Biochemical Changes in Root Exudate and Xylem Sap of Tomato Plants Infected with *Meloidogyne incognita*[1]

ERNIE L. H. WANG and G. B. BERGESON[2]

Abstract: Under two monoxenic culture techniques of growing plants (filter paper and silica sand cultures), sugar in root exudate from *Meloidogyne incognita*-infected tomato increased 133 to 836% over controls. In contrast, amino acids were moderately reduced 52 to 56%. Chromatographic analysis showed that galled root exudate contained three sugars, twelve amino acids, and three organic acids, whereas healthy root exudate contained four sugars, fifteen amino acids, and four organic acids. Polysaccharide was responsible for the large increase of sugars in galled root exudates. The concn and the absolute amount of total sugars in the infected plant xylem sap were greater than in healthy plant xylem sap up to 6 wk after inoculation, whereas amino acids were moderately lower than in controls throughout the test period. Chromatographic analysis showed that xylem sap from both healthy and infected plants at 4 wk after inoculation contained four sugars and five organic acids. We identified 18 and 17 amino acids in the healthy and infected plant xylem sap, respectively. The concn of sugar increased as the nematode inoculum increased at 2, 4 and 6 wk after inoculation. The amino acids in all samples from the infected plant moderately decreased with an increase of nematode inoculum. We suggest that changes in total sugars and amino acids of infected plant xylem sap and root exudate are a probable mechanism by which tomato plants are predisposed to Fusarium wilt. *Key Words:* root-knot, *Lycopersicon esculentum,* disease physiology, disease complex, Fusarium wilt.

The interaction between root-knot nematodes and fungi causes synergistic increases in the disease severity of many plants. It has been concluded that galled root tissue serves as an enriched medium for supporting the colonization and growth of fungal pathogens (4, 5, 16, 24). Melendez and Powell (16) found hyphae of Fusarium wilt fungus growing vigorously within the vascular system well above the soil line and far from the site of nematode infection. The relationship between nutrients in xylem sap and susceptibility of plants to vascular pathogens has been considered in silver-leaf disease of fruit trees (2). Bergeson et al. (5) reported a consistent increase in *Fusarium oxysporum* f. sp. *lycopersici* and a decrease in actinomycetes in the rhizosphere of tomato infected with *Meloidogyne javanica*. Since activity of *Fusarium* spp. in the rhizosphere is strongly influenced by the host root exudates, and since root-knot infection could alter root exudates, the rhizosphere may be the site of the first step in a series of interactions leading to synergistic disease enhancement by root-knot nematodes. Therefore, this research was conducted to determine whether root-knot nematode infection induced chemical changes in xylem sap and root exudate of tomato.

Received for publication 18 February 1974.

[1]Portion of a Ph.D. dissertation submitted by the senior author to Purdue University. Journal Paper No. 5380, Purdue University Agricultural Experiment Station.

[2]Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907. Present address of senior author: Department of Agriculture and Fisheries, P.O. Box 834, Hamilton, Bermuda.

MATERIALS AND METHODS

Plant culture for collection of root exudates: Tomato seeds, *Lycopersicon esculentum* Mill. 'Bonny Best', were surface-sterilized in 0.5% sodium hypochlorite and germinated in a moist chamber. Seedlings were transferred to a sterile growth chamber consisting of a 400-ml beaker with three layers of filter paper on the bottom and a petri dish cover on the top. The beakers were placed under a combination of fluorescent and incandescent light with a 16-h photo-period at 21-23 C. Enough 20% Hoagland's solution was added every 48 h to keep the filter papers wet. When seedlings were 12 days old, they were inoculated with 400 surface-sterilized larvae of *Meloidogyne incognita* (Kofoid and White) Chitwood per seedling. Larvae were obtained from sterilized egg masses.

In a second filter paper culture experiment, the above method was modified by placing the beaker containing 10 inoculated seedlings on a petri plate and enclosing it with a glass cylinder covered with cheesecloth at the upper end. Sterile deionized water and Hoagland's solution were added through the cheesecloth with a sterile syringe to keep the filter paper wet. Beakers showing fungal growth or discoloration due to bacterial contamination on the filter paper were discarded. Two weeks after inoculation, the beakers were rinsed twice with 50 ml of sterile deionized water, and the rinsing solution saved. Intact plants were removed from the beaker, and the filter paper cut into small pieces and transferred to a flask containing 100 ml of sterile deionized water and placed on a shaker for 30 min. The filter paper suspension was combined with the rinsing solution, passed through a sterile Millipore filter (1.2 μm) and stored at −20 C until chemically analyzed.

Silica sand culture: Three-day-old seedlings were transferred to a sterile growth chamber containing 18 leak-proof pots made of heavy duty aluminum foil (6.5 × 5 × 5 cm). A glass tube was inserted into each pot to serve as an entry for nematode inoculum, sterile water, and nutrients. The sterile chamber was a framework of wood dowels (36.5 cm long × 20 cm wide × 32.5 cm high) placed on a porcelain tray. The chamber was covered by four layers of cheesecloth with one side which could be rolled up to allow access to the pots. The chamber containing silica-sand-filled pots was autoclaved twice for 1 h. After transplanting, the cheesecloth on the top was replaced with a surface-sterilized plastic sheet, and the chamber was placed under the same lighting as described. Eighteen days after transplanting, the seedlings were inoculated with 2,500 larvae/plant. Care was taken to prevent waterlogging during the daily watering. Two weeks after inoculation the pots were punctured on the botton with a sterile needle, and sterile deionized water was added until 50 ml of leachate had drained from each pot. The maximum water-holding capacity of the pot of sand was 45 ml. The degree of contamination of leachates from both infected and healthy plants was determined by counting the microbial colonies on PDA plates inoculated with a 1-ml aliquot of leachate before filtration and storage at −20 C.

Another silica sand culture experiment was conducted in a greenhouse using 1-wk-old seedlings transplanted to 10.2-cm (4-inch) diam surface-sterilized plastic pots which were suspended above the bench in metal racks. Two weeks after transplanting, plants in each pot were inoculated with 2,500 sterile larvae. Just enough water was added daily to avoid drainage from the pots. At 3, 7, and 9 wk after inoculation, 50 ml of leachate was drained from each pot and saved. The leachates were processed as described.

Plant culture for the collection of xylem sap: In experiment A, 4-wk-old tomato seedlings grown singly in 10.2-cm (4-inch) diam plastic pots in a greenhouse were inoculated with 5,000 larvae/seedling. After inoculation, xylem sap was collected at weekly intervals over an 8-wk period as described below. In experiment B, seedlings of the same age were inoculated with 10,000 larvae or 100,000 larvae per seedling and xylem sap was collected at 2, 4, and 6 wk. Prior to collection, soil was brought to field capacity and the plant stem was surface-sterilized 4 cm above the soil line and the shoot was cut off at this height. The pots were inverted, suspended on a rack, and a sterile test tube containing 5 ml absolute ethanol placed under the stem for 48 h to collect the exuded sap. In experiment B, the xylem sap was collected in a moist chamber and since the chamber was enclosed, contamination was reduced and ethanol was not required. After collection, the volume of xylem sap from inoculated and control plants was measured before filtration and storage as described.

TABLE 1. The influence of *Meloidogyne incognita* infection on the total amino acid and sugar content of tomato root exudate.

Culture method	Infection period (wk)	Total amino compounds (μg per plant per day)			Total sugars (μg per plant per day)		
		Healthy	Galled	Difference (%)	Healthy	Galled	Difference (%)
Filter paper[a]	2	13.0	6.2	−52	1.0	9.8	+863
Silica sand[b]	2	11.4	4.9	−56	10.5	24.6	+133
Silica sand[c]	3	8.2	2.8	−66	14.6	7.3	−50
	7	5.0	0.5	−89	8.8	2.1	−76
	9	11.2	0.5	−95	13.2	0.6	−95

[a]Average of 32 plants (monoxenic cultures).
[b]Average of 18 plants (monoxenic cultures).
[c]Average of 18 plants (nonaxenic cultures).

TABLE 2. The influence of *Meloidogyne incognita* infection on sugar composition of tomato root exudate.[a]

Sugars	Healthy Plants		Galled Plants	
	(μg per plant per day)[b]	% of total	(μg per plant per day)[b]	% of total
Glucose	2.28	27	...[d]	...
Fructose	3.06	36	3.95	20
Sucrose	2.50	30	4.50	23
Polysaccharide[c]	0.60	7	11.24	57
Total	8.44	100	19.69	100

[a]Plants were grown in silica sand under near-sterile conditions and inoculated with 2,500 larvae. Exudate was collected 2 wk after inoculation.
[b]Average of 18 plants.
[c]Each value represents the yield of monosaccharides after acid hydrolysis of polysaccharide from a thin-layer chromatogram.
[d]Glucose was not detected in exudate of galled roots.

Fractionation of root exudates and xylem sap: Stored samples of root exudate and xylem sap were again passed through a sterile Millipore filter (0.45 μm and 0.22 μm), concd in a rotary evaporator at 45 C, adjusted to pH 7, and passed sequentially through a column (1 × 4 cm) of Dowex 50 (H^+) (29) and a column of Dowex 1 (formate) (9) at a flow rate of 1 ml/min. The cationic fraction (amino acids) was retained on the Dowex 50 column. The effluent from the Dowex 50 column was passed through the Dowex 1 column which retained the anionic fraction (organic acids). The effluent from the Dowex 1 column contained the neutral fraction (sugars). The cationic fraction was obtained by eluting the Dowex 50 column with 2N NH_4OH, and the anionic fraction with 4N HCOOH. All three fractions were evaporated to dryness at 45 C and dissolved in sterile deionized water, then stored for further analysis.

Chemical analysis of root exudates and xylem sap: Total amino acids were determined by the ninhydrin method (30) using leucine as a standard. Total sugar was determined by the anthrone method (19) using glucose as a standard. Total amounts of reducing sugars were determined by the method of Nelson (20), with glucose as a standard, and for non-reducing sugar by the method of Roe (26) with sucrose as a standard.

Amino acids, organic acids, and sugars were identified by their R_f values, color, location, and by comparison with known samples on two-dimensional thin-layer chromatographs (12, 14, 22). These compounds were estimated quantitatively by the method of Purdy and Truter (25).

RESULTS

Change in root exudates of galled tomato grown in monoxenic cultures: In exudates from galled roots, collected 2 wk after inoculation, total amino acids decreased about 52% and total sugars increased 863% over controls (Table 1). In silica sand culture, the decrease of total amino acids (−56%) and the increase of sugars (+133%) followed the same pattern as that from the filter paper culture method, but the sugar increase was

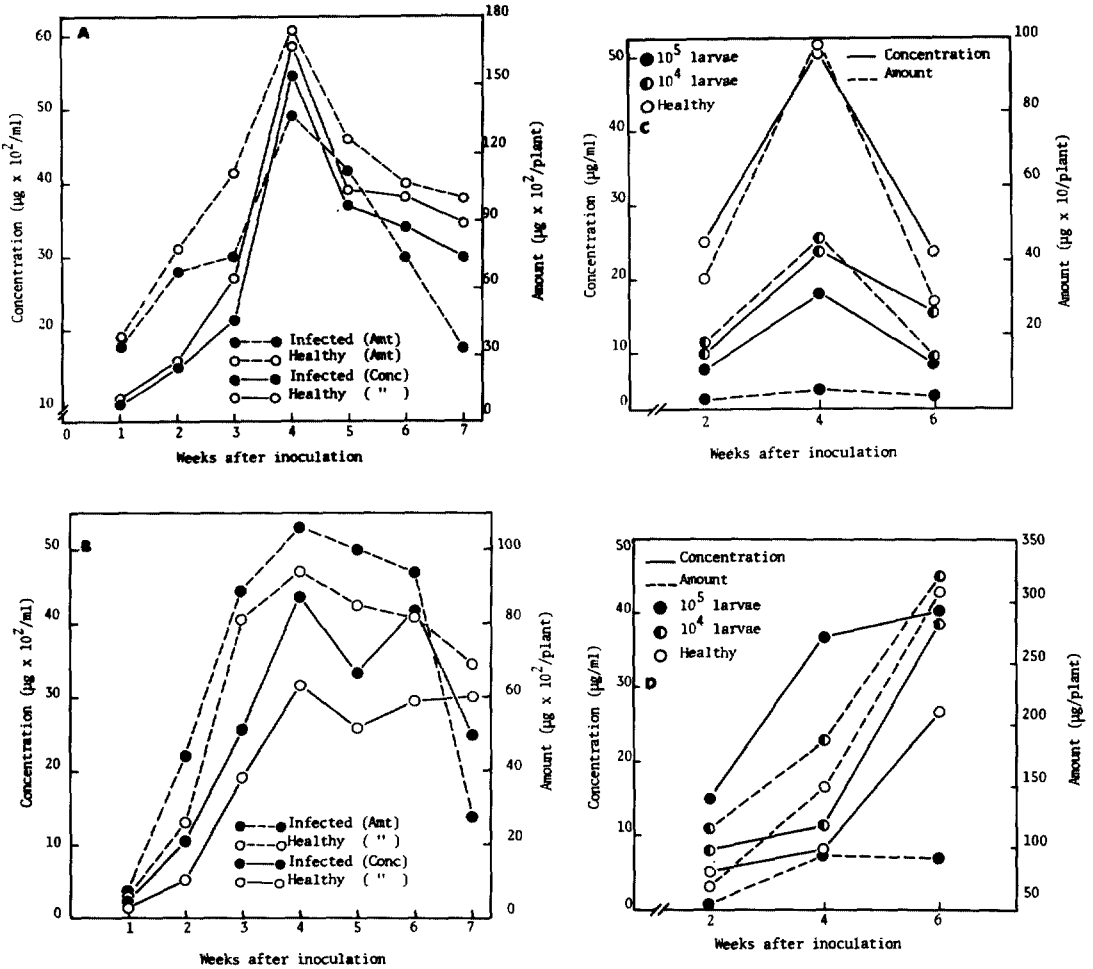

FIG. 1. **A)** Effect of *Meloidogyne incognita* on the concn and amount of amino compounds in xylem sap of tomato. **B)** Effect of *M. incognita* on the concn and amount of sugars in xylem sap of tomato. **C)** Effect of *M. incognita* inoculum level on concn and amount of amino compounds in xylem sap of tomato. **D)** Effect of *M. incognita* inoculum level on concn and amount of sugars in xylem sap of tomato.

considerably less. Since the microbial colonies (estimated from PDA plate counts) from galled and healthy root exudates in this experiment were less than 10 colonies/ml of root exudate, the few contaminants were considered negligible and the metabolities in root exudates probably were not significantly altered by microbial activity.

Changes in root exudates of galled tomato grown nonaxenically in silica sand cultures: Root exudates at 3, 7, and 9 wk after inoculation were heavily contaminated. The microbial colonies per ml of root exudate from galled plants were 18×10^3 and 124×10^3, whereas those from healthy plants were 3×10^3 and 6×10^3 after 7 and 9 wk, respectively. Total amino acids in exudates from galled roots decreased 66, 89, and 95% after 3, 7, and 9 wk, respectively. A striking difference that was never observed in monoxenic plant cultures was the decrease of total sugars amounting to 50, 76, and 95% after 3, 7, and 9 wk, respectively (Table 1).

Chromatographic analysis of amino acids, organic acids, and sugars in galled and healthy root exudates: Similar qualitative results were obtained from both monoxenic cultures, so only the chromatographic data from the silica sand cultures are presented. In root exudate from control plants, 15 amino acids were identified, whereas only 12 were identified from galled roots. The three amino acids in healthy but not in galled root exudates were threonine, serine, and histidine. Total amino

TABLE 3. Influence of *Meloidogyne incognita* infection on amino acid composition of tomato xylem sap.[a]

Amino Acids	Healthy Plants			Galled Plants		
	Concn (μg/ml)	Amount (μg per plant)	% total	Concn (μg/ml)	Amount (μg per plant)	% of total
Leucine	51.4	154.3	9.0	11.0	27.6	2.7
Isoleucine	23.5	70.6	4.1	25.6	64.0	6.3
Phenylalanine	19.0	56.9	3.3	20.6	51.6	5.0
Valine	11.5	34.5	2.0	26.2	65.4	6.4
γ-amino butyric acid	129.6	388.9	22.6	97.2	243.1	23.7
Tyrosine	16.3	48.8	2.9	24.8	62.0	6.1
Alanine	25.8	77.3	4.5	9.2	23.1	2.3
Proline	13.5	40.6	2.4	6.2	15.6	1.5
Threonine	20.7	62.1	3.6	18.2	45.5	4.4
Glutamic acid	43.7	131.1	7.6	32.6	81.6	8.0
Glycine	6.7	20.0	1.2	5.6	14.1	1.4
Serine	21.4	64.2	3.8	19.2	47.9	4.7
Citrulline	46.9	140.6	8.2	43.3	108.3	10.6
Aspartic acid	15.6	46.7	2.7	23.3	58.1	5.6
Lysine	47.5	142.4	8.3	26.4	66.1	6.4
Histidine	27.1	81.2	4.7	9.7	24.3	2.4
Arginine	7.6	22.9	1.3	10.3	25.7	2.5
Asparagine	44.8	134.5	7.8	...[b]	...	...
Total	572.6	1,717.6		409.4	1,024.0	

[a]Each value represents the average of 12 plants.
[b]Not present at a detectable level.

TABLE 4. The influence of *Meloidogyne incognita* infection on sugar composition of tomato xylem sap.[a]

Sugars	Healthy Plants			Galled Plants		
	Concn ($\mu g \times 10^2$/ml)	Amount per plant ($\mu g \times 10^2$)	% of total	Concn ($\mu g \times 10^2$/ml)	Amount per plant ($\mu g \times 10^2$)	% of total
Glucose	11.0	33.0	41.1	15.0	37.5	37.5
Fructose	7.0	21.1	26.2	13.4	33.6	33.6
Sucrose	5.9	17.6	22.0	8.1	20.2	20.2
Deoxyribose	2.9	8.6	10.7	3.5	8.8	8.7
Total	26.8	80.3		40.0	100.1	

[a]Average of 12 plants.

acids in root exudates from galled plants were 3.7 μg per plant per day vs. 9.1 μg per plant per day for healthy plants–a decrease of 60%. Three organic acids, fumarate, succinate, and α-keto glutarate were common to galled and healthy root exudates, whereas citrate was absent in galled roots. α-Keto glutarate and succinate were the most abundant in exudates from both galled and healthy roots. Total organic acids from galled root exudates were 4.2 μg per plant per day, whereas those from healthy root exudates were 9.6 μg per plant per day–a decrease of 56%.

Exudates from galled and healthy roots contained fructose, sucrose, and polysaccharide; healthy roots, in addition, contained glucose (Table 2). Polysaccharide constituted 57% of the total sugars in exudate from galled roots, but only 7% in healthy roots. Thus, the major differences between the exudates were the presence of abundant polysaccharide and the absence of glucose in galled roots. Quantitatively, fructose, sucrose, and polysaccharide in galled root exudates increased by 28, 18, and 1,773%, respectively. Total sugars in the galled root exudates were 19.6 μg per plant per day, and those in healthy root exudates were 8.4 μg per plant per day–an increase of 133%. The characteristics of the polysaccharide were identified as follows: it (i) was water-soluble; (ii) did not move when chromatographed in the second solvent system (*n*-butanol-ethyl acetate-isopropanol-acetic acid-water (35:100:60:35:30, v/v); (iii) moved only slightly on the thin-layer chromatogram in the first solvent system (isopropanol: water 4:1, v/v); and (iv) formed a napthoresorcinol-

positive bright blue strip which was widely separated from the fructose, glucose, and sucrose spots. The formation of the strip, a possible result of over concn of the polysaccharide or the existence of few oligosaccharides, was absent from healthy root exudates, but a napthoresorcinol-positive substance with very light blue color was present where the sample was spotted on the chromatogram. The area containing polysaccharide was removed from the silica gel plates, eluted with water, and hydrolyzed for 3 h in boiling 1 N HCl (17). The hydrolyzate was analyzed colorimetrically for total sugars and by thin-layer chromatography for sugar identification. The chromatogram showed a major spot which was glucose and a small unidentified napthoresorcinol-positive spot. A quantitative determination was also run on healthy root exudate; however, no qualitative determination was made due to the small quantity. The amount of polysaccharide in root exudates was also confirmed by calculating the difference between the total sugar and the sum of reducing sugars and nonreducing sugars. In all samples, this method also indicated abundant polysaccharide in galled root exudates.

Weekly changes in total amino acids and sugars in xylem sap of infected plants: In experiment A, the volume of xylem sap from infected plants was 8 to 50% less than from healthy plants. The concn of total amino acids decreased 2 to 13% in the first 6 wk and 33% at 7 wk after inoculation (Fig. 1-A). The amount of total amino acids decreased 14 to 35% during the first 6 wk and 68% at 7 wk after inoculation. The greatest differences in both concn and the amount of total amino acids between infected and healthy plants was at 3 and 7 wk after inoculation. Sap from both healthy and infected plants contained the highest concn and amount of total amino acids at 4 wk after inoculation. The concn and amount of total sugars in sap from infected plants were greater than that from healthy plants up to 6 wk after inoculation (Fig. 1-B). The concn of total sugars in the infected xylem sap increased by 30 to 92%, while the amount of total sugars increased by 10 to 69% over controls. The greatest rate of increase was observed 2 wk after inoculation. At 7 wk after inoculation, both the concn and the amount of total sugars in the sap of infected plants rapidly decreased to a level that was lower than that in healthy xylem sap.

Chromatographic analysis: Since both amino acids and sugars reached their highest level 4 wk after inoculation, these samples were analyzed qualitatively and quantitatively by chromatography for amino acids, organic acids, and sugars. Because the volume of sap from healthy and infected plants was not equal, both the concn and the amount of the compounds were calculated. We reasoned that the concn of a compound in xylem sap may be the more important to a vascular pathogen, whereas the net amount of a compound may be more important to the growth of a plant.

Eighteen and 17 amino acids were identified in sap from healthy and infected plants, respectively (Table 3). Asparagine was not detected in infected plants. Among the 17 common amino acids, the concn and the amounts of valine, tyrosine, aspartic acid, and arginine in the infected plants were greater than those from controls, whereas only the concns, but not the amounts, of isoleucine and phenylalanine in the infected plants was higher than in the control. All other amino acids decreased in sap from infected plants. Overall, total amino acids in the sap from the infected plants decreased 28% in concn and 40% in amount.

Five organic acids in xylem sap from both infected and healthy plants were identified. In both infected and healthy plants, citrate constituted more than 86% of the total, whereas fumarate, succinate, α-keto glutarate, and malate made up less than 14% of the total. In the infected plants the conc and the amount of the five organic acids decreased by 29 and 53%, respectively. Xylem sap from both infected and healthy plants contained glucose, fructose, sucrose, and deoxyribose. The concn of these sugars in sap from infected plants increased 36, 91, 37, and 25%, respectively (Table 4). As a whole, infection increased the concn of total sugars by 50%, whereas the amount of total sugars increased 25% over controls.

Effect of nematode inoculum level on total amino acids and sugars in xylem sap: In experiment B, at 2 wk after inoculation, the volume of xylem sap (ml per plant per 48 h) collected at an inoculum level of 10,000 larvae was about the same as in controls, whereas at an inoculum level of 100,000 it decreased 78%. At 4 and 6 wk, the volume of sap at the 10,000 inoculum level decreased 6 and 28%,

respectively, while at the 100,000 level the decrease was 85 and 78%, respectively (Fig. 1-C).

The concn and the amount of amino acids in all sap samples from infected plants were lower than from healthy plants, and it decreased as the nematode inoculum increased. The concn decrease was 33 to 58% at 10,000 larvae and 61 to 68% at 100,000 larvae, whereas the amount of amino acid decrease was 53 to 57% at 10,000 and 92 to 95% at 100,000. The highest concn and amount of amino acids in both infected and healthy xylem sap were at 4 wk after inoculation.

The increase in concn of sugars was correlated with nematode inoculum increase 2, 4, and 6 wk after inoculation (Fig. 1-D). However, the amount of sugars in the xylem sap increased only at an inoculum level of 10,000 larvae, whereas it decreased at an inoculum level of 100,000 larvae. Sugar concn at 10,000 larvae at 2, 4, and 6 wk increased 52, 34, and 45%, respectively, whereas at 100,000 larvae at 2, 4, and 6 wk it increased 200, 354, and 49%, respectively. In general, the concn and the amount of total sugars in sap from both infected and healthy plants tended to increase with time after inoculation.

DISCUSSION

A wide range of organic compounds exude from intact roots (11, 15). We determined only amino acids, organic acids, and sugars because of their important roles in the ecological succession of microorganisms colonizing roots in disease complexes. Quantitative variations (especially sugars) between the controls in the three experiments were judged to be associated with the different experimental conditions; i.e., filter paper vs. sand, monoaxenic vs. nonaxenic, container size, growth conditions, etc. Therefore, only intra-experiment comparisons were considered.

We found reduced amounts and kinds of amino and organic acids in galled root exudates from plants grown in monoxenic cultures; however, sugars increased quantitatively, but decreased qualitatively. Owens and Rubinstein (21) showed that the rate of intermediary metabolism is accelerated in galled roots, especially pathways leading to synthesis of protein and nucleic acids. De Mott (10) reported that the activity of hexose monophosphate pathway was increased 1.4 to 1.8 times in galled roots. The enzyme activities of several dehydrogenases of the citric acid cycle in galled tomato roots increased several fold over healthy roots (13). From those findings, a reduction of amino and organic acids, and the absence of glucose in exudates from galled roots could be caused by a rapid turnover rate of these metabolites as a result of the increased metabolic activities in galled roots. Since exudation of organic metabolites must move through the cytoplasm across root membranes and walls into the rhizosphere, the permeability of root cells, if altered by infection, could also cause the differences described. Neutral fractions of galled root exudates contained abundant polysaccharide. Secretion of a highly hydrated hexose polysaccharide is mediated by the golgi apparatus in corn roots (18). Since giant cells of galled tomato roots contain more golgi apparatus (6), this could account for the increase in polysaccharide in galled root exudates. Since hydrolyzates of polysaccharide from galled root exudates contained primarily glucose, we suggest that the absence of glucose in galled root exudates is related to the increase in polysaccharide synthesis.

In nonaxenic silica-sand cultures, total sugars in exudates from galled roots decreased markedly after 3 wk. The amount of amino acids was also extensively reduced. These reductions are probably associated with corresponding increases in the microbial populations in the root exudates. In galled root exudates, there was a 6-fold increase in microbial colonies at 7 wk and a 20-fold increase at 9 wk over controls. We believe that the decline in the amount of sugars and amino acids was a result of microbial consumption.

Schroth et al. (27) reported that root exudates containing amino acids and sugars from the host appear to provide the stimulus necessary for *Fusarium* chlamydospore germination and mycelial growth. They suggested that carbon (carbohydrates) is a major factor limiting chlamydospore germination in soil. Bergeson et al. (5) showed that propagules of *Fusarium* increased and that those of actinomycetes (an antagonist of *Fusarium*) decreased in the rhizosphere of galled roots. They suggested that the root-knot infection may have the dual effect of stimulating plant pathogens and inhibiting their antagonists. Actinomycetes develop

more slowly than most fungi and bacteria, a characteristic suggestive of their inability to be effective competitors and of their lack of prominence when the nutrient level is high and the pressure of competition great (1). From our data the stimulation of microbial populations around galled roots under nonaxenic conditions could explain the decrease of actinomycetes in the galled root rhizosphere. Also, the stimulation of plant pathogens may result from the increase of sugars in exudates from galled roots noted in our experiments.

In experiments A and B, the volume of xylem sap from infected plants was less than from controls. As the root-knot infection progressed, the volume of xylem sap gradually decreased, probably as a result of a reduction in absorbing surface area of galled roots (3) and injury to vascular tissue (7).

For colorimetric and chromatographic data, the quantitative differences in amino acids, organic acids, and sugars between xylem sap from healthy and infected plants coincided with those of root exudates and may be caused by the same factors. However, the increase of sugars in xylem sap from infected plants may be due to intensified photosynthesis of infected plants which results in excessive sugar translocation to galled roots. Bodrova (8) reported more intense photosynthetic activity in plants infected with *Meloidogyne* sp., and more reducing sugars than in control plants.

The increase in total sugar is directly related to the level of nematode inoculum. Below a certain level of inoculum, the root-knot infection appears to increase the concn and the amount of sugar in the xylem sap; e.g., 10,000 larvae in experiment B and 5,000 larvae in experiment A. When the nematode inoculum exceeds 10,000 to 100,000 larvae, only the concn of sugar is increased due to a reduced volume of sap.

Higher sugar and amino acid content in both healthy and infected plants in experiment A vs. B may be due to the fact that collections from A were made at night while collections from B were made at noontime. After completing the experiments, we noted in the literature (28) that during rapid transpiration and water uptake the solute concn of xylem sap decreases. Nonetheless, differences between healthy and infected plants in both experiments were still apparent.

Hyphae of Fusarium wilt fungus grow vigorously within the xylem elements of galled plants (16). In considering the mechanism of disease enhancement by root-knot, the increase in sugar in infected xylem sap would be an important factor in predisposing plants to secondary invasion by *Fusarium*. The decrease in amino acids in infected xylem sap may be unimportant for the growth of Fusarium wilt pathogen in the xylem elements. Van Andel (31) reported that the growth of *F. oxysporum* f. *lupini* was usually less in a liquid median containing various concns of amino acids than in media containing equimolar sugar concns. Asparagine, alanine, and leucine were unsuitable substrates for this fungus. He obtained similar results with a strain of *F. oxysporum* f. *callistephi*. Therefore, the absence of asparagine and a decrease in alanine and leucine in the xylem sap as determined in our experiments would not reduce the suitability of the plant as a host to the fungus, and may make it more suitable. Porter and Powell (23) reported that tobacco plants were maximally predisposed to Fusarium wilt by *M. incognita* 3-4 wk after infection. The time interval appears so important to the host that the incubation period necessary for Fusarium wilt is more nearly related to the length of nematode infection than to that of fungus. This implies time-related physiological changes induced by nematode infections and may relate to our results which indicate that sugars in the xylem sap reach maximum concns 4 wk after inoculation. We propose that changes in total sugars and amino acids in the xylem sap and root exudate of nematode-infected plants contribute to the enhancement of Fusarium wilt.

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