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Carbon Partitioning in Soybean Infected with *Meloidogyne incognita* and *M. javanica*[1]

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Abstract: Seven-day-old seedlings of two cultivars (Cristalina and UFV ITM1) of *Glycine max* were inoculated with 0, 3,000, 9,000, or 27,000 eggs of *Meloidogyne incognita* race 3 or *M. javanica* and maintained in a greenhouse. Thirty days later, plants were exposed to $^{14}CO_2$ for 4 hours. Twenty hours after $^{14}CO_2$ exposure, the root fresh weight, leaf dry weight, nematode eggs per gram of root, total and specific radioactivity of carbohydrates in roots, and root carbohydrate content were evaluated. *Meloidogyne javanica* produced more eggs than *M. incognita* on both varieties. A general increase in root weight and a decrease in leaf weight with increased inoculum levels were observed. Gall tissue appeared to account for most of the root mass increase in seedlings infected with *M. javanica*. For both nematodes there was an increase of total radioactivity in the root system with increased levels of nematodes, and this was positively related to the number of eggs per gram fresh weight and to the root fresh weight, but negatively related to leaf dry weight. In most cases, specific radioactivities of sucrose and reducing sugars were also increased with increased inoculum levels. Highest specific radioactivities were observed with reducing sugars. Although significant changes were not observed in endogenous levels of carbohydrates, sucrose content was higher than reducing sugars. The data show that nematodes are strong metabolic sinks and significantly change the carbon distribution pattern in infected soybean plants. Carbon partitioning in plants infected with nematodes may vary with the nematode genotype.

Key words: carbohydrates, carbon partitioning, *Glycine max, Meloidogyne incognita, Meloidogyne javanica,* nematode, photoassimilate translocation, root growth, soybean.

After the penetration of the root by the *Meloidogyne* second-stage juvenile (J2), some cells of the vascular parenchyma around the J2 become hypertrophied, and are generally referred to as giant cells (Huang, 1985). Hypertrophy seems to be a response to the feeding activity of the nematode.

There has been some disagreement regarding the importance of nematodes as metabolic sinks. Wallace (1974) exposed tomato plants infected with *Meloidogyne javanica* to $^{14}CO_2$ and concluded that root galls did not constitute a metabolic sink. Bird and Loveys (1975), however, observed intense incorporation of radioactivity in nematodes and egg masses in tomato infected with *M. javanica*. The peak of radioactivity was coincident with the commencement of egg-laying, suggesting a high demand for nutrients at this stage, presumably translocated from the leaves via phloem. McClure (1977) exposed tomato plants infected with *M. incognita* to $^{14}CO_2$ and, using autoradiography, showed that more radioactivity was located at the infection site than in uninfected parts of the root.

Further evidence that nematodes can be significant metabolic sinks was recently obtained by Böckenhoff et al. (1996) when they used radioactive and fluorescent probes to investigate the role of syncytia as transfer cells in plants infected by cyst nematodes (Böckenhoff et al., 1996). A similar conclusion was obtained for *M. incognita* infecting tomato (Dorhout et al., 1993).

Obviously, since energy and organic nutrients are required to support nematode growth and egg production (Meon et al., 1978), some degree of competition for photoassimilates must exist between host and parasite. However, it is not clear whether diversion of photoassimilates to the parasite is important to shoot growth when compared with effects of root dysfunction on water and nutrient uptake.

Poskuta et al. (1986) measured photosyn-

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thesis, photorespiration, and respiration of soybean infected with *Heterodera glycines* and concluded that growth reduction of infected plants was directly related to reduced leaf area, reduced photosynthesis per unit of leaf area, and altered dry matter partitioning.

Considering that the growth in size of adult females of *M. incognita* in a moderately resistant *Vitis vinifera* cultivar was comparable with growth in a susceptible cultivar (Melakeberhan and Ferris, 1988), Melakeberhan et al. (1990) investigated the possibility that resistant and susceptible cultivars might partition energy differently, either for defense or for repair of damage caused by nematode infection. It was observed that moderately resistant and susceptible cultivars expended similar amounts (15% and 10%, respectively) of the assimilated energy. The authors concluded that the defense mechanisms in the resistant cultivar may have a high energy price and that the difference in growth of inoculated and uninoculated susceptible plants might be explained by high demand of energy for nematode growth and reproduction. Our objective in this study was to compare carbon partitioning in two soybean cultivars differing in their susceptibility to *M. incognita* and *M. javanica.*

Materials and Methods

Plant culture and nematode inoculation: Seeds of the soybean cultivars Cristalina and UFV ITM1 were germinated in pots containing a mixture of soil and sand (1:1) that had been autoclaved (120 °C, 60 minutes) followed by methyl bromide treatment. Seven-day-old seedlings with two expanded leaves were inoculated with 0, 3,000, 9,000, or 27,000 eggs of *M. javanica* or *M. incognita* race 3. Eggs were extracted from infected tomato plants according to Taylor and Sasser (1978). Until treatment with $^{14}CO_2$, the pots were kept in the greenhouse and received 100 ml of complete nutrient solution every week (Hoagland and Arnon, 1950). During the growth period the temperature inside the greenhouse varied between 18 °C and 28 °C. There was no control of light. Each treatment was replicated five times.

$^{14}CO_2$ treatment: Each pot was placed inside another plastic pot, which had been filled with water (Fig. 1). The water level reached the middle of the inner pot. A layer of cooking oil was included on the surface of the water to avoid diffusion of $^{14}CO_2$ into the water. A small beaker containing an aqueous solution of 85 kBq of $NaH^{14}CO_3$ was placed on the substrate, and a transparent plastic jar was inverted and lowered over the pots so that the open end became immersed in the water. With a syringe, the plastic jar was perforated and 200 µl of 4 N HCl was added to the $NaH^{14}CO_3$ solution in the beaker. Immediately after removing the needle, the hole was closed with adhesive plastic tape. The whole system was assembled on the day of exposure to $^{14}CO_2$. $^{14}CO_2$ release was initiated at 8:00 a.m. on a sunny day. The seedlings were kept under these conditions for 4 hours, when the jars were removed and the pots transferred to the bench. The exposed plants were harvested at 8:00 a.m. on the next day.

Collection and analyses: Shoots were cut 1 cm above the ground and the leaves were dried at 80 °C to determine dry weight. The roots were washed with running tap water, and, after brief blotting with filter paper, they were cut into small pieces, mixed, and divided into two portions and the fresh weight determined. One portion was used to estimate the number of nematode eggs, and the other was used for chemical analysis. Eggs were extracted from the roots and

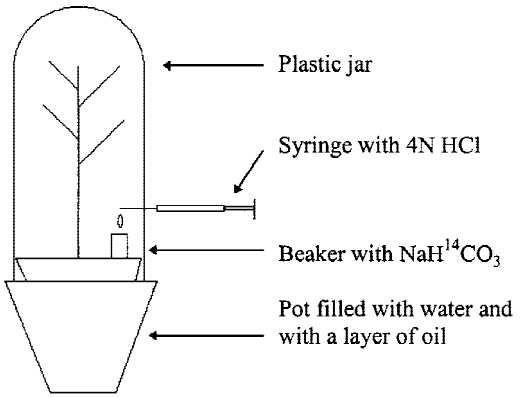

Fig. 1. Diagram of the apparatus used to expose soybean seedlings to $^{14}CO_2$.

evaluated according to Taylor and Sasser (1978). The reproduction factor was calculated by dividing the total number of eggs per root system by the number of inoculated eggs. The portions reserved for chemical analysis were frozen in liquid nitrogen and extracted with 12 ml of methanol with a Politron cell disrupter (Kinematika AG Littau, Switzerland). The extracts were centrifuged at 4 °C, and the supernatant was collected for analysis. A 1-ml aliquot was taken from the extracts, transferred to scintillation vials, and dried at 80 °C. Radioactivity was determined after addition of 1 ml methanol and 5 ml of organic scintillation fluid. In order to determine counting efficiency, a known amount of radioactivity of a labeled aqueous solution of $NaH^{14}CO_3$ was added to the flasks and counting was repeated. Reducing-sugar concentration in the methanolic extracts was determined with Nelson's reagent (Chaplin, 1986) and sucrose according to Dubois et al. (1956). Methanolic extracts (1 ml) belonging to the same treatments were combined, and the methanol was partially eliminated by flushing the samples with N_2 and drying under vacuum. The residue was solubilized in 3 ml of distilled water and mixed with 0.5 g of Dowex 1 – $HCOO^-$ and 0.5 g of Dowex 50 – H^+ (Sigma Chemical Co. St. Louis, USA) ion exchange resins in order to eliminate amino acids and organic acids. After gentle agitation at 4 °C overnight, the Dowex resins were eliminated by centrifugation, and sucrose and reducing sugars were determined as described. Radioactivity was determined after drying a 500-µl aliquot at 80 °C followed by the addition of 1 ml methanol and 5 ml of organic scintillation fluid.

Statistical design and analysis: The pots were distributed randomized on the bench in the greenhouse. The data for each nematode were analyzed separately as a double factorial (cultivar × inoculum level) with five replicates. Means were compared by Tukey ($P = 0.05$) if the F value was significant.

Results

Plant growth and egg production: Meloidogyne javanica reproduction seemed to be better than that of *M. incognita* in both cultivars. Egg production per gram root fresh weight was greater for *M. javanica,* particularly on cultivar Cristalina (Table 1). In addition, the nematode reproduction factors calculated for seedlings inoculated with 3,000 eggs were 0.19 for *M. incognita* on UFV ITM1, 0.29 for *M. incognita* on Cristalina, 0.33 for *M. javanica* on UFV ITM1, and 0.44 for *M. javanica* on Cristalina.

Considering that eggs are the ultimate sink for photoassimilates, earlier egg production by *M. javanica* would lead to greater translocation of labeled products to roots infected with this nematode, as shown later. Consequently, comparison of plants infected with *M. javanica* and *M. incognita* would be misleading. Therefore, the data were statistically analyzed as a double factorial (2 cultivars × 4 inoculum levels) for each nematode.

Although not statistically discriminated at the cultivar × inoculum interaction level, for both nematodes there was a trend to associate increase of root fresh weight and reduction in leaf dry weight with increased levels of inoculum (Table 1). These differences were greater with *M. javanica.* Compared with the control seedlings, the reduction of leaf dry weight of Cristalina infected with *M. javanica* was more pronounced than for other treatments. For example, at the highest inoculum level (27,000 eggs) there was a 60% reduction, which is coincident with the higher number of eggs per gram root fresh weight. However, when UFV ITM1 infected with *M. javanica* was compared with Cristalina infected with *M. incognita,* the two varieties showed similar leaf dry-weight reductions (~30%) at 27,000 eggs per plant, even though number eggs per gram root fresh weight was much lower for Cristalina, suggesting that plant-growth alterations may not be directly related to nematode reproduction.

The data on endogenous contents of sucrose and reducing sugars were not consistent (Table 2). While there was an increase of carbohydrates in some interactions, the opposite occurred in others. Significant statistical differences were observed only with

TABLE 1. Number of eggs per gram fresh root weight, root fresh weight, and leaf dry weight of seedlings of soybean cultivars UFV ITM 1 and Cristalina inoculated with *Meloidogyne incognita* and *M. javanica.*

Inoculum level	*M. incognita*			*M. javanica*		
	UFV ITM1	Cristalina	Mean	UFV ITM1	Cristalina	Mean
			Eggs per gram fresh root weight			
0	0 d	0 d	0 C	0 c	0 c	0 C
3,000	123 bc	22 d	73 B	194 bc	243 bc	218 BC
9,000	157 ab	35 cd	96 AB	375 bc	667 b	521 B
27,000	230 a	50 cd	140 A	773 b	1,719 a	1,246 A
Mean	128 A	27 B		335 B	657 A	
			Root fresh weight (grams)			
0	3.29	3.53	3.41 B	3.53	4.07	3.80 C
3,000	4.64	5.25	4.95 A	3.88	4.60	4.24 BC
9,000	4.92	4.61	4.77 A	5.02	5.19	5.11 AB
27,000	5.03	4.01	4.52 A	5.58	5.26	5.42 A
Mean	4.47	4.35		4.50	4.78	
			Leaf dry weight (milligrams)			
0	324	342	333 AB	349	316	332 A
3,000	366	390	378 A	283	232	257 AB
9,000	363	310	336 AB	281	195	238 B
27,000	321	244	282 B	243	127	185 B
Mean	344	322		289 A	217 B	

[a] Different capital letters indicate significant difference ($P \leq 0.05$) among means at the cultivar or nematode level, and small letters indicate significant difference ($P \leq 0.05$) among treatments at the cultivar × inoculum interaction level. Data are means of five replicates.

sucrose in seedlings inoculated with *M. javanica:* there was an increase in UFV ITM1 and a decrease in Cristalina. Sucrose content was three to four-fold higher than reducing sugars.

Carbon partitioning: There generally was increased radioactivity with increased levels of inoculum (Table 3). This result was more evident for total radioactivity in the root system than for root-specific radioactivity (radioactivity per gram root). Total radioactivity per root system was positively correlated with root fresh weight ($r = 0.45$, $P = 0.0040$ for *M. incognita;* $r = 0.43$, $P = 0.0058$ for *M. javanica*) and number of eggs per gram root weight ($r = 0.55$, $P = 0.002$ for *M. incognita;* $r = 0.48$, $P = 0.0016$ for *M. javanica*), but negatively correlated with leaf dry weight ($r = -0.30$, $P = 0.061$ for *M. incognita;* $r = -0.42$, $P = 0.0072$ for *M. javanica*). The highest radioactivity data found for *M. javanica* indicate that the roots of the seedlings infected with this nematode were stronger sinks than those infected with *M. incognita.*

The specific radioactivities of sucrose and reducing sugars were calculated before and after partial purification with Dowex resins and, as expected, specific radioactivity increased after purification. Highest radioactivity was found in reducing sugars and, for most of the cases, statistically significant differences were observed at the inoculum level (Table 3). Taken together, data on radioactivity of sucrose and reducing sugars are in agreement with the total radioactivity detected in the roots; i.e., highest specific radioactivity was observed at the highest inoculum level. This was more evident for seedlings inoculated with *M. javanica.* Comparing inoculum levels of 0 and 27,000 eggs/plant, the higher increases of specific radioactivity were observed with this nematode.

DISCUSSION

In our study, plants were exposed to $^{14}CO_2$ 30 days after introducing nematodes because that is when egg-laying begins. The commencement of egg-laying was chosen for $^{14}CO_2$ exposure because Bird and Loveys (1975) observed that tomato plants at this stage had the highest nutrient demand when infected with *M. javanica.* Our evalua-

TABLE 2. Specific radioactivity of the roots, total radioactivity in the root system, and endogenous contents of reducing sugars and sucrose in the roots of seedlings of the soybean cultivars UFV ITM 1 and Cristalina inoculated with *Meloidogyne incognita* and *M. javanica*.

Inoculum level	*M. incognita*			*M. javanica*		
	UFV ITM1	Cristalina	Mean	UFV ITM1	Cristalina	Mean
Specific radioactivity of the roots (kcpm per gram fresh root)						
0	155	137	146	191	186	188 B
3,000	150	131	141	306	227	266 AB
9,000	188	162	175	300	244	272 AB
27,000	200	185	193	354	396	375 A
Mean	173	154		288	263	
Total radioactivity in the roots (kcpm)						
0	195	202	198 B	319	257	288 C
3,000	239	252	245 AB	376	343	359 C
9,000	282	301	291 A	564	417	490 B
27,000	314	280	297 A	666	587	626 A
Mean	258	259		481 A	401 B	
Reducing sugars (milligrams per gram root fresh weight)						
0	2.18	1.02	1.60	1.09	1.09	1.09
3,000	2.13	1.12	1.63	1.43	1.21	1.32
9,000	1.68	1.04	1.36	1.34	1.17	1.27
27,000	1.82	0.98	1.40	1.45	1.42	1.44
Mean	1.95 A	1.04 B		1.33	1.22	
Sucrose (milligrams per gram root fresh weight)						
0	4.19	4.24	4.22	3.03 c	3.64 abc	3.34
3,000	4.60	4.28	4.44	3.24 bc	3.39 abc	3.32
9,000	4.75	3.62	4.19	4.33 ab	3.37 abc	3.85
27,000	4.11	3.13	3.62	4.42 a	2.65 c	3.54
Mean	4.41	3.82		3.81 A	3.26 B	

[a] Different capital letters indicate significant difference ($P \leq 0.05$) among means at the cultivar or nematode level, and small letters indicate significant difference ($P \leq 0.05$) among treatments at the cultivar × inoculum interaction level. Data are means of five replicates.

tions started before massive egg production; therefore, the reproduction factors were lower than expected, especially for plants inoculated with *M. javanica,* which were extensively galled. However, the calculated reproduction factors and the number of eggs per gram root fresh weight showed that nematode reproduction in seedlings inoculated with *M. javanica* was greater than that of *M. incognita*. Since this difference in egg production could be misleading, the data for each nematode were statistically analyzed separately.

A significant increase of root growth in response to inoculum level was observed. Despite some variation, principally with Cristalina infected with *M. incognita,* both cultivars exhibited a tendency to increase root growth in response to nematode inoculation. This was not necessarily related to the number of eggs produced per gram root; for example, UFV ITM1 seedlings had a similar root weight increase with both nematodes, but fewer eggs were found in roots of seedlings infected with *M. incognita*. Although we did not count galls, seedlings inoculated with *M. javanica* undoubtedly had more galls than those inoculated with *M. incognita*.

Few galls were observed on the roots inoculated with *M. incognita,* and the number of eggs per gram root was low. Therefore, two components may have been responsible for increases in root weight: gall formation and secondary root proliferation. Galls probably were the main component in seedlings infected with *M. javanica*. There are previous reports that low levels of inoculum can stimulate root growth (Abrão and Mazzafera, 1998; Wallace, 1971), likely through secondary root proliferation (Lordello, 1986).

Suppression of photosynthesis by nematodes has been reported. Compared to our work, higher inoculum levels were used in

TABLE 3. Specific radioactivity of reducing sugars and sucrose before and after purification of the extracts with Dowex resins. Extracts were from roots of seedlings of the soybean cultivars UFV ITM 1 and Cristalina inoculated with *Meloidogyne incognita* and *M. javanica.*

Inoculum level	*M. incognita*			*M. javanica*		
	UFV ITM1	Cristalina	Mean	UFV ITM1	Cristalina	Mean
			Reducing sugars before Dowex (kcpm per milligram)			
0	85	171	128 B	241	214	228
3,000	88	143	115 B	257	242	250
9,000	143	212	177 AB	304	262	283
27,000	153	269	211 A	293	256	275
Mean	117 B	199 A		274	244	
			Sucrose before Dowex (kcpm per milligram)			
0	49	52	51 B	91	62	77
3,000	46	38	42 B	114	81	98
9,000	49	55	52 B	90	86	88
27,000	69	81	75 A	94	123	109
Mean	53	56		97	88	
			Reducing sugars after Dowex (kcpm per milligram)			
0	148	217	183 B	402	176	289 B
3,000	146	240	194 B	430	368	399 AB
9,000	246	314	281 A	484	417	450 A
27,000	248	438	343 A	493	430	462 A
Mean	197 B	303 A		452 A	348 B	
			Sucrose after Dowex (kcpm per milligram)			
0	82	89	86 B	137	103	120
3,000	76	64	70 B	149	127	138
9,000	84	84	84 B	142	142	142
27,000	129	135	132 A	161	204	183
Mean	93	93		147	144	

[a] Different capital letters indicate significant difference ($P \leq 0.05$) among means at the cultivar or nematode level, and small letters indicate significant difference ($P \leq 0.05$) among treatments at the cultivar × inoculum interaction level. Data are means of five replicates.

previous studies. Loveys and Bird (1973) observed reduction of photosynthesis in tomato plants infected with 30,000 to 50,000 J2 of *M. javanica* 22 days after inoculation. Schans (1991) inoculated two potato cultivars with J2 of *Globodera pallida* (the resistant cultivar with 260,000 and the susceptible with 60,000) and observed reduction of photosynthesis just 3 days after inoculation. The reductions were 43% and 28% in the resistant and susceptible cultivars, respectively. On the other hand, photosynthesis was little affected in soybean plants infected with *H. glycines,* even at high inoculum levels (Koenning and Barker, 1995). Only part of the soybean yield reduction was attributed to altered photosynthesis induced by the nematode.

Despite a reduction in leaf weight, the amount of radioactivity incorporated into the root system of seedlings inoculated with *M. javanica* suggests that photosynthesis was stimulated by nematode infection. However, this inference may not be true. Poskuta et al. (1986) observed that although leaf area was reduced in soybean infected with *H. glycines,* in vivo kinetic parameters of ribulose bisphosphate carboxylase (Rubisco) were not affected, suggesting that CO_2 exchange was not altered. If photosynthesis was also unaffected in our study, then the high radioactivity present in the roots of Cristalina and UFV ITM1 infected with *M. javanica* appears to be due to increased partitioning of photoassimilates into the roots, reducing the assimilates available for shoot growth.

The higher specific radioactivity of reducing sugars suggests that sucrose was readily reduced to glucose and fructose after phloem unloading. However, compared to sucrose, the low endogenous content of reducing sugars indicates that they were quickly metabolized. Indeed, on average, the specific radioactivities increased ap-

proximately 60% after Dowex treatment, indicating that other compounds had assimilated the labeled carbon from carbohydrates. The significant, highest specific radioactivity of reducing sugars in the highest inoculum level (27,000 eggs) might be due to increased root respiration, as energy demand was high. Root respiration increases have been observed with compatible and incompatible nematode-plant interactions, and were higher in the former (Poskuta et al., 1986).

Melakeberhan et al. (1990) observed a lowered sucrose level in roots of a grape cultivar resistant to *M. incognita,* and an increased level in a susceptible cultivar. No alteration was observed for reducing sugars. These authors concluded that diminished photosynthate translocation to the nematode feeding site occurred in the resistant cultivar. Based on the calculated reproduction factors, if it is assumed that Cristalina is more susceptible than UFV ITM1 to both nematodes, then our results do not support this generalization because sucrose content was not significantly changed in Cristalina. On the other hand, significant increase was observed with UFV ITM1 infected with *M. javanica.* Therefore, carbohydrate variation in roots infected by nematodes seems to be dependent on the plant and nematode species. Decrease of carbohydrates was also obtained with susceptible tomato inoculated with *M. incognita, Paratrichodorus minor,* and *Pratylenchus scribneri,* where inoculated plants showed lower contents of glucose, starch, and sucrose (Anwar, 1995). Reduction in carbohydrates was also observed in other plants susceptible to nematode infection (Nasar et al., 1980; Singh et al., 1980).

In summary, our results support the interpretation that nematodes are strong sinks and that their effects on carbon partitioning in plants depend on the plant and nematode species involved. Our results confirm previous reports that shoot reduction is strongly influenced by increased partitioning of carbohydrates to the roots. Our results also suggest that formation of new secondary roots may contribute substantially to root growth in plants infected with nematodes. However, the relative participation of secondary roots and galling as photoassimilate sinks has still to be determined.

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