The Incorporation of Photosynthates by Meloidogne javanica

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Abstract: The root-knot nematode, *Meloidogyne javanica,* incorporated ^{14}C from its host after exposure of the plant to $^{14}CO_2$. This uptake was relatively slow and was not detected in nematodes exposed to a labelled plant for periods of 2 and 4 h, but was after 24 h. Nematodes were grown in plants previously infected at weekly intervals to provide animals at various stages of growth. Plants were harvested 24 h after exposure to the label and the rate of incorporation per unit area of nematode was measured. This rate was found to be related to the nematode's physiological age and reached its peak at the time egg-laying commenced, after which it started to decline. The results support the hypothesis that the nematode functions as a metabolic sink. *Key Words:* autoradiography, transfer cell, syncytia, photosynthesis, galls, egg mass, sink.

It has been suggested (3, 4) that syncytia (giant cells) induced by nematodes are multinucleate forms of transfer cells. This nematode-induced transfer cell has a distinct pattern of growth which, not unexpectedly, can be closely correlated with the physiological age of the nematode (2). Normal transfer cells are thought to supply organic nutrients to plant tissues which are actively growing or secreting, but nematode-induced transfer cells are thought to supply organic nutrients to the nematode.

Pate and Gunning (5) pointed out that there is little information on the physiological role of transfer cells. Indeed their role as efficient exchangers of solutes with their extracellular environment has been deduced from their ultrastructure but not proven in physiological terms. We have set out to obtain this proof using nematodes dissected from roots.

MATERIALS AND METHODS

The host plants, beans *(Vicia faba* L., 'Early Long Pod') and tomato *(Lycopersicon esculentum,* Mill; 'Tatura Dwarf') were divided into six batches, and a different batch was exposed to approximately 5,000 freshly hatched larvae of *Meloidogyne javanica* each week for six weeks. Plants were grown in pots 15 cm diam. containing University of California (U.C.) mix, housed in a glasshouse at temperatures that fluctuated between a night mean minimum of 17 C and a day mean maximum of 34 C.

Plants containing nematodes of specific ages were allowed to photosynthesize in an

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atmosphere of $^{14}CO_2$ for 30 min. This was achieved by liberating 500μCi of $^{14}CO_2$ by treating aqueous sodium bicarbonate with an excess of 1.0N sulphuric acid. The $^{14}CO_2$ was contained in a bag of nylon sheeting having negligible CO_2 permeability. This was sealed to the pot and completely enclosed the plant. The $^{14}CO_2$ was pumped into, around and out of the bag by manual compression of a bulb which was attached to the container from which the $^{14}CO_2$ was being liberated. This process was monitored with a portable Geiger counter.

After 30 min the bag was removed and the plants were left in the glasshouse for 2 and 4 h and 1 and 5 days before being harvested.

Galls were examined in the fresh state or after fixing for 48 h at 5 C in either acetic:ethanol (1:3) or in 4% phosphate-buffered formaldehyde (pH 7.3). After fixation, the galls were rinsed in distilled water and the nematodes dissected out in distilled water. Care was taken that the nematodes were taken from galls containing only a single nematode to avoid differences in size caused by competition. Nematodes damaged during dissection were discarded.

Five nematodes were placed in each scintillation vial and this was replicated at least three times for each fixative. These measurements were also made on whole galls with egg masses attached in the fresh state and on ungalled adjacent root and dissected egg masses.

One-half milliliter of solubilizing agent was added to each scintillation vial which then was incubated at 60 C for 1 h and cooled to 20 C; 5 ml of scintillation fluid (Packard Instagel) then was added to each vial. Samples were placed in a liquid scintillation spectrometer (Packard model 3320 Tricarb) and allowed to equilibrate for 1 h before counts were started. The numbers of disintegrations per minute (dpm) were calculated using the channels ratio method of quench correction and are presented on a unit area basis. The areas of nematodes lying in a horizontal optical plane were measured by a planimetric technique that has been described in detail elsewhere (1), and root material and egg masses were weighed.

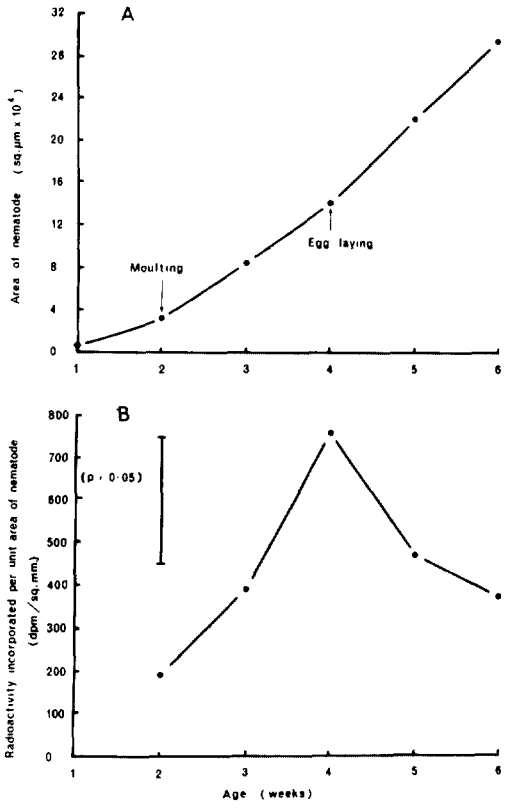

FIG. 1. *Meloidogyne javanica* fixed in 4% phosphate buffered formaldehyde and dissected from its host *Vicia faba* at weekly intervals. **A**) Growth curve of the nematode. **B**) Uptake of ^{14}C into the nematode exposed to this label for a period of 24 h.

RESULTS

Initial experiments showed that the uptake of radioactivity by the nematode was relatively slow and that, for periods of 2 h and 4 h after exposure of the plant to $^{14}CO_2$, no significant uptake of ^{14}C into the nematodes was detected. We allowed at least a 24-h period for the accumulation of translocated photosynthates in subsequent experiments. Galls and egg masses from plants which were harvested 5 days after exposure to $^{14}CO_2$ contained about 6 times as much ^{14}C as did adjacent root (2,210 dpm/mg FW compared with 365 dpm/mg FW, difference significant, $P = 0.01$) and about half of this activity was located in the egg mass.

Surprisingly, the amount of ^{14}C in fixed nematodes was not significantly less than that in fresh unfixed nematodes, so that the 2-day period of fixation at 5 C did not lead to elution of the label. Even treatment of the galls in 80% ethanol at 75 C for 20 min failed to remove significant amounts of the ^{14}C, which must, therefore, have become incorporated into relatively insoluble compounds within the nematode.

The nematodes grew rapidly after molting, which took place 2 weeks after infection of the plants (Fig. 1-A). Differences in size between each harvest were significant ($P = 0.05$). The rate at which *M. javanica* incorporated ^{14}C on a per nematode unit area basis reached a peak at approximately four weeks after infection (Fig. 1-B), when it was greater than at other ages.

One reason for a lower incorporation at ages greater than four weeks is that a portion of the label is incorporated into the egg mass which the nematode exudes onto the surface of the root. The uptake of label into these structures over a period of 5 days is shown in the autoradiograph (Fig. 2).

DISCUSSION

Our results show that these nematodes act as sinks, that their uptake of carbon compounds increases at a time when there is maximum growth and activity of the transfer cell-like syncytia and its components (2), and that the organic nutrients required by these nematodes originate at least in part from the products of current photosynthesis and would thus presumably be translocated to the nematode via the phloem.

In conclusion, we must emphasize the importance of selecting the right stage of development of the nematode if comparative experiments are to be made. Its ability to function as a sink will vary considerably depending on its physiological age, and the time over which its metabolic activities are being measured.

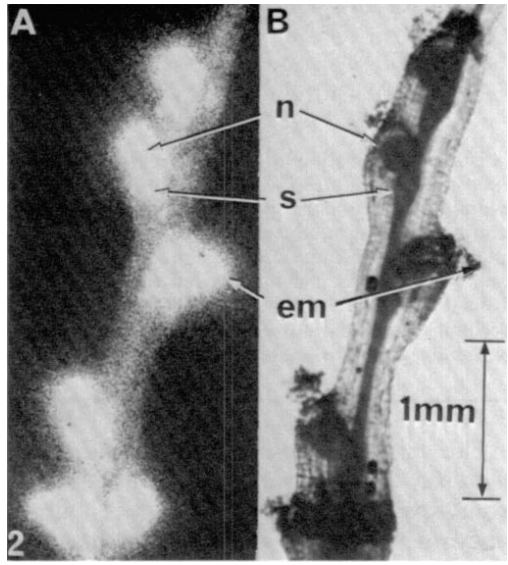

FIG. 2. Root of *Lycopersicon esculentum* parasitized with 7-week-old *Meloidogyne javanica* and harvested five days after exposure to $^{14}CO_2$. **A)** Autoradiograph of freeze-dried root showing accumulation of labelled photosynthate in region of nematode (n), egg mass (em), and syncytia (s). **B)** Root removed from surface of X-ray film and treated at 60 C for 18 h in lactophenol containing a trace of cotton blue showing position of nematode (n), egg mass (em) and syncytia (s).

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