The Nature and Role of Metabolic Leakage from Root-knot Nematode Galls and Infection by Rhizoctonia solani

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Abstract: A severe root rot of tomato caused by *Meloidogyne incognita* and *Rhizoctonia solani* was associated with nutrient mobilization into gall tissue and root exudation. Root decay did not develop when root exudates were continuously removed by leaching. When leachates were collected from *M. incognita*-infected and control roots and applied to roots of tomatoes inoculated with *R. solani* alone, roots receiving leachates from *M. incognita*-infected roots developed a severe rot while roots receiving leachates from control roots were free of decay. During the fourth and fifth weeks following nematode infection, an increased mobilization of ^{14}C labelled compounds to nematode-infected roots occurred. Higher counts of water soluble nonvolatile ^{14}C labelled exudates leaked from nematode-infected roots than from control roots at each weekly sampling period. Higher concentrations of Ca, Mg, Na, K, Fe, and Cu were found in exudates from nematode-infected roots than from the control roots. During the first 14 days following nematode infection, carbohydrates were the major organic constituents in exudates leaking from nematode-infected roots. Fourteen days after nematode infection, nitrogenous compounds become the major organic constituents leaking from roots. Shifts in C/N ratio of root exudates from nematode-infected roots were associated with parasitic development of *R. solani* in tomato roots.
Key Words: fungus-nematode interactions, *Meloidogyne incognita,* root exudates, C/N ratios.

Meloidogyne spp. induce major morphological and physiological changes within roots. Owens and Specht (12) found that carbohydrates, pectins, cellulose, and lignin decreased in nematode-infected roots. Marked increases in hemicelluloses, organic acids, free amino acids, proteins, nucleotides, RNA, DNA, lipids, and minerals were found in nematode-infected roots. Physiological alterations in plant roots induced by *Meloidogyne* spp. or exudates emanating from infected roots could be of major importance in the predisposition of plants to infection by other soilborne organisms (5, 15). This study tested the hypotheses that root-knot nematode galls leaked more nutrients than nongalled roots, that these exudates stimulated and attracted the fungus to gall tissue, and that these exudates contributed nutritionally to the ability of the fungus to overcome the natural plant resistance and cause root rot.

METHODS AND RESULTS

Statistical Analysis: Data were subjected to analysis of variance (ANOVA). The treatment means were compared by Duncan's multiple range test at appropriate levels of significance as obtained from the ANOVA.

Leaching techniques: A double-root technique was developed by stimulating a secondary root system and by using standard aerial layering techniques on the main stem of 4-week-old 'Pixie Hybrid' tomato plants, *Lycopersicon esculentum* Mill. The secondary root system was used to provide nutritional support to the plant while various experiments were performed on the primary root system. Sterilized soil was used in all experiments. The normal root system was inoculated by injection of 10,000 freshly hatched and surface sterilized second-stage larvae of *M. incognita* into the soil around the roots. After 72 h, roots were removed from the soil and washed gently to remove adhering soil and larvae that had not penetrated the roots. The infected primary root system was transplanted into sterilized silica sand contained in 946-ml plastic pots with perforations in the bottom. Next, the secondary root system was transplanted into a 473-ml (16 oz) plastic cup that was modified by slits in the side and bottom of the cup. The cup was slipped around the stem and sealed with caulking rope to make the containers leak-proof. The container holding the secondary root system was filled with moist vermiculite and irrigated daily with single strength Hoagland's solution. The container holding the primary root system was then fitted into a leachate collection

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vessel. The plants were subsequently grown in the greenhouse for 5 weeks. The primary root system of each plant was leached continuously by a drip irrigation system that delivered approximately 400 ml of distilled water/pot/day. The leachates were collected daily in the attached bottom collection container. Leachates were filtered through 0.45-μm bacterial filters, concentrated to a 100-ml volume in teflon beakers over a hot water bath, and applied to plants as described for subsequent experiments.

The effects of continuous removal of root exudates on the interaction of *Meloidogyne incognita* and *Rhizoctonia solani* were studied by leaching the rhizosphere of nematode-infected tomato roots inoculated with *R. solani*. Inoculations with *R. solani* consisted of culturing one mycelial mat on potato dextrose broth blended with 100 ml of distilled water and pouring the mixture onto the surface of the silica sand in each of the pots that contained the primary root system. The fungal mycelium was covered with additional moist, sterilized sand. Ten tomato plants inoculated with *R. solani* daily received 100 ml of the concentrated leachate from the roots infected wth root-knot nematodes. The concentrated leachates were the only liquids that the primary root systems received for the duration of the experiment. Tomato roots inoculated with *M. incognita* alone, with *R. solani* alone, and with *M. incognita* + *R. solani* and subjected to continuous leaching for 5 weeks were free of root rot. When leachates were collected from *M. incognita*-infected and control roots, concentrated, and applied to roots of tomatoes inoculated with *R. solani* alone, the roots that received leachates from *M. incognita*-infected roots developed severe rot, whereas roots receiving leachates from control roots were free of rot. When tomato roots inoculated with *M. incognita* alone, *R. solani* alone, and *M. incognita* + *R. solani* were not subjected to leaching, severe rot developed on roots inoculated with *M. incognita* + *R. solani,* whereas roots from the other treatments were free of rot. Plants that received both the nematode + fungus inoculum were stunted.

Carbon-14 techniques: The effect of *M. incognita* parasitism on translocation and exudation of organic compounds from roots was determined by using Carbon-14 as a marker. Four-week-old 'Pixie Hybrid' tomato plants growing in steam-sterilized silica sand in paper cups were inoculated with 10,000 freshly hatched surface sterilized second-stage larvae. Seventy-two hours after inoculation, the roots were washed gently and transferred to solution cultures of single strength Hoagland's solution contained in plastic pots equipped with aeration tubes connected to needle valves to control the air flow and a filter to remove any airborne contaminants. The liquid level within each pot was maintained at 600 ml. At weekly intervals, the solution cultures were renewed.

The $^{14}CO_2$ was generated from $Ba^{14}CO_3$. One mCi of $Ba^{14}CO_3$ was emptied into the barrel of a 2-ml hypodermic syringe containing 6 mg of 'cold' $BaCO_3$. The $^{14}CO_2$ gas was generated when 1 ml of 10% perchloric acid reacted with the $Ba^{14}CO_3$ in the syringe. The acid was expressed from the hypodermic syringe and the $^{14}CO_2$ gas injected into a holding vessel equipped with a rubber serum stopper. (Personal communication, Roy Young, University California, Riverside.)

Four nematode-infected and four control tomato plants were pulse-labelled with $^{14}CO_2$ over a 42-day period at 7-day intervals following nematode inoculation. The foliage of each tomato plant was enclosed in a polyacetate bag which was tied securely around the main plant stem and sealed with caulking rope. Twenty μCi of $^{14}CO_2$ gas was removed from the holding vessel with a 50-μliter syringe and injected directly into the bag. The needle hole was covered immediately with cellophane tape. Plants were allowed to photosynthesize $^{14}CO_2$ for 2 h in full sunlight, after which the polyacetate bags were removed. After labelling, the duration of each weekly experiment was 32 h, of which 21 h were light hours.

At 8-h intervals for a period of 32 h after labelling was completed, two replicate 1-ml portions of the solution culture were removed from each pot. The samples were transferred to liquid scintillation vials containing 15 ml of Bray's solution (3). A 5-ml portion of the culture solution was removed from each pot at 8-h intervals and transferred to a 20-ml screw top vial. Since the experiment was not designed to study

volatile compounds, $^{14}CO_2$ and other volatiles were removed from the 5-ml sample solution by acidifying the sample with 0.5 ml of 0.1-N HCl and shaking each vial for 5 min. Two replicate 1-ml portions of this solution were processed as just described. The exudate samples were counted in a liquid scintillation spectrometer.

Thirty-two hours after being labelled with ^{14}C, the plants were removed from the pots. Each plant was divided into three samples: roots, stems, and leaves. The plant samples were dried at 60 C for 48 h and total dry weights were determined for each sample. The dried sample was broken into small pieces by using a mortar and pestle, and a 250-mg portion of each sample was placed on a small sheet of cotton fibers (about 50 mg) for combustion. The oxygen combustion method used for determination of total ^{14}C in tomato plants was described by Buyske et al. (4).

The distribution of ^{14}C within *M. incognita*-infected and control tomato plants 32 h after supplying $^{14}CO_2$ to the foliage is presented in Table 1. Only minor changes in ^{14}C mobilization occurred within nematode-infected and control roots during the first 14 days following nematode infection. At 21 days, there was increased translocation of labelled compounds from the aboveground portions to roots in all plants (P @ 0.01). Increases in ^{14}C mobilization to nematode-infected roots occurred from 28 to 35 days following nematode infection (P @ 0.01). Twenty-eight-day nematode-infected roots contained 53.9% more ^{14}C labelled compounds than control roots did. Forty-two days following nematode infection, higher levels of labelled compounds were detected in control roots than in nematode-infected roots (P @ 0.01).

The extent of leakage of ^{14}C labelled compounds from *M. incognita*-infected and control roots is presented in Figure 1. Higher counts of water soluble nonvolatile ^{14}C labelled exudates leaked from nematode-infected roots than from control roots ($P < 0.01$). Concurrently, there was a general decline in the total amount of ^{14}C that leaked from roots of both plant groups as they aged ($P < 0.01$). The greatest total leakage occurred in young plants and nematode-infected roots which leaked 36% and 21% more water-soluble, nonvolatile ^{14}C labelled compounds than did control roots at 7 and 14 days, respectively (P @ 0.05). The total leakage from all plants at days 28, 35, and 42 was from five- to six-fold less than during the early growth period ($P < 0.01$).

Electrolyte measurements: Electrolyte

TABLE 1. The effects of *Meloidogyne incognita* on the distribution of ^{14}C within the tomato plant at intervals after inoculation.

Plant part and treatment	Days after nematode inoculation					
	7	14	21	28	35	42
	Per cent of total radioactivity within plant[a]					
Leaf						
Nematode						
Infected Plants	87.94zy	87.10zy	81.73zy	83.36zy	79.20y	78.95y
Control Plants	91.00z	90.02z	82.65zy	86.79zy	87.60zy	66.57x
Stem						
Nematode						
Infected Plants	6.16wv	6.43wv	6.76xwv	6.55wv	10.63yx	11.77y
Control Plants	3.84v	4.98wv	7.03xwv	8.55yxw	7.32xwv	17.75z
Roots						
Nematode						
Infected Plants	5.88w	6.46xw	11.49y	10.07y	10.15y	9.27yx
Control Plants	5.14w	5.86w	10.30y	4.64w	5.07w	15.67z

[a]Means of four replications. Means without a common alphabet letter after the value differ statistically at the level of probability indicated. Comparison of means within a plant part by Duncan's multiple-range test. Levels of statistical significance (P) are as follows: Leaf @ 0.01, Stem @ 0.05, and Roots @ 0.01.

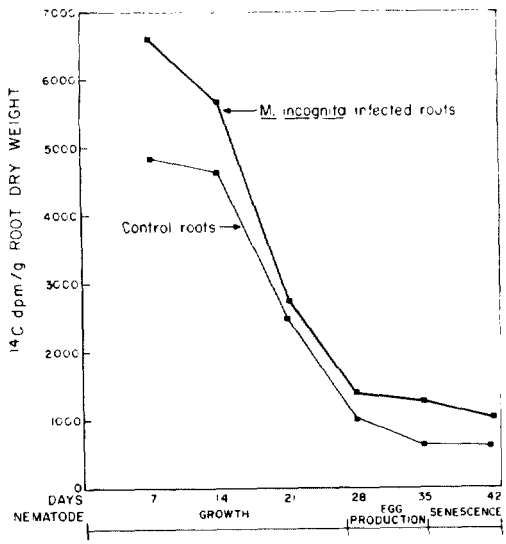

FIG. 1. Exudation of water soluble, nonvolatile ^{14}C labelled compounds as measured in disintegrations/minute from *Meloidogyne incognita*-infected and control tomato roots on 7, 14, 21, 28, 35, and 42 days following nematode inoculation.

leakage was measured from three nematode-infected and three control root systems washed thoroughly in sterile distilled water and blotted dry with sterile filter paper at 7-day intervals for a period of 42 days following inoculation. Individual galls from the nematode-infected roots were excised and the cut ends were sealed with nonionic stopcock grease. Portions of roots of the same age were excised from control plants and cut ends sealed with nonionic stopcock grease. The excised root sections were transferred to beakers containing 50 ml of sterile glass-distilled water and placed on a shaker maintained at 25 C in a temperature controlled room. The electrical conductance of the bathing solutions was determined at 3-h intervals over an 18-h period. The amount of electrolyte leakage was expressed as the change in conductance, micromhos/gram dry weight of root. At the end of the experiment, the root pieces were removed from the beakers and dried at 60 C for 24 h and weighed.

In addition to the excised root technique, electrolyte leakage from whole root systems of plants was measured, beginning 7 days after inoculation and at 7-day intervals thereafter, for a period of 42 days following nematode infection. Four replicate nematode-infected and four control plants were harvested at each interval for study. The silica sand was gently washed from the roots with distilled water and the root systems were severed from the plant, transferred to plastic containers which contained 400 ml of glass-distilled water, and treated in the same manner as the root sections.

The levels of electrolytes leaking from intact nematode-infected and control roots, in addition to excised galls and control roots after 8 and 9 h, respectively, are presented in Figure 2. During the period of maximum nematode growth within the root, i.e., 7 to 21 days following infection, leakage of electrolytes occurring from nematode-infected roots increased progressively in comparison with leakage of controls. All differences shown for the excised root technique are statistically significant at $P < 0.01$, and those shown using the whole-root technique at and after 21

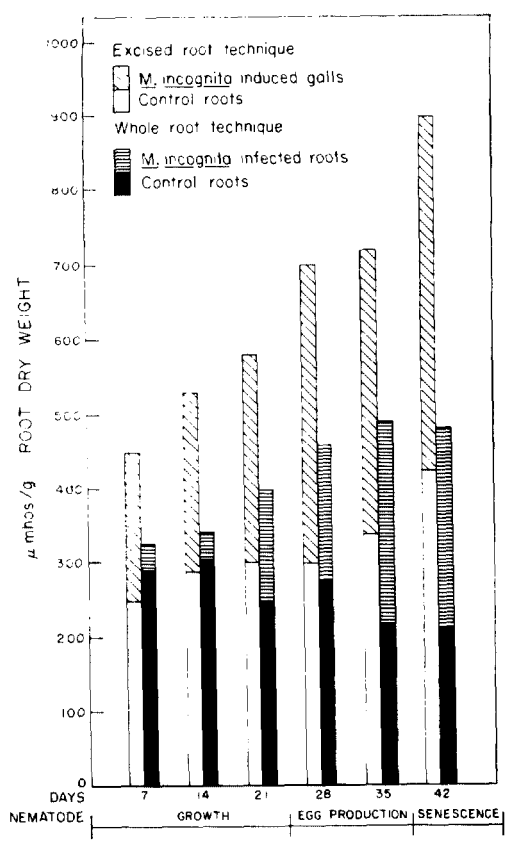

FIG. 2. Exudation of electrolytes from *Meloidogyne incognita*-infected and control tomato roots on 7, 14, 21, 28, 35, and 42 days following nematode inoculation.

days are statistically significant at $P < 0.01$. Total amounts of electrolytes leaking from intact nematode-infected root systems were not as great as those leaking from excised galls ($P < 0.01$). This finding, however, can be explained by the dilution effect created by nongalled portions of intact nematode-infected roots. Leakage of electrolytes from excised galls and intact roots from 28 days through 42 days was 1.5 to 2 times greater than that of controls ($P < 0.01$).

Root exudate production: A workable experimental system which would permit replicated, long-term production and recovery of root exudates from *M. incognita*-infected and control tomato roots was devised by using a three-tiered container column to partition the various components of the system.

Tomato seeds were surface disinfested in aqueous 0.1% HgCl solution for 2 min and then rinsed five times for 2 min in sterile, distilled water. Seeds were planted in sterile 10-cm diam plastic pots containing moist, steam-sterilized vermiculite. The pots were placed in aluminum flats that contained steam-sterilized, acid-washed silica sand. The bottom of the plastic pots had 10-mm holes which permitted roots to grow from the vermiculite into the sand below (Fig. 3). The seedlings were watered daily with sterile single-strength Hoagland's solution. After 5 weeks, roots growing in the sand were inoculated with 12,000 freshly hatched, surface sterilized, second-stage larvae. Seventy-two hours after inoculation, the silica sand was washed from the roots with distilled water. The plastic pot with the tomato plant growing in vermiculite hereafter is referred to as "Unit A" and the root exudate collection container as "Unit B." Unit A was equipped with a circular plastic ring containing a glass aeration tube (Fig. 3). The ring was fitted snuggly around Unit A 2.5 cm above the root exit holes and was snapped onto the top of Unit B to provide an airtight seal for root exudate collection. Unit B contained 600 ml of sterile glass-distilled water in Experiment I, and in Experiment II, it contained steam-sterilized, acid washed, 20-mesh silica sand. In Experiment I the water in Unit B was aerated with air that was filtered through a 14-μm millipore filter and then through a 0.45-μm filter. Exudate samples were combined from 10 *M. incognita*-infected roots and 10 control roots. In Experiment II, 20 *M. incognita*-infected and 20 control root systems were held in steam-sterilized, acid washed, silica sand instead of water. Six hundred ml of sterile glass-distilled water was passed through the sand at 48-h intervals to leach out the exudates.

Exudate solutions in both experiments were collected in "Unit C" (Fig. 3). Exudate solutions in Unit C were decanted, filtered, and concentrated *in vacuo* at 50 C at 48 h-intervals. Forty-eight-hour samples from nematode-infected roots and samples from control roots were combined into weekly samples and analyzed for organic and nonorganic chemicals. Root exudates were collected for 5 weeks following nematode inoculation.

This technique permitted: long-term production and recovery of root exudates (i.e., for the duration of the nematode's life cycle within roots), study of both inorganic and organic metabolites leaking from the roots, and experimental roots to gain their nutrition from the support root system held in vermiculite. Every precaution was taken to keep contamination to a minimum, i.e. vermiculite and sand steam-sterilized prior to use, seed and nematode inoculum surface disinfested, air filtered prior to use, and exudate samples filtered through bacterial filters immediately after collection. One-ml portions of the exudate solution from each container were streaked onto nutrient agar plates at each collection period to check for bacterial contamination. Bacterial counts were found to be low at each collection period. The system was considered reasonably free of contaminating organisms which might either contribute metabolites of their own or metabolize exudates prior to analysis. Weekly exudate samples were adjusted to a volume of 500 ml with glass-distilled water prior to chemical analysis.

Analysis of root exudates: Beginning 3 days after nematode infection and continuing for 42 days, major and trace elements were analyzed on root exudates collected from *M. incognita*-infected and control roots. The concentration of macroelements and microelements in exudate samples was determined by using a direct reading spectrograph. In the hydroponic experiments (Experiment I) and in the silica sand

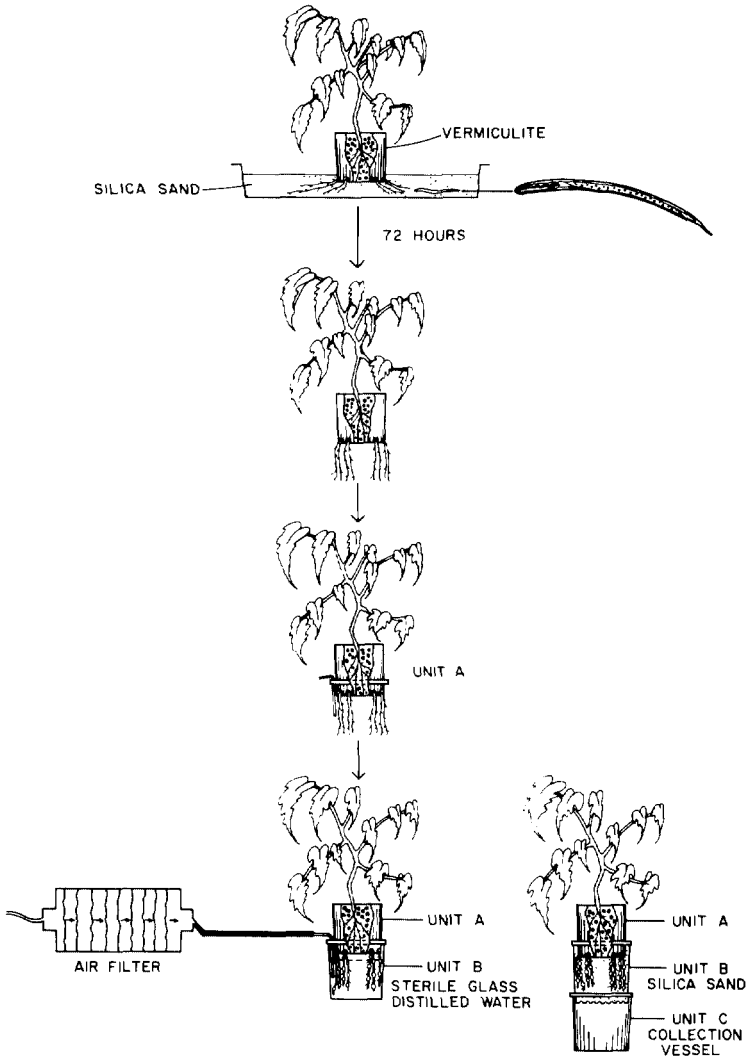

FIG. 3. Diagram of the apparatus designed for long-term production and recovery of root exudates from *Meloidogyne incognita*-infected tomato roots. Plants in plastic pots (Unit A) were set in sand and extending roots were inoculated with nematodes, removed from the sand, washed, and placed in a second plastic container (Unit B). A third container (Unit C) acted as a collection vessel for root exudates.

experiments (Experiment II), higher concentrations of Ca, Mg, Na, K, Fe, and Cu were found in the exudates collected from *M. incognita*-infected roots than from control roots. Maximum leakage of minerals from *M. incognita*-infected roots occurred between days 28 to 35 (P @ 0.01). The increase in leakage of minerals after 21 days contributed to the increase in electrolytes shown in Figure 2. During this period, leakage from nematode-infected roots, as compared with that from control roots, was increased in silica sand by the following: 149% Ca, 139% Mg, 46% Na, 226% K, 65% P, 112% Fe, and 102% Cu. The high levels of mineral leakage observed coincide with the period prior to and during the time of nematode egg production.

Total nitrogen on 1-ml portions of the exudate solutions was determined by using Nesseler's reagent after the sample was digested at 310-320 C for 2-3 h according to the method of Lang (6). Ammonium sulfate was used as the nitrogen standard. Total protein in exudate samples was determined by using the method of Lowry

et al. (7). Bovine serum albumin was used as the protein standard. Quantitative estimates of the amino acids in the exudate solutions were obtained by using the method of Yemm and Cocking (18).

Total carbohydrates were determined by using the method of Mokrasch (8). Total reducing sugars were determined by using the method of Nelson (11). Glucose was used as the standard in each case. Glucose was determined by using Glucostat (1) with glucose as the standard. Total carbohydrates and total N data obtained from root exudates collected continuously (from 7 to 42 days following nematode infection) from *M. incognita* and control roots are presented in Figure 4. During the first 14 days following nematode infection, relatively more carbohydrates than nitrogen occurred in exudates from nematode-infected roots. Nematode-infected roots leaked 48% greater concentration of carbohydrates than did control roots during the 7- to 14-day period following nematode infection (P @ 0.01). Reducing sugars, 17% of which were glucose, made up 30% of the total carbohydrates in the exudates collected from 3-7 days after nematode infection. Reducing sugars, 16% of which were glucose, made up 29% of the total carbohydrates collected between days 7 to 14 after nematode infection. No significant differences were found in the amount of carbohydrates in the exudates collected during the period from 21 to 42 days after nematode infection. Fourteen days after nematode infection and until the termination of the experiment, control and nematode-infected roots exuded low concentrations of total carbohydrates in about equal amounts (P @ 0.01). Nematode-infected roots leaked higher levels of total N between 21 to 42 days than did the control roots (P @ 0.05). Total carbohydrate leakage decreased after the first 14 days following nematode infection and remained low for the duration of the nematode's life cycle within roots (P @ 1%). In contrast, the greatest increase in total nitrogen leaking from nematode-infected roots was during the later stages of the nematode's developmental cycle within roots (P @ 0.05) and consisted about equally of protein and amino acids.

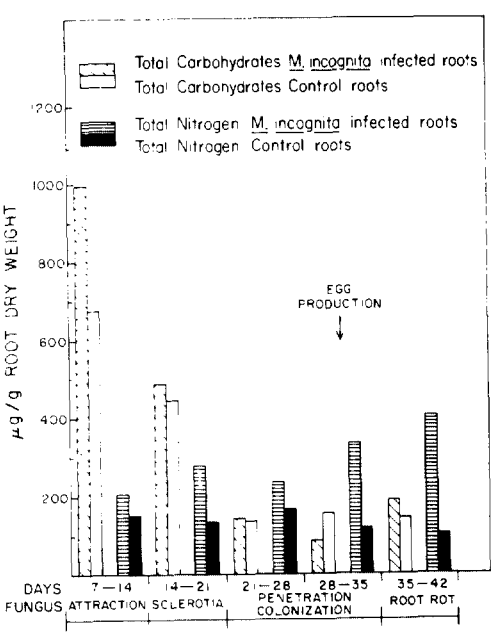

FIG. 4. Total carbohydrates and total nitrogen occurring in tomato root exudates collected by utilizing a silica sand leaching technique from *Meloidogyne incognita*-infected and control roots from 7 to 42 days following nematode inoculation.

DISCUSSION

A study of the prepenetration response of *R. solani* to stimuli originating from *M. incognita*-infected roots and passing through a semipermeable cellophane membrane suggested that root exudates were responsible for attracting the fungus to the galls and for initiating sclerotial formation (5). If the stimuli inducing *R. solani* attack of nematode-infected roots were root exudates, removal of the exuded substances as they were produced should reduce the severity of root disease. When tomato plants were inoculated simultaneously with *M. incognita* and *R. solani* and subjected to continuous leaching utilizing a continuous drip irrigation system, no root decay occurred. In contrast, when leachates were collected from *M. incognita*-infected roots and applied to roots of tomatoes inoculated with *R. solani* alone, a severe rot developed on roots receiving leachates. A severe rot developed when tomato roots were inoculated with *M. incognita* + *R. solani* and not subjected to leaching.

Increases in ^{14}C mobilization into nematode-infected roots occurred on days 28 and 35 following infection. This result corresponds to the time when the female nematodes were actively producing eggs

and placing high demands upon the plant for nutrition. The higher levels of labelled compounds detected in control roots after 42 days occurred at a period when the nematode had either completed or was nearing the completion of its life cyle and there was little nematode activity within these roots. These results and those of Owens and Specht (12) and Bergeson (2) clearly demonstrate that there is increased mobilization and accumulation of nutrients in gall tissue.

In addition to the increased levels of carbon compounds in galled roots, there were also increases in ^{14}C labelled metabolites in exudates from nematode-infected roots in comparison with increases from control roots, particularly during the first 2 weeks after nematode infection. Carbohydrates were the major ^{14}C labelled constituents in the exudates recovered between 3 and 14 days. The high concentrations of carbohydrates exuding from nematode-infected roots may be due to increased amylase, cellulase, and pectinase production by the nematode during the time of penetration and migration through the root to their feeding sites and during giant cell initiation.

The major differences between ^{14}C labelled metabolites exuding from *M. incognita*-infected roots and control roots on days 28, 35, and 42 were in the amounts of nitrogenous compounds (primarily protein and amino acids). The exact source of these nitrogenous leakage products is still not fully known. In addition to possible leakage from giant cells, the adult female nematode discharges nitrogenous waste products and/or secretes stylet exudates, both of which are high in nitrogen content (9, 10). The excretion of nitrogenous compounds such as urea and amino acids from the nematode within the roots could also have a marked effect on osmotic relationships and permeability of the plant cells.

The levels of electrolytes leaking from nematode-infected roots increased progressively, following infection, from days 7 to 42, with maximum leakage occurring after 21 days. Following nematode infection, the period between days 28 and 35 coincided with the period when the swollen females ruptured the cortex and epidermis of the roots and deposited their eggs in a gelatinous matrix on the root surfaces. Minerals and amino acids were the major electrolytes detected in root gall exudates. Nematode-induced giant cells are located in the xylem of the root where mineral concentrations are high and the rupturing of cortical cells by the nematode at 28-35 days may provide a natural channel for xylem fluids to leak directly into the rhizosphere.

The results appear to be in disagreement with those of Wang et al. (15) who found the electrolyte leakage lower from *M. incognita*-galled tomato roots than from healthy roots. The fact that their method of calculation was in percentages rather than absolute measurements may account for the differences in their data. They did find that the concentration of electrolytes in the galled root homogenate was 60% greater than in healthy root homogenate, a confirmation that galls do act as nutrient sinks.

In view of the etiological sequence of events that occurred in this complex disease, *R. solani* was stimulated in the rhizosphere and attracted to nematode-infected roots during the first 14 days, a time when total carbohydrates in exudates were more abundant from roots with nematodes and the C/N ratio was high. Although the precise events that triggered sclerotial initiation by *R. solani* are not known (13), carbon source and its concentration are known to influence the number, size, coloration, and distribution of sclerotia *in vitro* (14). Sclerotia were formed directly on gall surfaces from 14-21 days following nematode infection, a time when total nutrients in root exudates were declining and there was a balanced C/N ratio. Twenty-eight days after nematode infection, the sclerotial masses on gall surfaces were large, abundant, and beginning to germinate. At this time, nutrients in exudates from galled roots were primarily nitrogenous compounds and minerals that resulted in a shift to a low C/N ratio. Weinhold et al. (16, 17) found that the nutrient requirement for maximum virulence of *R. solani* was greater than for vegetative growth. They reported that an increase in asparagine content of the medium on which *R. solani* was grown resulted in a corresponding increase in virulence. When asparagine content was reduced to 0.5 gm/liter, the resulting

mycelium was avirulent, regardless of the glucose concentration in the medium. Therefore, a critical level of nitrogen was required for maximum virulence of *R. solani*.

The present studies show that, at 3 to 4 weeks after nematode infection, the nutritional balance on the root surface favors a parasitic rather than a saprophytic development of the fungus. The host resistance of the root is probably weakened by nematode parasitism and thereby gives way to a strengthened facultative parasite. Once within the gall tissue, the fungus is surrounded by highly active cells containing high levels of carbohydrates, amino acids, and minerals which provide a luxuriant medium for rapid fungus growth and enzyme production. Indeed, the fungus did rapidly and extensively colonize the nematode giant cells. It then moved into the surrounding xylem elements of the roots and eventually induced tyloses and vascular browning of the stem tissue.

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