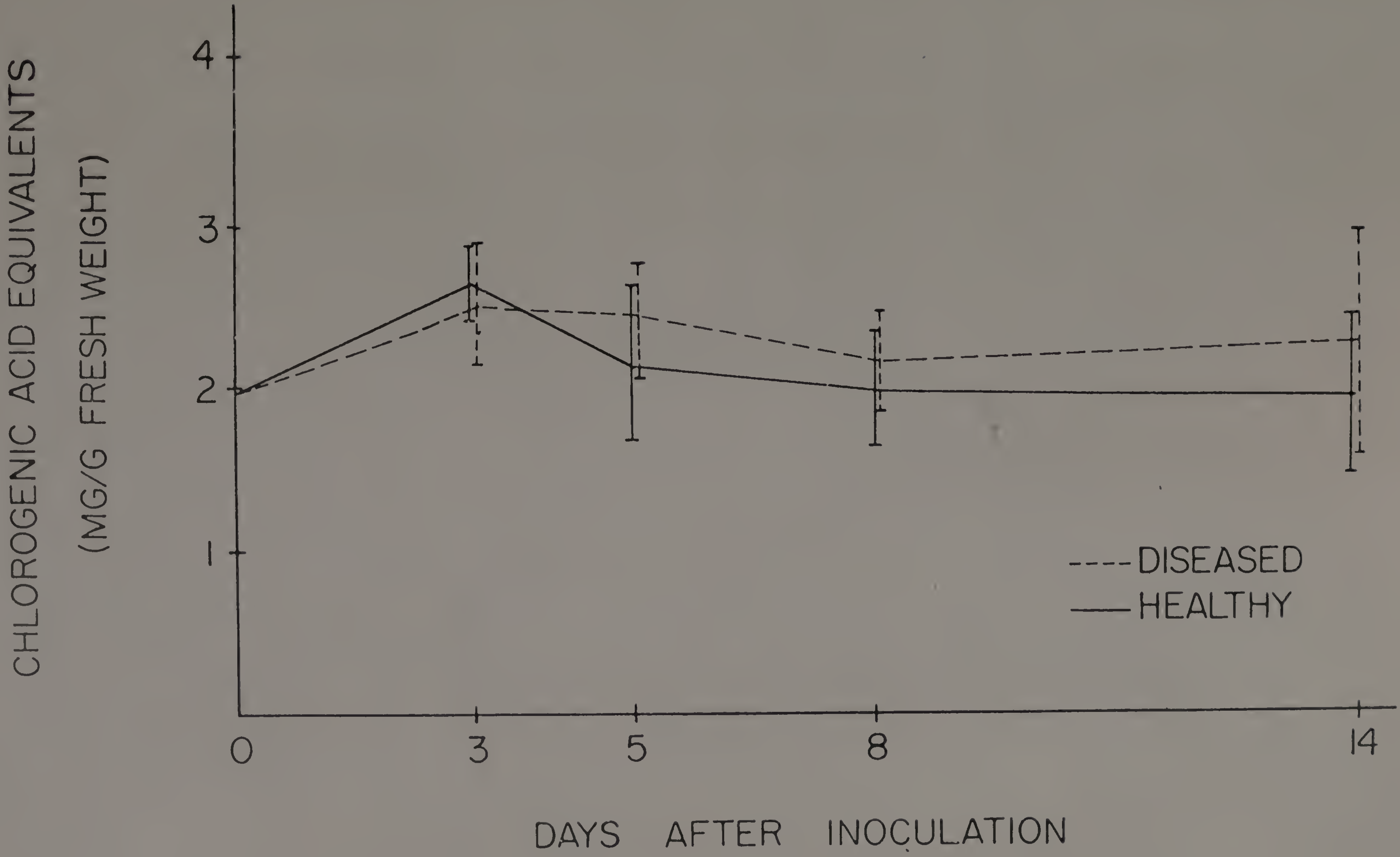

CHLOROGENIC ACID EQUIVALENTS
(MG/G FRESH WEIGHT)
4
3
2
1
0
3
5
8
14
DAYS AFTER INOCULATION
---- DISEASED
—— HEALTHY

Fig. 2. Total soluble phenols in leaves of healthy and nematode-infected 'Rutgers' tomato. Nematode-infected plants were inoculated with 1,400 *P. penetrans*. Each point represents the mean of 5 samples. The 95% confidence limits are indicated for each mean.

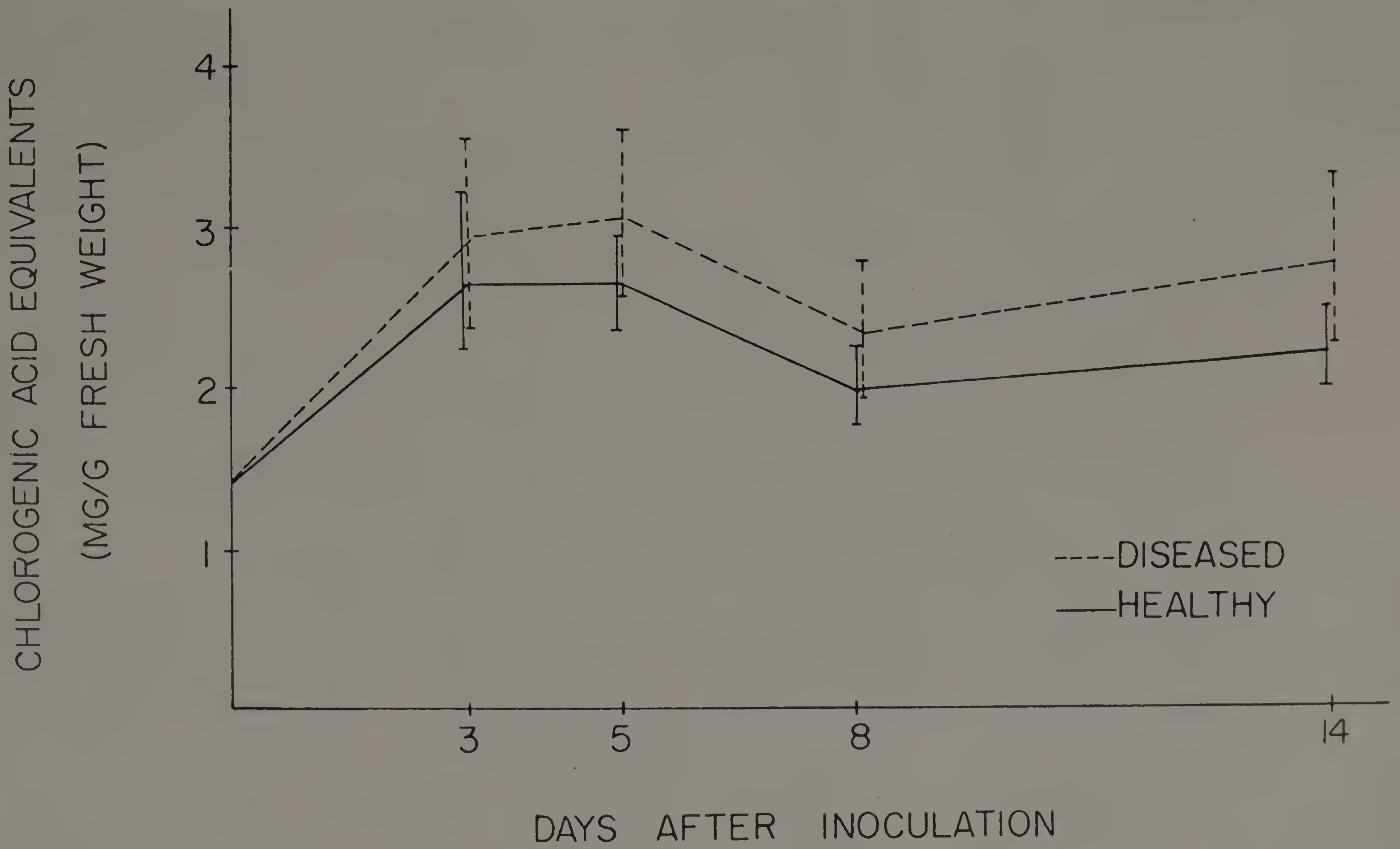

CHLOROGENIC ACID EQUIVALENTS
(MG/G FRESH WEIGHT)
4
3
2
1
3
5
8
14
DAYS AFTER INOCULATION
----DISEASED
——HEALTHY

Fig. 3. Total soluble phenols in leaves of healthy and nematode-infected 'Manalucie' tomato. Nematode-infected plants were inoculated with 2,200 *P. penetrans*. Each point represents the mean of 5 samples. The 95% confidence limits are indicated for each mean.

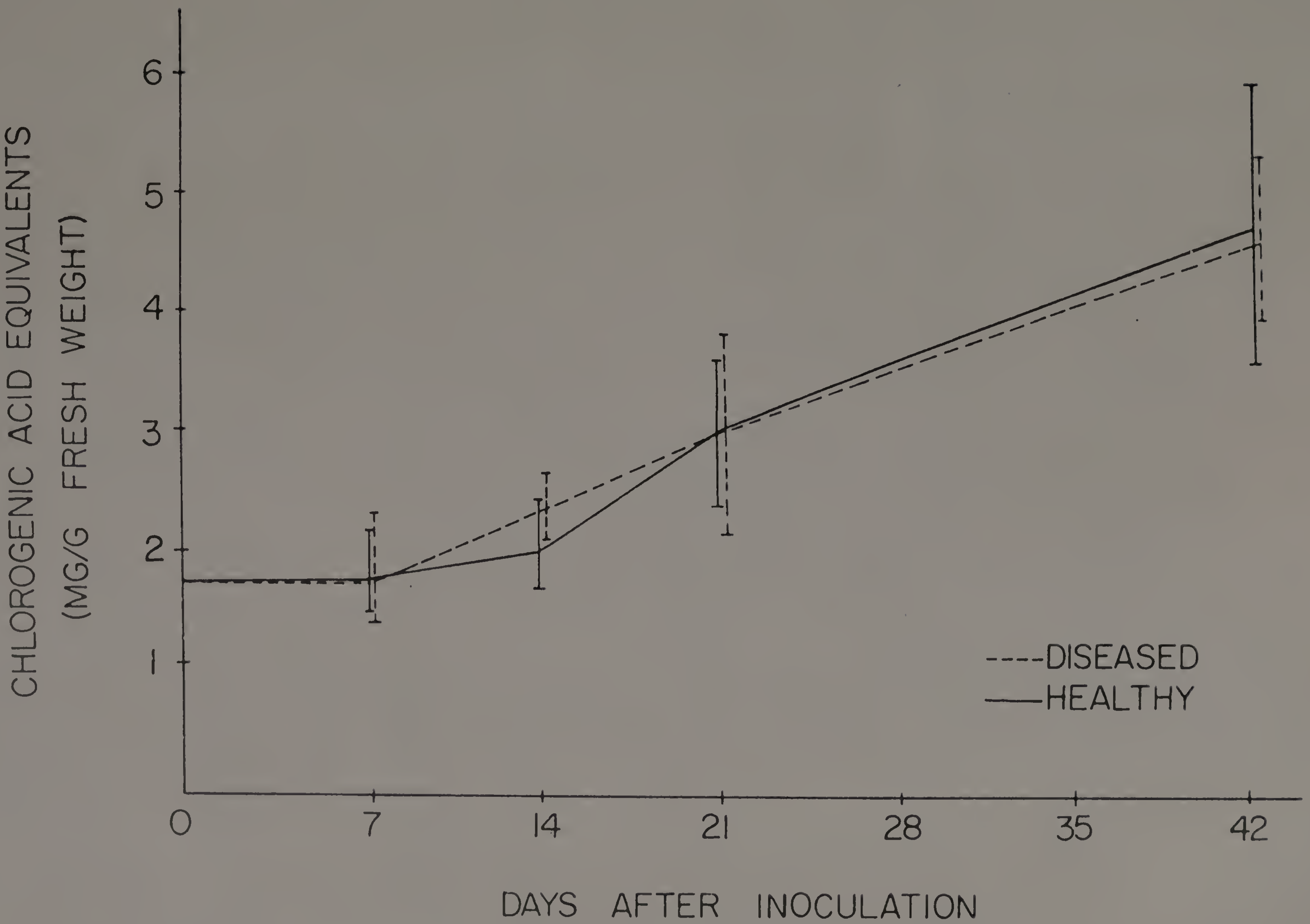

CHLOROGENIC ACID EQUIVALENTS
(MG/G FRESH WEIGHT)
6
5
4
3
2
1
0
7
14
21
28
35
42
DAYS AFTER INOCULATION
----DISEASED
——HEALTHY

Fig. 4. Total soluble phenols in leaves of healthy and nematode-infected 'Rutgers' tomato. Nematode-infected plants were inoculated with 2,200 *P. penetrans*. Each point represents the mean of 5 samples. The 95% confidence limits are indicated for each mean.

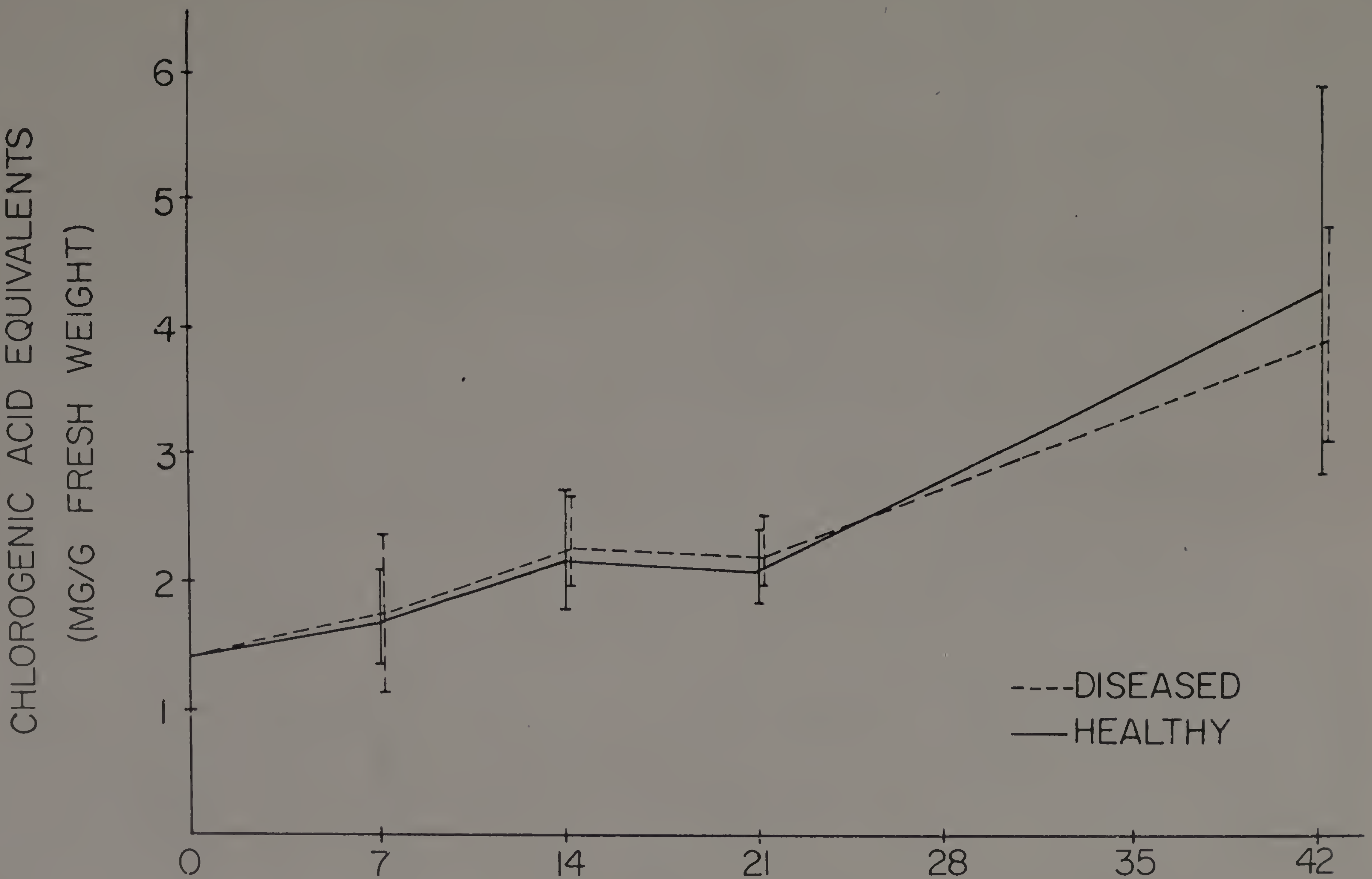

CHLOROGENIC ACID EQUIVALENTS
(MG/G FRESH WEIGHT)
6
5
4
3
2
1
0
7
14
21
28
35
42
DAYS AFTER INOCULATION
----DISEASED
—HEALTHY

Fig. 5. Chromatograms of bound phenols of 'Manalucie' tomato 28 days after inoculation. Several spots show increases in size and intensity of fluorescence under ultraviolet light as a consequence of *P. penetrans* infection.

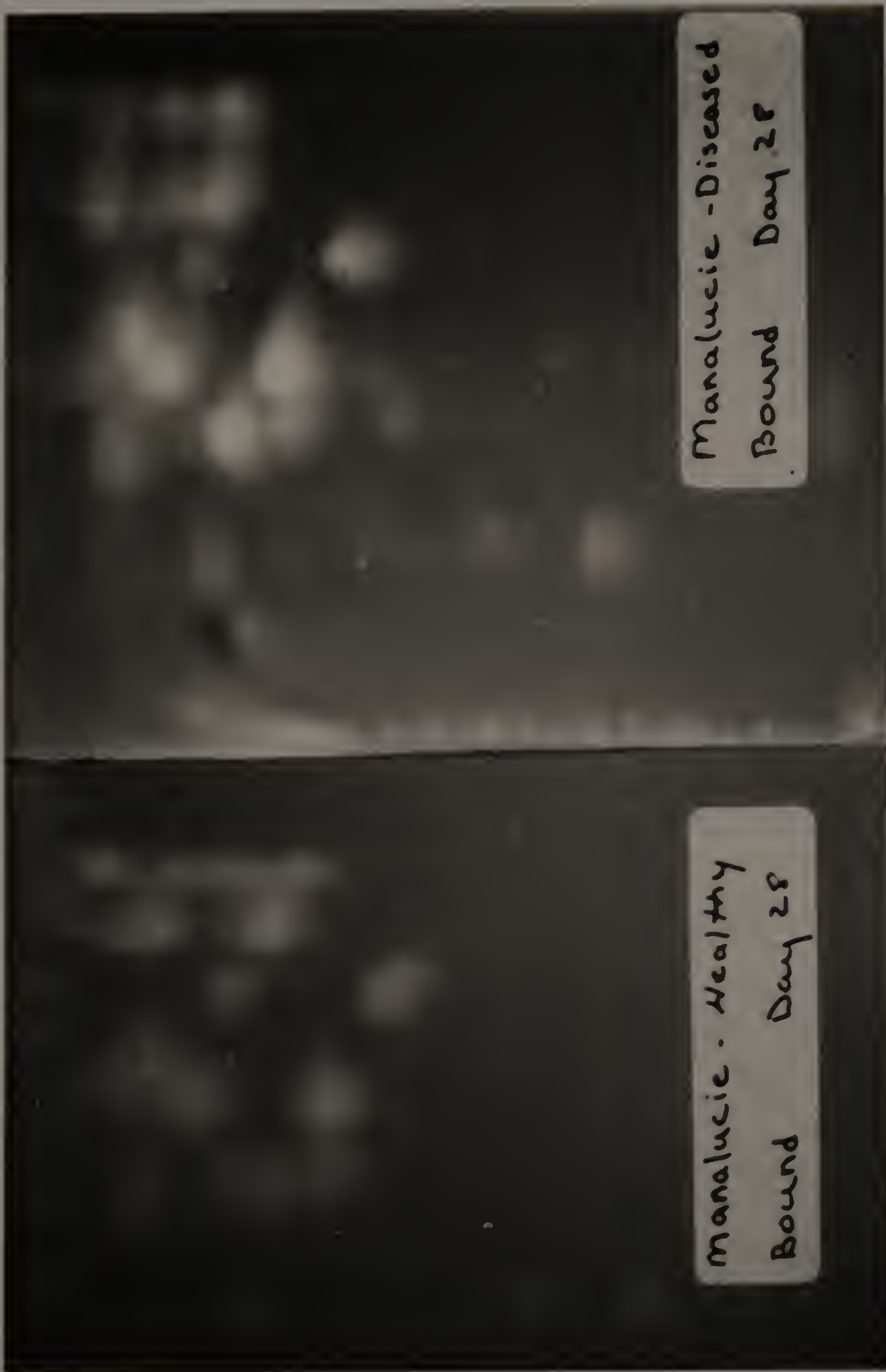

Manalucie - Diseased
Bound Day 28
Manalucie - Healthy
Bound Day 28

Fig. 6. Chromatograms of bound phenols of 'Rutgers' tomato 28 days after inoculation. Several spots show increases in size and intensity of fluorescence under ultraviolet light as a consequence of *P. penetrans* infection.

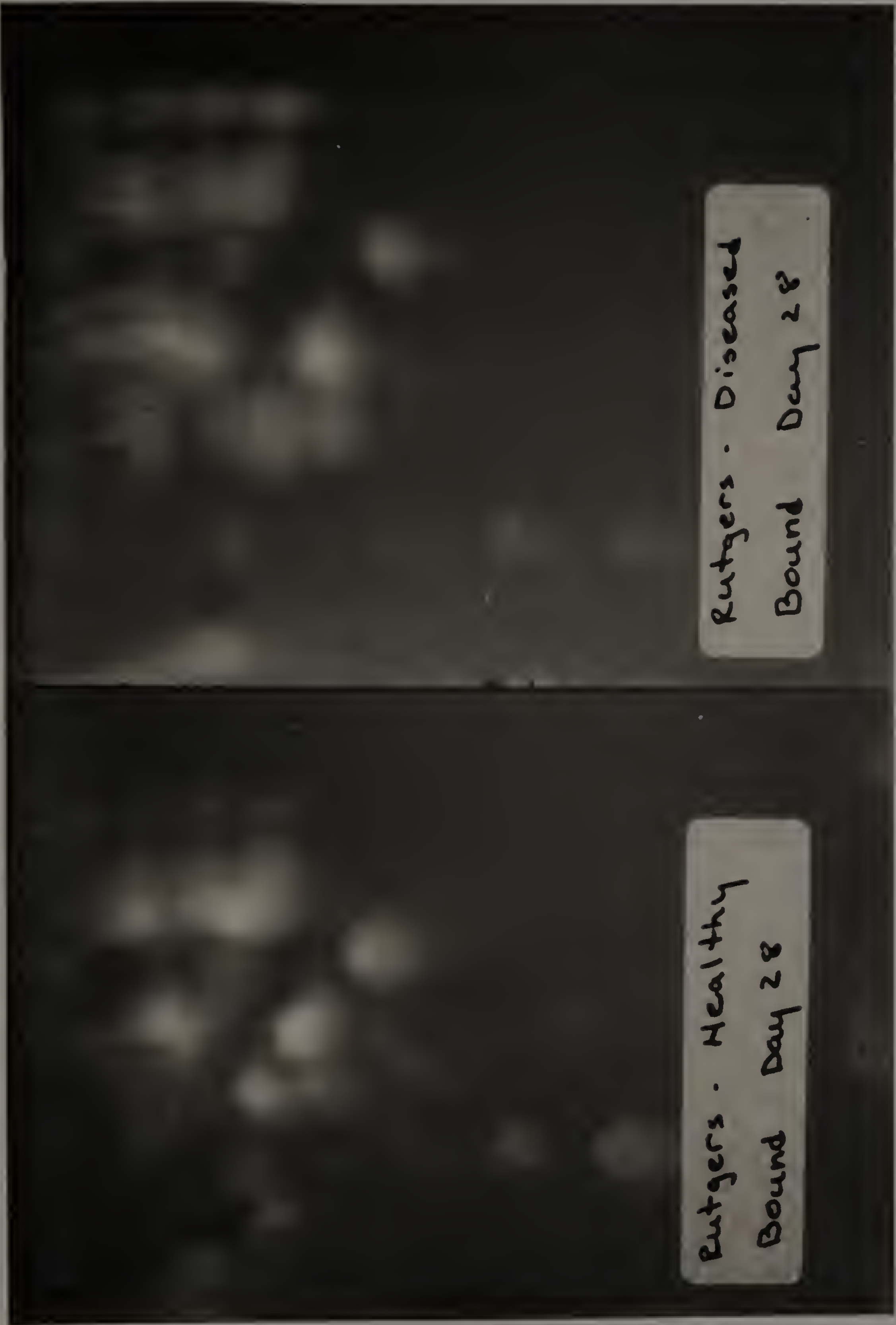
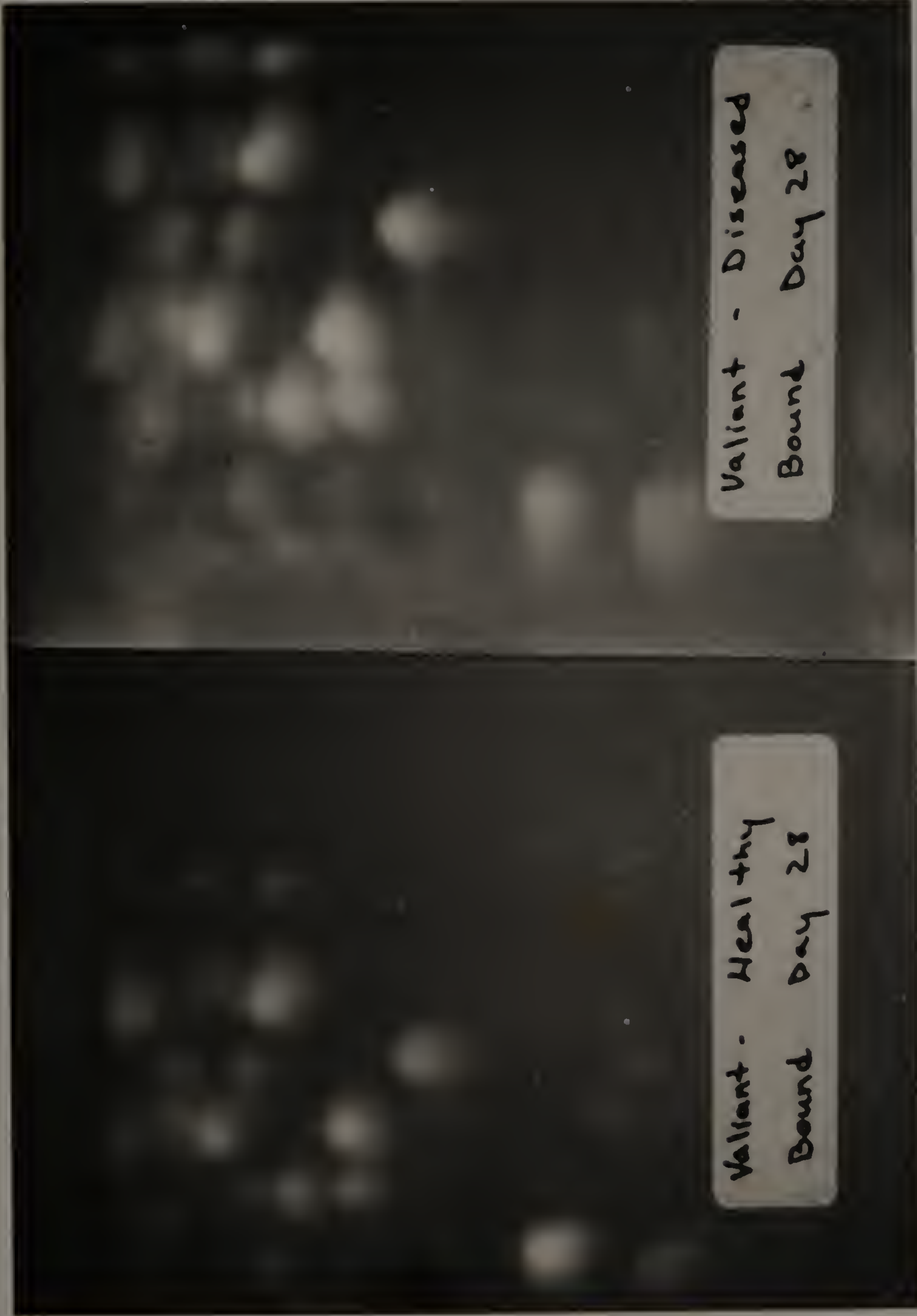

Rutgers · Diseased
Bound Day 28
Rutgers · Healthy
Bound Day 28

Fig. 7. Chromatograms of bound phenols of 'Valiant' tomato 28 days after inoculation. Several spots show increases in size and intensity of fluorescence under ultraviolet light as a consequence of *P. penetrans* infection.


Valiant - Diseased
Bound Day 28
Valiant - Healthy
Bound Day 28