BIOCHEMICAL CHANGES INDUCED IN ROOTS AND XYLEM SAP OF BLACK PEPPER BY *MELOIDOGYNE INCOGNITA* *

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ABASTRACT

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Gas-liquid chromatography (GLC), two-dimensional paper chromatography (TDPC) and thin layer chromatography (TLC) analyses of healthy black pepper (*Piper nigrum* L.) plants and those infested with *Meloidogyne incognita* showed marked differences in the concentrations and presence of amino acids, organic acids and sugars in galled roots and xylem sap of galled plants. The amino acid lysine was detected in roots of infected plants but not in roots of healthy ones. Serine and glumatic acid decreased 29 and 20% respectively and aspartic acid increased 10% in infected roots, compared to healthy ones. In contrast to the roots, concentrations of amino acids in xylem sap from infected plants were higher than in sap of healthy ones. Alanine, glycine, aspartic acid and glutamic acid increased 219, 207, 80 and 37% in xylem sap from infected plants compared to healthy ones. Tyrosine was detected only in xylem sap from infected plants, either in samples analysed by GLC or TDPC. As for organic acids, citric and malic acids increased 223 and 243% in infected roots compared to healthy ones. Glycolic acid decreased 19% in roots of infected plants. Glyveric acid was found in extract of infected roots but was not detected in extract of healthy ones. The sole organic acid identified in samples of xylem sap was glycolic acid which increased 84% in xylem sap from infected plants compared to healthy

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ones, when samples were analysed by GLC. Glycolic and glyoxilic acids were detected by TLC in xylem sap from both infected and healthy plants. Gas-liquid chromatography (GLC) of sugars from root extract of infected plants revealed the presence of low concentrations of fructose and glucose. Thin-layer chromatography (TLC) of root extract from infected plants showed the occurrence of fructose, glucose and sucrose. In xylem sap from infected plants, fructose showed an increase of 8% compared to its concentration in xylem sap from healthy plants. Glucose, on the other hand, decreased 80% in xylem sap from infected plants compared to its concentration in xylem sap from healthy plants.

RESUMO

Alterações bioquímicas em raízes e seiva de pimenta-do-reino causadas pelo parasitismo de *Meloidogyne incognita*

Anâlises de plantas sadias de pimenta-do-reino e plantas infestadas por *Meloidogyne incognita*, realizadas através de cromotografia gas-liquido (CGL), cromatografia bi-dimensional de papel (CBDP) e cromotografia de camada delgada (CCD), revelaram diferenças marcantes quanto à concentração e presença de aminoácidos, ácidos orgânicos e açúcares em raízes e seiva de plantas infestadas pelo nematóide. O aminoácido lisina foi encontrado no extrato de raízes infestadas mas não no extrato de plantas sadias. As concentrações de serina e ácido glutâmico decresceram em 29 e 20%, respectivamente, e a de ácido aspártico aumentou em 10% em raízes infestadas, em comparação com raízes sadias. Ao contrário das raízes, as concentrações dos aminoácidos na seiva de plantas infestadas aumentaram em relação às concentrações na seiva de plantas sadias. Alanina, glicina, ácido aspártico e ácido glutâmico tiveram suas concentrações aumentadas em 219, 207, 80 e 37%, respectivamente, na seiva de plantas infestadas em comparação às plantas sadias. Tirosina foi detectada somente na seiva de plantas infestadas, independente de as amostras terem sido analisadas por CGL ou CBDP. Com relação aos ácidos orgânicos, os ácidos cítrico e málico exibiram aumentos em suas concentrações equivalentes a 223 e 143%, respectivamente, em raízes infestadas em comparação às raízes sadias. A concentração do ácido glicólico descresceu em 19% em raízes infestadas. O ácido glicérico foi encontrado em extrato de raízes infestadas mas não em extrato de raízes de plantas sadias. O único ácido orgânico encontrato em amostras de seiva quando as amostras foram analisadas por CGL foi o ácido glicólico, o qual teve sua concentração aumentada em 84% na seiva de plantas infestadas em comparação às plantas sadias. Os ácidos glicólico e glioxílico foram detectados através de CCD, tanto na seiva de plantas infestadas como nas sadias. Cromatografia gas-líquido (CGL) do extrato de raízes infestadas revelou a presença de baixas concentrações dos açúcares frutose e glucoce. Cromatografia de camada delgada (CCD) de extrato radicular de plantas infestadas revelou a ocorrência de frutose, glucose e sucrose. Na seiva de plantas infestadas a frutose exibiu um aumento de 8% na sua concentração, comparado à sua concentração na seiva de plantas sadias. A glucose, ao contrário, decresceu sua concentração em 80% na seiva de plantas infestadas em comparação à sua concentração na seiva de plantas sadias.

INTRODUCTION

Giant cells formation induced by root-knot nematodes, *Meloidogyne* spp., represents a complex host response involving biochemical and physiological changes of the infected roots. Biochemical effects of *Meloidogyne* spp. in plant roots have been summarized by Bird (1961) and Krusberg (1963).

Alterations in several organic and inorganic components of tomato roots infected with *Meloidogyne* spp., compared to normal roots, have been reported by Owens & Specht (1966). Biochemical changes in xylem sap of tomato plants infected with *M. incognita,* compared to xylem sap from normal plants, have been detected by Wang & Bergeson (1974). Drastic changes in phenol, protein, amino acids, carbohydrates and other components of plant tissues, as a result of infection with root-knot nematodes, have been reported by other workers (Myuge, 1956; Kannan, 1968; Saxena, 1972; Bird, 1975, 1979; Lewis & McClure, 1975; Masood & Husain, 1975; Alam *et al.*, 1976).

Since there have been no reports on biochemical alterations of black pepper plants parasitezed by root-knot nematodes this study was undertaken aiming to determine the changes in amino acids, organic acids and sugars in roots and xylem sap of black pepper plants infected with *M. incognita,* compared to healthy plants.

MATERIALS AND METHODS

Seedlings inoculation – Eight month-old black pepper seedlings, cv. Singapura, were kept in 10 cm diameter plastic pots, partially filled with an autoclaved loam: sand mixture (3: 2), and inoculated with 10,000 freshly hatched second-stage juveniles of *M. incognita.* Plants were kept in a heated glasshouse where temperatures ranged from 22°C to 37°C. Fifteen plants were inoculated and fifteen non-inoculated plants were kept as control.

Collection of xylem sap – One month after inoculation all plants were uprooted and the root systems washed in a stream of tap water to remove soil particles. The stems were cut at an oblique angle, with a sterilized blade, at 10 cm above the soil. Each plant was immediately covered with a sterilized specimen glass tube of 2.5 x 15 cm and placed into 1,000 ml capacity beakers containing 500 ml of distilled water, five plants per beaker. At intervals of one hour, for a total of six hours, sap was collected using sterilized 10 μl disposable micro-pipettes and placed into sterilized 5 ml capacity glass vials containing 3 ml of 80% ethanol. The vials were kept in a freezer, at – 20°C, to avoid any possible chemical alteration. The volumes of sap collected from galled plants and healthy plants were 200 μl and 240 μl, respectively.

Collection of root extracts – After sap collection, roots were chopped into pieces of 1 to 2 cm and 5 g of either galled or healthy roots were crushed in 10 ml of 80% ethanol, using a pestle and mortar. The extracts were filtered in four layers of muslin and centrifuged for 30 minutes at 3,000 rpm. Xylem sap and root extracts were serially passed through 35 μm and 10 μm nylon sieves, to give a preliminary cleaning, and then passed through autoclaved membrane filters of 5 μm and 0.2 μm pore size, placed back into sterilized 5 ml capacity glass vials and kept in a freezer, at – 20°C, until processing (Wang & Bergeson, 1974).

Fractionation of xylem sap and root extracts – Two ionic exchange resins were used throughout this experiment: a basic anion exchange resin Dowex – 1, chloride form, dry mesh 200 – 400, and a cation exchange resin Dowex 50 W – X8, hydrogen form, mesh 200 – 400. Before use, both resins were activated. After activation, four 6 x 1 cm glass columns were prepared for each resin. The original contents of xylem

sap and root extracts were separated into three fractions through the ion exchange resins. These three fractions were acid (mainly organic acids), basic (mainly amino acids), and neutral (mainly sugars). The original volumes of each xylem sap and root extract were passed, separately, through the columns of Dowex 50 W – X8. The effluents were collected in 100 ml capacity sterilized Erlenmeyer flasks and passed through the columns of Dowex – 1. The effluents of these columns constituted the sugar fraction. The amino acids were eluted from the Dowex 50 W – X8 resin with 50 ml of a 7N NH_4OH solution and the organic acids were eluted from the Dowex – 1 resin with 40 ml of a 4N HCOOH solution (Canvin & Beevers, 1961). Each sample of amino acid, organic acid and sugar fractions was split into two sub-samples, evaporated to dryness in a rotary evaporator at 45°C for approximately 15 minutes and kept in a freezer, at – 20°C, until required for chemical analysis. Amino acid samples were analysed by gas-liquid chromatography and by two-dimensional paper chromatography. Organic acid and sugar samples were analysed by gas-liquid chromatography and by thin-layer chromatography.

Gas-liquid chromatography of amino acids – All amino acid samples were taken up in 1 m of distilled water and then varying amounts were dried (100 to 500 µl) in reacti vials using dry air. The internal standard was prepared by dissolving 100 mg of norleucine in 100 ml of distilled water. A 2 ml volume of n-propanol-2N hydrochloric acid reagent was added to each sample and then placed in an oven at 110°C for 30 minutes, cooled and then evaporated at temperatures of 70°C – 80°C under a stream of dry nitrogen. Heptafluorobutyric anhydride (0.2 ml) plus acetonitrile (0.1 ml) were added to the dried residue and acylation was carried out at 150°C for five minutes. Samples were again dried down at room temperature under a stream of dry nitrogen for approximately two hours. All samples were made up in volumes of 50 µl in ethyl acetate plus 200 µg of norleucine (amino acid internal standard). For analysis, 10 µl of the mixture was drawn into a 10 µl Terumo microsyringe and then injected into the gas chromatograph (5710 a Gas Chromatograph, Hewlett-Packard, U.S.A.).

Chromatograph conditions – The glass column was 12 'x 3/8' I.D., packed with 3% (w/w SE-30 on 120 – 140 mesh Gas-Chrom Q. The carrier gas was carbon dioxide (CO_2) and gas flow rates were as follows: carbon dioxide, 30 ml/min; hydrogen 30 ml/min; and air, 60 ml/min. The injecton port temperature was 250°C, the detector temperature (FID) 300°C and the auxilary temperature 100°C.

The column conditions were as follows: initial isothermal (hold) period of eight minutes at 80°C, with the temperature increasing at 4°C per minute until reaching the final temperature of 250°C for eight minutes. The chart speed was 0.5 cm/min and a flame ionization detector was used (Kirkman, 1974).

Amino acids in test samples were identified by comparing the chromatograms obtained with chromatograms previously developed from known standard amino acids. The peak areas for each amino acid were determined on a chromatogram, through the print-out of a 304-50 Computing Integrator (Laboratory Data Control, Shannon, Ireland). Concentrations of amino acids in the samples were calculated by multiplying peak areas by the response factor and correction factor of each amino acid, multiplied by the area of internal standard added to the sample.

Two-dimensional paper chromatography of amino acids – Amino acid fractions from roots and xylem sap of galled and healthy plants of black pepper were applied on medium flow rate 3 mm sheets (46 x 57 cm) of Whatman chromatographic paper. Spots of 100 µl were applied 8 cm equidis-

tant from two perpendicular edges and dried under a stream of warm air. Samples were applied on chromatographic paper with the aid of 20 μ l disposable micro-pipettes and chromatograms were repeated for all samples. For the first direction samples were repeated for all samples. For the first direction samples were run in phenol-water solvent (phenol, detached crystals, 75g; distilled water, 25 ml) for 18 hours and the sheets dried in a fume cupboard for at least 12 hours. Samples were run in a second direction, at right angles to the first, in n-butanol, 80 ml; glacial acetic acid, 20 ml; distilled water, 20 ml. After running for 15 hours in the second solvent the sheets were again dried in a fume cupboard for 12 hors. Paper chromatography was performed at room temperature (around 23°C). Amino acids were located by spraying the sheets with 0.2% ninhydrin (2 g of crystalline ninhydrin in 100 ml of absolute alcohol). The different amino acids present in the samples were determined by comparing with standard chromatogram of known amino acids.

Gas-liquid chromatography of organic acids – Samples were dried at room temperature (around 23°C) under a stream of dry nitrogen and reacted with a mixture of 50 μ l of N,0 – Bis (trimethylsilyl) – trifluoroacetamide, 30 μ l of trimethylcholorosilane and 20 μl of pyridine.

The mixture was heated for 30 minutes at 80°C and a 10 μl aliquot was injected with a 10 μl Terumo microsyringe into the gas chromatograph. The internal standard was prepared by dissolving 100 mg of 3,3 – dimethyl glutaric acid in 100 ml of distilled water. Two hundred micrograms of internal standard were added to each samples.

Qualitative identification of organic acids occurring in test samples was achieved by comparing the retention time of the peaks from samples with the peaks from known standard organic acids. Chromatographic conditions and calculations of organic acids concentrations in samples were similar to those for amino acids.

Thin-layer chromatography of organic acids – Standard solutions of organic acids were prepared by dissolving 5 mg of each acid in 2 ml of 10% concentrated formic acid. The following organic acids were used as standard: citric, fumaric, glyceric, glycolic, glyoxilic, malic, oxalic, oxaloacetic, pyruvic, succinic, tannic, tartaric and α - ketoglutaric. Precoated silica gel plates (Sil G 25, Macherey, Nagel and Company, Düren, FRG) were dried at 105°C for one hour before being used. Spots of 1 μ l of each standard were applied 1 cm apart and along a line of origin marked out 2 cm above the lower edge of the plates. Spots were applied with 1 μ l disposable micropipettes and dried under a stream of warm air. A mixture consisting of 1 μ l of each organic acid was also applied beside the individual spots. Two solvent systems were used: amyl formate-chloroform-formic acid (70 : 15 : 15 ml) and amyl-formate – chloroform – formic acid (20 : 70 : 10 ml). To ensure that chromatography took place in an atmosphere saturated with solvent vapours, the freshly prepared solvent was poured into the chromatographic tank at least one hour prior to use. In each solvent, the standards were allowed to run 1 1/2 hours.

After the solvent front had migrated approximately 15 cm the plates were removed and dried in a current of air in a fume cupboard for 24 hours to remove all possible traces of formic acid. Spots of organic acids were located by dipping the plates for three minutes into a reagent consisting of 0.1 g of bromocresol green, 500 ml of absolute alcohol and 5 ml of 0.1 M sodium hydroxide (Hansen, 1976).

Spots of 1 μl of organic acid fraction obtained from roots and xylem sap of galled and healthy black pepper plants were applied on silica gel plates following the sa-

me procedures already described. A mixture of all standards was applied beside the plant samples. Organic acids were located using the same reagent and for each solvent system three plates were prepared.

Gas-liquid chromatography of sugars – Dried samples of sugars were silylated in reacti vials by adding to each sample 100 µl of Tri-sil 'Z'. This reagent is a mixture of Trimethylsilylimidazole in dry pyridine – 1.5 meq/ml, which has also been used for silylation of other compounds (Horning *et al.*, 1967). The mixtures were allowed to react at 40°C for 15 minutes and an accurately measured aliquot of 10 µl was drawn into a 10 µl Terumo microsyringe and then injected into the gas chromatograph.

Qualitative presence of sugars in root extracts and xylem sap was confirmed by comparing the chromatograms obtained from samples with the chromatograms from known standard sugars previously prepared. Chromatographic conditions were similar to those utilized for gas-liquid chromatography of amino acids and organic acids. Concentrations of sugars in the samples were determined using the same calculation for amino acids and organic acids, except for the internal standard which was not used in this case.

Thin-layer chromatography of sugars – Standard solutions of sugars were prepared by dissolving 10 mg of each of the following sugars in 5 ml of distilled water: fructose, glucose, maltose, mannitol, ribose, sorbitol, sucrose and trehalose. Aliquots of 10 µl of each standard were drawn into a 10 µl Terumo microsyringe and applied in bands of 1.5 cm long and less than 2 mm wide, 0.5 cm apart and along a line of origin marked out 1.5 cm above the lower edge of 20 x 20 cm pre-coated silica gel plates which had been previously cut into two equal halves. Bands were dried under a stream of warm air and two plates each time were placed upright in the chromatographic tank with the plastic backs leaning against opposite sides of the tank. The chromatograms were developed twice in the same direction using the same solvent: ethyl acetate 60 ml, pyridine 30 ml, glacial acetic acid 5 ml, and distilled water 15 ml. Each run took approximately 40 minutes and between runs the plates were removed to a fume cupboard for drying for 10 minutes. After the second run plates were dried for 30 minutes and sugars located by using two different reagent combinations.

A 100 ml stock solution of methanol and 10 g of 4-aminobenzoic acid (PABA) was prepared. The reagent for single-stage reaction was prepared with 14 ml of stock PABA solution, orthophosphoric acid (90 g/100 ml) 20 ml, and methanol to give a final volume of 100 ml. Plates were dipped into the reagent, absorbent surface facing downwards, in a plastic tray for three minutes, and allowed to dry for 10 minutes at room conditions. The colour reaction was produced in a oven at 130°C for 5 to 10 minutes.

Another reagent used was naphthoresorcinol 200 mg, orthophosphoric acid 3.2 ml and methanol to give a solution volume of 100 ml. After being dipped into the reagent for three minutes, plates were dried at room temperature and then heated at 130°C in a oven for 5 to 10 minutes (Menzies & Mount, 1975).

Sugar fraction obtained from roots and xylem sap of galled and healthy black pepper plants were applied on silica gel plates, following the same procedures for the sugar standards, and located using the same reagents. Test samples were applied beside a mixture of standards and three half plates were used for each sample.

RESULTS

Amino acids in roots – Gas-liquid chromatography (GLC) of amino acid fraction obtained from infected and healthy roots of black pepper revealed the presence

of ten and nine amino acids, respectively (Table 1). Lysine was detected in roots of infected plants but not in roots of healthy ones. Serine and glutamic acid decreased 29 and 20% respectively and aspartic acid increased 10% in infected roots, compared to healthy ones.

Analysis of amino acid fraction undertaken by two-dimensional paper chromatography (TDPC) showed the presence of eleven and ten amino acids in infected and healthy roots, respectively (Table 2). Proline which was not detected by GLC appeared as faint spots on chromatograms of TDPC. Several small peaks were observed on the GLC chromatograms suggesting the presence of more amino acids in extracts from infected and healthy roots. As their concentrations in the root extracts were very low the specific component of each peak was not resolved and identified. Determination by TDPC also showed faint spots of compounds with positive reaction to ninhydrin indicating the occurrence of other amino acids in the samples.

Amino acids in xylem sap – Six amino acids were found in xylem sap from infected plants and five in xylem sap from healthy plants when samples were analysed by GLC. In contrast to the roots, concentrations of amino acids in xylem sap from infected plants were higher than in sap from healthy ones. Alanine, glycine, aspartic acid and glutamic acid increased 219, 207, 80 and 37% in xylem sap from infected plants compared to healthy ones (Table 1). Tyrosine was detected only in xylem sap from infected plants, either in samples analysed by GLC or by TDPC (Tables 1 and 2).

Table 1. Effect of infestation by *M. incognita* on the amino acid concentration in roots and xylem sap of black pepper. As detected by gas-liquid chromatography 1

Amino Acids	Healthy Plants (μg/ml of the Whole Sample)		Galled Plants (μg/ml of The Whole Sample) Roots	Sap
	Roots	Sap	(Change %)	(Change %)
Alanine	–	35.4	–	113.2 (+ 219)
Arginine	–	*	–	*
Aspartic	1003.3	51.2	1099.6 (+ 10)	92.5 (+ 80)
Glutamic	274.8	5.6	228.8 (– 20)	7.7 (+ 37)
Glycine	–	13.8	–	42.5 (+ 207)
Lysine	*	*	–	*
Proline	*	*	*	*
Serine	336.9	–	260.2 (– 29)	–
Threonine	–	*	–	*
Tyrosine	–	*	–	–
Valine	–	*	–	*

1 Mean of fifteen plants.
– Present but concentrations not calculated (see text)
* Not present at a detectable level.

Table 2. Effect of infestation by *M. incognita* on the amino acid concentration in roots and xylem sap of black pepper.

As detected by two-dimensional paper chromatography 1

Amino Acids	Healthy Plants		Galled Plants	
	Roots	Sap	Roots	Sap
Alanine	–	–	–	–
Arginine	–	*	–	*
Aspartic	–	–	–	–
Glutamic	–	–	–	–
Glycine	–	–	–	–
Lysine	*	*	–	*
Proline	–	*	–	*
Serine	–	–	–	–
Threonine	–	*	–	*
Tyrosine	–	*	–	–
Valine	–	*	–	*

1 Mean of fifteen plants.
– Present in the samples.
* Not present at a detectable level.

Analyses carried out by TDPC also showed the presence of six and five amino acids in xylem sap from infected and healthy plants, respectively. The amino acids were the same as those determined by GLC (Table 2). Once more, unidentified compounds were present in small amounts in xylem sap from infected and healthy plants but their peaks on chromatograms obtained by GLC were too close or too small to be resolved and identified.

Organic acids in roots – Four and three organic acids were present in roots of infested and healthy plants, respectively, in samples analysed by GLC. Citric and malic acids increased 223 and 143% in infected roots compared to healthy ones. Glycolic acid decreased 19% in roots of infected plants. Glyceric acid was found in extracts from infected roots but was not detected in extracts from healthy ones (Table 3). When samples were analysed by thin-layer chromatography (TLC) seven organic acids were found in root extracts of both infected and healthy plants (Table 4).

Organic acids in xylem sap – The sole organic acid identified in samples of xylem sap was glycolic acid which increased 84% in xylem sap from infected plants compared to healthy ones, when samples were analysed by GLC (Table 3). Glycolic and glyoxilic acids were detected by TLC in xylem sap from both infected and healthy plants (Table 4). Gas-liquid chromatography analysis

of root extracts and xylem sap from infected and healthy plants revealed the presence of small peaks on the chromatograms. Although this indicates the occurrence of other organic acids, qualitative and quantitative determinations were not possible since the concentrations in the samples seemed to be extremely low.

Sugars in roots – Gas-liquid chromatography (GLC) of sugars from root extract of infected plants revealed the presence of low concentrations of fructose and glucose. Some unidentified technical problem with the sugar sample from root extract of healthy plants prevented the determination of sugars present in this sample (Table 5). Thin-layer chromatography (TLC) of root extract from infected plants showed the occurrence of fructose, glucose and sucrose (Table 6). Again the root extract from healthy plants did not present reliable results, although some spots had appeared near the positions of fructose, glucose and sucrose. However, the presence of these three sugars in this particular sample was not confirmed.

Sugars in xylem sap – In xylem sap from infected plants, fructose showed an increase of 8% compared to its concentration in xylem sap from healthy plants. Glucose, on the other hand, decreased 80% in xylem sap from infected compared to its concentration in xylem sap from healthy plants. These were the only sugars positively identified and quantified in xylem sap when samples were analysed by GLC (Table 5). In samples analysed by TLC the qualitative results were similar to those obtained by GLC, which confirmed the presence of fructose and glucose in xylem sap from infected and healthy plants (Table 6). Few peaks formed on chromatograms from GLC indicated the presence of other compounds, probably sugars, but it was not possible to identify them.

Table 3. Effect of infestation by *M. incognita* on the organic acid concentration in roots and xylem sap of black pepper.

As detected by gas-liquid chromatography 1

Organic Acids	Healthy Plants (μg/ml of the Whole Sample)		Galled Plants (μg/ml of The Whole Sample)	
	Roots	Sap	Roots (Change %)	Sap (Change %)
Citric	182.8	*	591.9 (+ 223)	*
Glyceric	*	*	17.7	*
Glycolic	94.1	25.3	78.5 (– 19)	46.8 (+ 84)
Malic	89.7	*	218.7 (+ 143)	*

1 Mean of fifteen plants.
* Not present at a detectable level.

Table 4. Effect of infestation by *M. incognita* on the organic acid concentration in roots and xylem sap of black pepper.

As detected by two-dimensional paper chromatography 1

Organic Acids	Healthy Plants		Galled Plants	
	Roots	Sap	Roots	Sap
Citric	–	*	–	*
Glyceric	–	*	–	*
Glycolic	–	–	–	–
Glyoxilic	–	–	–	–
Malic	–	*	–	*
Succinic	–	*	–	*
Tartaric	–	*	–	*

1 Mean of fifteen plants.
– Present in the samples.
* Not present at a detectable level.

Table 5. Effect of infestation by *M. incognita* on the sugar concentration in roots and xylem sap of black pepper.

As detected by gas-liquid chromatography 1

Sugars	Healthy Plants (μg/ml of the Whole Sample)		Galled Plants (μg/ml of The Whole Sample)	
	Roots	Sap	Roots (Change %)	Sap (Change %)
Fructose	**	2.5	2.1	2.7 (+ 8)
Glucose	**	6.3	1.9	3.5 (– 80)

1 Mean of fifteen plants.
** Apparently present but not confirmed (seex text).

Table 6. Effect of infestation by *M. incognita* on the sugar concentration in roots and xylem sap of balck pepper.

As detected by thin-layer chromatography 1

Sugars	Healthy Plants		Galled Plants	
	Roots	Sap	Roots	Sap
Fructose	**	–	–	–
Glucose	**	–	–	–
Sucrose	**	*	–	*

1 Mean of fifteen plants.
– Present in the samples.
* Not present at a detectable level.
** Apparently present but not confirmed (see text).

DISCUSSION

Amino acids – The biochemical changes in amino acids induced in roots and xylem sap of black pepper plants by *M. incognita* differed somewhat from those previously reported for other host plants (Myuge, 1956; Owens & Novotny, 1960; Owens & Specht, 1966; Owens & Rubinstein, 1966; Wang & Bergeson, 1974). As no information is available about the biochemical effects of plant parasitic nematodes on black pepper, the results obtained in this study are discussed in relation to other plants.

A substancial increase in amino acids in roots of plants infected with root-knot nematodes has been reported by many workers (Myuge, 1956; Owens & Novotny, 1960; Owens & Specht, 1966; Owens & Rubinstein, 1966; Alam *et al.*, 1976). Although only three amino acids were quantified in roots of black pepper parasitized by *M. incognita*, the only one which showed an increase compared to healthy roots was aspartic acid. Serine and glutamic acid, on the other hand, decreased their concentrations in infected roots. In xylem sap from infected plants of black pepper the amino acids alanine, glycine, aspartic and glutamic increased their concentrations. The results are in contrast to those of Wang & Bergeson (1974) who found a decrease in the total concentration and amount of amino acids in xylem sap from tomato plants infected with *M. incognita*, compared to uninfected plants.

The parasitism of *M. incognita* in black pepper seems to cause a rather drastic reaction in this plant leading to a decrease in some amino acids in galled roots and an increase of these compounds in xylem sap, while in other hosts the general trend is to increase the concentration of free amino acids in roots and decrease it in xylem sap from plants infected with root-knot nematodes.

Masood & Husain (1975) observed an increase in amino acid concentration and a decrease of protein content in roots of two varieties of tomato, resistant and moderately resistant to *M. incognita.* A third variety, highly susceptible to this nematode, showed a decrease in amino acid concentration when infected. The authors suggested that the increase in amino acid followed by the decrease in protein content occurred due to the protein breakdown by the proteolytic enzymes secreted by *M. incognita.* On the other had, the decrease in amino acid concentration in the third variety could be related to its high susceptibility to *M. incognita.* The decrease in the concentration of some free amino acids in roots of black pepper infected with *M. incognita* could be an indication of its high susceptibility to this nematode. In roots of tomato parasitized by *Meloidogyne* spp., however, Owens & Specht (1966) found increases of 304 and 80% in free amino acids and proteins, respectively.

The absence of lysine in healthy roots of black pepper and xylem sap from infected and healthy plants suggests that its synthesis occurred only when roots were parasitied by *M. incognita;* If tthis amino acid is present in normal plants of black pepper it was not detected either by GLC or by TDPC. Lysine could also have been released from the nematode's body when galled roots were crushed for collection of the extract. As demonstrated by McClure (1977), females of *M. incognita* do act as a metabolic sink, accumulating high amounts of nutrients. Lysin was not present in healthy roots of jute but was detected in root extracts from galled plants and in females of *M. javanica* (Saxena, 1972), again suggesting that this amino acid is associated with the feeding sites of root-knot nematodes and with the nematodes themselves. Bird (1979) pointed out that the normal technical difficulties in making reliable measurements involving whole plants have led many workers to base their studies on plant's response to parasitism by *Meloidogyne* on homogenized extracts or dissected materials, causing the inevitable mixture of nematode and plant materials.

The absence of tyrosine in xylem sap from healthy plants could be explained by its normal low concentration. In xylem sap from tomato plants infected with *M. incognita* Wang & Bergeson (1974) found tyrosine in xylem sap from both healthy and infected plants although its concentration was higher in xylem sap from infected plants.

According to Bird (1979), plants of tomato respond to parasitism by *M. javanica* by decreasing their photosynthetic rate and accumulating free proline. In infected roots of tomato, proline accumulates mainly in egg masses and galls while its concentration decreases in the tops of these plants. If this mechanism does occur in plants of black pepper infected with *M. incognita,* proline concentration had already decreased to very low levels during the collection of xylem sap and root extracts.

The possibility that variations in amino acids, as observed in this work, were caused by differences in host physiology cannot be ruled out. Moreover, the method of collecting the xylem sap by keeping the root systems immersed in water could have affected the concentration of amino acids in the root tissues, as the upwards movement of water may have forced the translocation of compounds from roots to stems. Also, root extracts were obtained by crushing these same roots after sap had been collected.

Unfortunately, very little is known about the metabolism of amino acids in plant parasitic nematodes, so that the results obtained from this sort of study are discussed without involving the actual influence of the relationship on the nematodes themselves. *Meloidogyne* sp. has been reported to synthetize tryptophan, an essential amino acid, which implies the presence of metabo-

lic pathways previously not associated with animal metabolism (Nicholas, 1975). How the nematodes utilize amino acids inside the roots and what pathway of syntheses are involved remain unsolved. Irrespective of host physiology or method used, the results from this work reveal a marked influence on the composition of amino acids in black pepper plants due to the parasitism by *M. incognita.*

Organic acids – Concentrations of two organic acids namely citric and malic increased in galled root extract of black pepper plants. Increase in concentrations of citric and malic acids was reported by Owens & Specht (1966) and by Owens & Rubinstein (1966) in roots of tomato infected with *Meloidogyne* spp. Owens & Specht (1966) also reported a total increase of 67% in organic acid fraction from infected roots of tomato. Glyceric acid was found in infected roots of black pepper in low concentration but was not detected in healthy roots when the sample was analysed by GLC. In samples analysed by TLC seven organic acids, namely citric, glyceric, glycolic, glyoxilic, malic. succinic and tartaric were found in root extracts from both infected and healthy plants of black pepper.

The absence of citric, glyceric, malic, succinic and tartaric acids in root extracts and xylem sap from infected and healthy plants, in samples analysed by GLC and TLC, indicates that only very low levels of these acids occur naturally in black pepper plants. Glycolic was the sole organic acid detected in xylem sap from infected plants. Its concentration increased in xylem sap in contrast to the roots. The increase could have been caused by the translocation of this acid from the roots to the stems during the collection of xylem sap.

According to Owens & Specht (1966), decrease of organic acids in infected tissues suggests the blockage of the normal pathway responsible for their synthesis or acceleration of reactions in which they are converted to other intermediates. Increase of some organic acids in infected roots could be explained by an inverse mechanism in which their synthesis is normal but conversion to other intermediates is retarded.

Sugars – Only low concentrations of fructose and glucose were detected in extracts from galled roots of black pepper. These same sugars could not be found in extracts from healthy roots as the presence of an unidentified compound impaired the qualitative and quantitative analyses of any possible sugar present in this sample.

In roots of tomato infected with *Meloidogyne* spp., the reducing sugars fructose, glucose, trehalose and ribose decreased to little more than half, compared to their concentrations in roots of normal plants, except in the case of sucrose which decreased only about 20%. All phosphorylated sugars increased in galled tissues (Owens & Specht, 1966). In plants of *Acalypha indica* infected with *M. incognita*, Kannan (1968) found that sugars decreased in leaves and roots compared to uninoculated. The total carbohydrate in roots of tomato increased when plants were infected with *M. incognita* (Alam *et al.*, 1976). These authors did not mention which sugars among the carbohydrates increased in galled roots. Owens & Specht (1966) stated that the overall pattern of carbohydrate composition in altered tissues falls within the range for normal cells, except for starch which disappears in plant roots infected with *Meloidogyne* spp As for xylem sap of black pepper again the sugars fructose and glucose were the only compounds detected in samples from both infected and healthy plants.

Analysis of xylem sap from infected plants of tomato parasitized by *M. incognita* showed an increase of sugars compared to healthy plants. The concentration of sugars increased as the inoculum of *M. incognita* increased at 2, 4 and 6 weeks after inoculation. The greatest rate of increase was two weeks after inoculation. At the seventh week

both concentration and amount of total sugars in xylem sap from infected plants decreased to levels lower than those in xylem sap from healthy plants (Wang & Bergeson, 1974). The same workers suggested that the increase of sugars in xylem sap from infected plants was caused by intensive photosynthetic activity of infected plants which led to a significant translocation of sugars to the galled roots. Sugar increase in plants infected with *Meloidogyne* caused by acceleration of photosynthetic acitivty was demonstrated by Bodrova (1961) (cited by Wang & Bergeson, 1974).

The qualitative and quatitative differences observed in sugars from roots and xylem sap of black peppr infected with *M. incognita* show the ability of this nematode to change the normal chemical composition of host plants.

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