PHENOLICS INVOLVEMENT IN SWEET POTATO RESISTANCE TO *Meloidogyne incognita* AND *M. javanica*

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ABSTRACT

The possible involvement of phenols in sweet potato resistance to root-knot nematode was shown by the reduced number of eggs hatched and high larval mortality of *Meloidogyne incognita* and *M. javanica* exposed to root extracts of the resistant cultivars even as low as 10% concentration. The compounds present in the extracts were heat resistant, of low molecular weight and water soluble.

Thin layer chromatography (TLC) revealed two distinct groups of blue fluorescent spots in susceptible and resistant sweet potato extracts with Rf values corresponding to chlorogenic acid, scopoletin and esculin. More phenolics accumulated in root extracts of resistant sweet potato cultivars following infection as indicated by the stronger color intensity of the fluorescent spots.

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KEY WORDS: Sweet potato. *Meloidogyne incognita. M. javanica.* Thin layer chromatography (TLC). Chlorogenic acid. Scopoletin. Esculin. Phenolics.

INTRODUCTION

The root-knot nematodes, ***Meloidogyne*** spp. are among the most important pests of sweet potato. They cause galling on the feeder roots, roughening and frequent cracking of tubers, and general decay of the entire fibrous root system, thus reducing yield and quality of tubers. In a pot experiment, reduction in yield as high as 60% due to ***Meloidogyne incognita*** was reported in susceptible sweet potato cultivar, BNAS-51 (Gapasin

and Valdez, 1979). Other investigators confirmed the ability of root-knot nematodes to cause damage to sweet potato (Elliot, 1918; Poole and Schmidt, 1927).

Pre- and post-infectional mechanisms of resistance to ***Meloidogyne*** spp. occur in sweet potato. In several host-nematode combinations, the occurrence of substances responsible for host resistance has long been recognized (Giebel, 1970; Feldman and Hanks, 1968; Hung and Rohde, 1973). Post-infectional resistance was indicated by delayed or retarded development of the nematodes after penetration in Jasper and Jewel sweet potato varieties or non-development to maturity of nematodes in W-86 (Gapasin, 1984).

This study aimed to determine the chemical substances in resistant sweet potato cultivars responsible for their resistance to the root-knot nematodes, ***Meloidogyne incognita*** and ***M. javanica.***

MATERIALS AND METHODS

Culture. Cuttings of sweet potato cultivars which are susceptible (Binicol and UPR) and resistant (Jasper, Jewel and W-86) to root-knot nematodes were obtained from the Philippine Root Crop Research and Training Center, ViSCA, Baybay, Leyte.

Isolates of ***Meloidogyne incognita*** and ***M. javanica*** were obtained by removing an egg mass from galled roots of tomato, and placing it in a watch glass with a few drops of water. The species was identified and the culture maintained in susceptible tomato (variety VC-11) plants for 45 days. The nematode species were increased and maintained separately in susceptible tomato plants for several generations as source of inocula.

Nematode Inocula. Eggs and larvae were used as inocula. Eggs were collected from infected VC-11 tomato roots following the procedure described by Hussey and Barker (1973). The desired inoculum density was obtained by computing the average of three counts of 1 mL sample each.

The nematode larval inocula were obtained from the light brown egg masses in infected roots and were placed on a piece of wire mesh lined with tissue paper in a petri dish. Distilled water just enough to saturate the tissue paper was poured into the plate. After 12 hr, larval suspensions were pipetted from the plates and transferred to a beaker. Larvae were handpicked singly from the suspension.

Inoculation. Cuttings (30 cm long) of the five sweet potato cultivars were planted separately in clay pots (10 cm dia.) filled with baked sandy loam soil. After 10 days, each plant was inoculated with 5000 eggs of the specified nematode species.

Preparation of Root Extracts. Extracts were prepared by grinding 20 g fresh roots of 30-day old resistant or susceptible sweet potato cultivars with mortar and pestle. The extracted sap was centrifuged at 10,000 rpm for 15 min in a Sorvall RC5 using an SS34 rotor and the clear supernatant was

collected. Half-mL aliquots of the root extracts were placed separately in small dishes. One mature egg mass each of *M. incognita* and *M. javanica* was immersed in either the root extract or distilled water (control). The larvae that emerged from the egg mass after 24 hr were counted using a stereomicroscope.

To determine the effect on larval mortality, 20 larvae each of *M. incognita* and *M. javanica* were handpicked singly and placed in separate petri dishes containing distilled water (control) or 10, 50, 70 and 100% extract. Larval mortality was determined after 24 hr incubation.

Characterization of the Root Extract. Several tests were conducted to determine the substances present in the root extract. The root extracts were subjected to 60 and 100°C for 5 min and allowed to cool for 1 hr. Then 20 larvae of each nematode species were placed in 0.2 mL aliquots of each extract and incubated at room temperature (27°C). Larval mortality was determined after 24 hr.

Two mL each of root extracts from resistant and susceptible varieties were seperately dialyzed twice in dialysis bags (molecular weight, 12,000) against distilled water for 24 hr at 4°C. The undialyzed root extracts were prepared following the procedure described earlier. The activity of both dialyzed and undialyzed extracts was determined on larval mortality after 24 hr.

Roots (10 g each) of resistant and susceptible cultivars were washed and blotted dry. The roots were cut into small pieces and macerated in 5 mL chloroform with mortar and pestle. The extracts were centrifuged at 10,000 rpm for 15 min. The chloroform and water fractions of the extracts were separated and their activities tested on larval mortality. For the chloroform control, 0.2 mL chloroform evaporated to dryness served as control.

Phenol Extraction. Thirty days after inoculation with *M. incognita* and *M. javanica,* the roots of each resistant and susceptible sweet potato cultivar were cut into pieces and kept in the freezer for 24 hr or until ready for use. The samples were lyophilized, immediately powdered, stored in sample bottles and kept in the refrigerator.

Samples were extracted and fractionated following the procedure of Uritani et al. (1982). The solvents were evaporated from aliquots of Fractions I, II, III, and IV and the residues were individually dissolved in small amounts of ethyl alcohol for analysis using thin layer chromatography (TLC).

Thin Layer Chromatography of Fractions. Forty μL samples were applied as spots on silica gel plates with gel type 60 GF 254. Chlorogenic acid, p-coumaric acid, caffeic acid, scopoletin and esculin were used as standards. The plates were developed in tanks saturated with n-butanol: acetic acid: water (4:1:2 v/v/v) solvent system. Following evaporation of the solvent, the plates were scanned under ultraviolet light. Fluorescent spots observed were marked and the color intensity was recorded.

RESULTS AND DISCUSSION

Effect of Root Extracts on Egg Hatching and Larval Mortality

Root extracts of resistant sweet potato cultivars inhibited hatching of eggs of both nematode species with W-86 giving the lowest number of eggs hatched (1.4) followed by Jasper, 17.2 and Jewel, 27.4 (Table 1). Both the susceptible varieties Binicol and UPR allowed the largest number of eggs hatched (98.8 and 88.4, respectively) which were not significantly different from the control (distilled water).

Larval mortality values of both test nematode species were significantly higher in root extracts of the resistant cultivars than in those of the susceptible ones (Table 1). The mean larval mortality of ***M. incognita*** in extracts of Jasper and W-86 was 14.0 and 20.0, respectively. These were significantly

Table 1. Effect of crude root extracts of susceptible and resistant sweet potato cultivars on egg hatching and larval mortality of two ***Meloidogyne*** spp.[1]

Nematode Species/ Cultivar	Number of Eggs Hatched[2]	Larval Mortality[3]
M. incognita		
Binicol (S)	98.8a	3.6b
Jasper (R)	17.2b	14.0c
W-86 (R)	1.4c	20.0c
Distilled water (control)	129.0a	0.2a
M. javanica		
UPR (S)	88.4a	3.8b
Jewel (R)	27.4b	11.8c
W-86 (R)	1.6c	20.0d
Distilled water (control)	115.4a	0.0a

[1] Means of 5 replicates. In a column, means followed by a common letter are not significantly different at 5% level, DMRT. Data were transformed to log (X + 1) for statistical analysis.

[2] Mean number of larvae, dead and alive which hatched from a single egg mass after 24 hr immersion.

[3] Mean of 20 second stage larvae per dish of extract.

different from extracts of Binicol and the control with larval mortality counts of 3.6 and 0.2, respectively. With *M. javanica,* larval mortality in extracts of Jewel and W-86 were 11.8 and 20.0, respectively.

The above findings indicate that some substances present in the crude sweet potato root extracts affect egg hatching and larval mortality. These chemical substances, while present in susceptible cultivars, may be lesser in quantity compared to the resistant ones. According to Sheperd and Clarke (1971), some phenols such as p-cresol are potent inhibitors of hatching. Kaul (1962) found that catechin inhibited hatching of cyst nematodes.

Dilution of extracts resulted in decreased larval mortality counts (Table 2). The root extract of the resistant variety W-86 was the most potent since 50 and 30% dilution did not lower its effect on larval mortality. For Jasper and Jewel which are also rated resistant, 30% dilution decreased the extracts' potency by 75%. The susceptible varieties Binicol and UPR lost their effect on larval mortality upon dilution.

Physical Property of the Extract

Stability. The root extract of the sweet potato cultivars still had considerable lethal effect on the larvae even

Table 2. Effect of varying concentrations of crude root extracts of resistant and susceptible sweet potato cultivars on larval mortality of ***Meloidogyne incognita*** and ***M. javanica.***[1]

Nematode Species/ Cultivar		Larval Mortality Counts — Concentration of Extract (%)				
		0	10	50	70	100
M. incognita						
Binicol	(S)	0.0a	0.0a	0.2a	0.2a	3.5b
Jasper	(R)	0.0a	0.0a	3.5b	8.0c	13.8d
W-86	(R)	0.0a	3.8b	18.8d	20.0d	20.0d
M. javanica						
UPR	(S)	0.0a	0.0a	0.0a	0.0a	4.0cd
Jewel	(R)	0.0a	0.0a	2.5b	7.8b	12.8a
W-86	(R)	0.0a	5.0d	20.0f	20.0f	20.0f

[1] Means of 4 replicates; 20 larvae immersed per concentration of extract for 24 hr. In a column or row within each nematode species, means followed by a common letter are not significantly different at 5% level, DMRT. Data were transformed to log (X + 1) for statistical analysis.

Table 3. Effect of varying temperatures on root extracts of resistant and susceptible sweet potato cultivars as determined by bioassay with ***Meloidogyne*** larvae.[1]

Nematode Species/ Cultivar	Larval Mortality Counts		
	27°C	60°C	100°C
M. incognita			
Binicol (S)	3.5b	2.2b	0.5a
Jasper (R)	13.8d	8.2c	0.8a
W-86 (R)	20.0d	16.8d	1.5b
Distilled water (control)	0.0a	0.0a	0.0a
M. javanica			
UPR (S)	4.0c	2.0c	0.0a
Jewel (R)	12.8e	8.8d	0.2a
W-86 (R)	20.0e	17.8e	1.0b
Distilled water (control)	0.0a	0.0a	0.0a

[1] Means of 4 replicates; root extracts heated for 5 min. In a column or row within each nematode species, means followed by a common letter are not significantly different at 5% level, DMRT. Data were transformed to log (X + 1) for statistical analysis.

after heating at 60°C (Table 3). The results indicate that the substance(s) present in resistant sweet potato cultivars are relatively heat-stable.

Size. Dialysis resulted in 80% loss of activity on larval mortality (Table 4) for both nematode species indicating low MW of the active factor. The residual activity left in the dialysis bag could be due to the binding or association of the small active factor with bigger molecules like proteins.

Solubility. The active compound present in the root extracts was water soluble (Table 5). The compound may have lesser affinity with chloroform and if present, it was not enough to affect the root-knot nematode larvae.

Production of Phenols in Response to Nematode Infection

Two distinct groups of fluorescent spots were observed in all cultivars (Fig. 1). Those in the upper row had blue color while those in the second row differed in color intensity depending on the cultivar. However, the blue

Table 4. Effect of dialysis on the activity of crude root extracts of resistant and susceptible sweet potato cultivars as determined by bioassay with *Meloidogyne* larvae.[1]

Nematode Species/ Cultivar	Larval Mortality Counts		
	Dialyzed Extract[2]	Undialyzed Extract	Distilled Water (control)
M. incognita			
Binicol (S)	0.5a	2.2c	0.0a
Jasper (R)	1.8b	9.5d	0.0a
W-86 (R)	3.8c	20.0e	0.0a
M. javanica			
UPR (S)	0.2a	2.2c	0.0a
Jewel (R)	1.0b	8.0d	0.0a
W-86 (R)	2.8c	20.0e	0.0a

[1] Means of 4 replicates; nematodes immersed in the treatments for 24 hr. In a column or row, means followed by a common letter are not significantly different at 5% level, DMRT. Data were transformed to log (X + 1) for statistical analysis.

[2] Crude extracts were dialyzed for 24 hr before assay.

color of the upper spots was more intense in resistant than in susceptible samples. Inoculation with nematodes resulted in more intense and darker blue spots indicating larger amounts of this factor in the infected samples.

When compared with the standard, the lower spot had Rf similar to esculin while the upper spot had Rf similar to. chlorogenic acid and scopoletin (Fig. 2). Caffeic and p-coumaric acids did not produce fluorescent spots.

The findings indicate that chlorogenic acid, scopoletin and esculin could be present in root extracts of both root-knot nematode-susceptible and —resistant sweet potato cultivars. The observation of more intense fluorescent spots in inoculated resistant cultivars further suggests that more of these compounds were produced in those samples than in the others (Fig. 2). The fluorescent spots in uninoculated resistant cultivars were more intense than those in uninoculated susceptible cultivars Binicol and UPR. This indicates that the compounds were already present in the sweet potato test plants before infection but were increased upon infection.

Table 5. Effect of water and chloroform fractions of crude root extracts of resistant and susceptible sweet potato cultivars on the solubility of the active compound as determined by bioassay with ***Meloidogyne*** larvae.[1]

Nematode Species/ Cultivar	Larval Mortality Counts	
	Water Fraction[2]	Chloroform Fraction
M. incognita		
Binicol (S)	3.3b	0.0
Jasper (R)	16.0c	0.0
W-86 (R)	20.0c	0.0
Distilled water (control)	0.0a	
Chloroform		0.0
M. javanica		
UPR (S)	5.8b	0.0
Jewel (R)	15.2c	0.0
W-86 (R)	20.0c	0.0
Distilled water (control)	0.0a	
Chloroform		0.0

[1] Means of 4 replicates; nematodes immersed in fractions for 24 hr. In a column, means followed by a common letter are not significantly different at 5% level, DMRT. Data were transformed to log (X + 1) for statistical analysis.

[2] A 0.2 mL aliquot of each extract was placed in a dish then evaporated. An equal amount of distilled water was placed before putting the nematode larvae. The same procedure was done with the chloroform fraction.

The compounds tentatively identified based on color of fluorescent spots and Rf values were probably responsible for larval mortality in this study. Chlorogenic acid has been implicated in several resistant reactions against nematodes (Van Gundy and Kirkpatrick, 1964; Sitaramaiah and

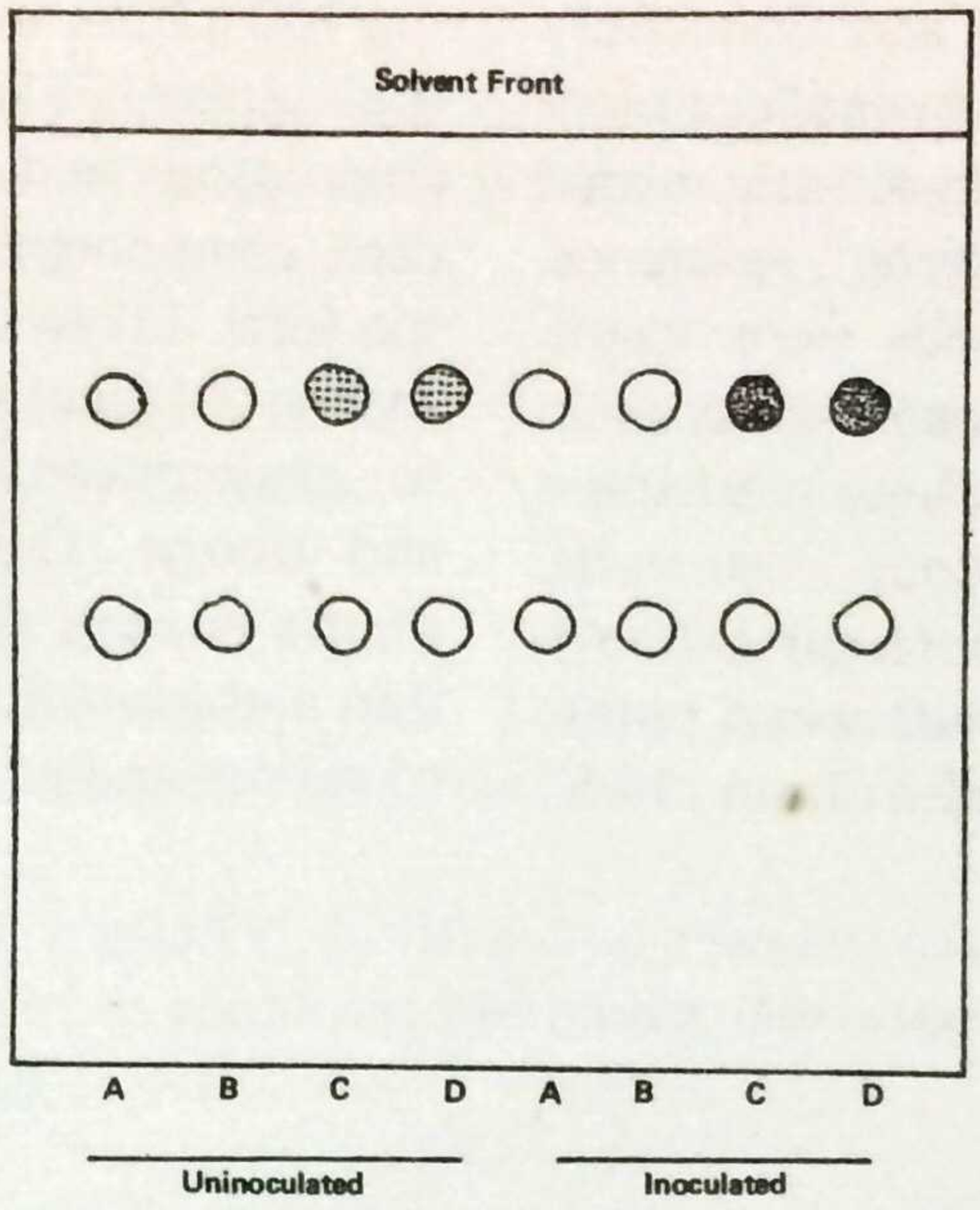

Figure 1. Schematic diagram of the distribution of fluorescent spots in root extracts of susceptible and resistant sweet potato cultivars using Fraction II on silica gel plates. Binicol (A), UPR (B), Jasper (C) and W-86 (D). Note intensity of fluorescent spots in resistant cultivars C and D. Solvent system: n-butanol: acetic acid: water (4:2:1 v/v/v).

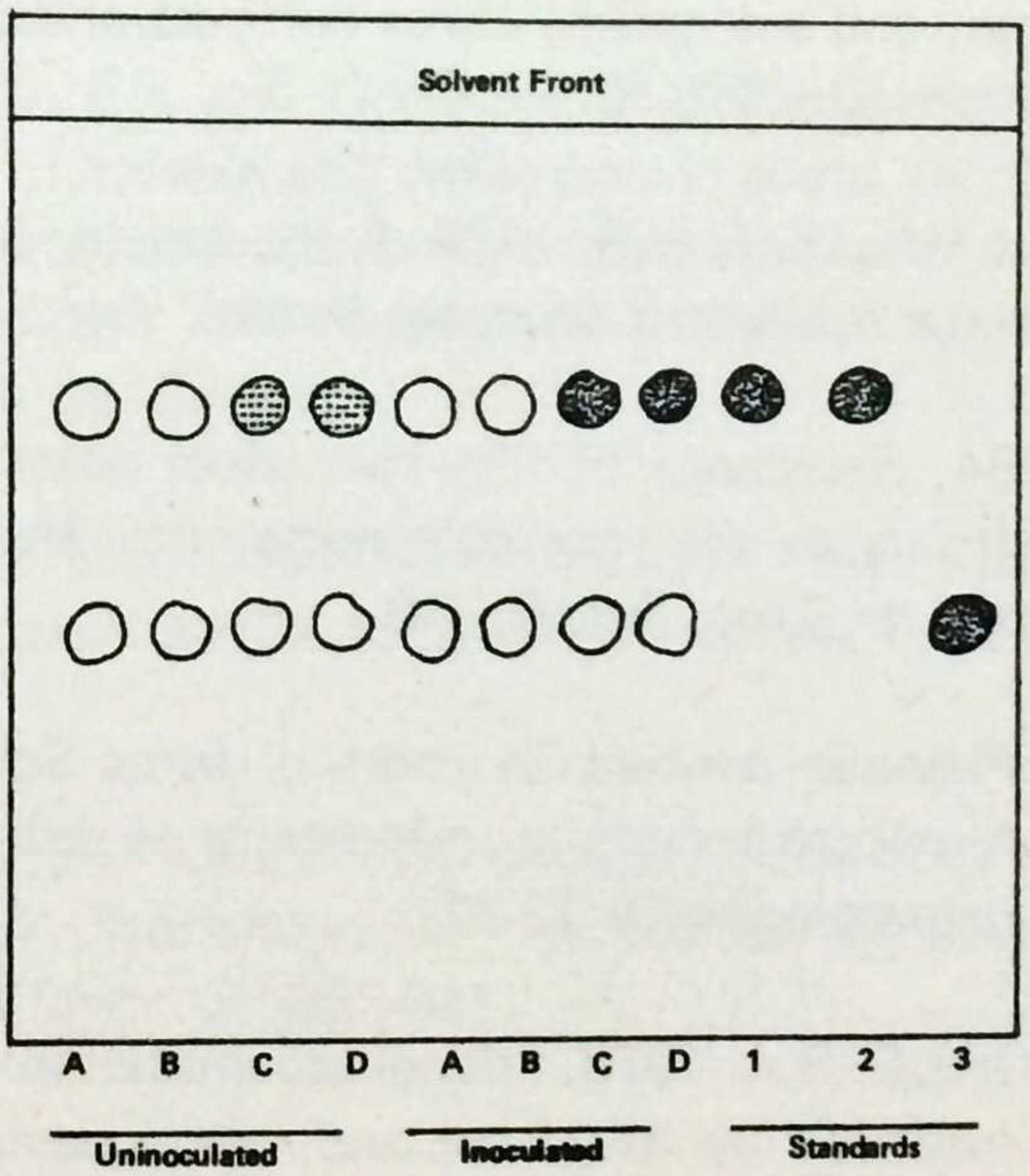

Figure 2. Schematic diagram of the distribution of fluorescent spots in root extracts of susceptible and resistant sweet potato cultivars using Fraction II on silica gel plates with standard. Binicol (A), UPR (B), Jasper (C) and W-86 (D). Chlorogenic acid (1), Scopoletin (2) and Esculin (3). Solvent system: n-butanol: acetic acid: water (4:2:1 v/v/v).

Pathak, 1979). Singh and Choudhury (1973) reported that phenolic content in tomato cultivars was directly related to root-knot nematode resistance.

Phenolic compounds were identified in sweet potato and these could play a role in the defense mechanism against the root-knot nematode. Walter et al. (1979) found caffeoylquinic acid esters in 10 sweet potato cultivars which ranged from 14-51 mg/100 g fresh weight depending upon the cultivar. Chlorogenic acid and isochlorogenic acid were the most abundant components comprising 30% of the total. Likewise, Thompson (1981) analyzed 14 cultivars of sweet potato for their phenolic content at harvest and storage. Qualitative examination of the phenols resulted in the separation and identification of four isomers of caffeoylquinic acid.

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