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Short communication

Carbon fixation and partitioning in coffee seedlings infested with *Pratylenchus coffeae*

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Abstract

The objective was to study CO_2 fixation and photoassimilate partition in coffee (*Coffea arabica*) seedlings infested with the lesion nematode *Pratylenchus coffeae*. Seedlings infested with 0, 1000 and 8000 *Pratylenchus coffeae* nematodes were exposed to $^{14}CO_2$ and the incorporation and distribution of radioactivity were followed in the roots, stems and leaves. Fresh mass, pigments, soluble sugars, sucrose and specific radioactivity of sucrose in the plant parts were determined. At the highest level of infestation almost all the parameters were significantly changed showing the carbon fixation in the leaves and partitioning to the roots were decreased. Since lesion nematodes are not sedentary and do not form feeding sites that could be characterised as metabolic sinks, it is suggested that their damage is more readily expressed by the leaves, through a reduction in photosynthesis and phloem transport.

The root lesion nematode, *Pratylenchus coffeae*, (Zimmermann, 1898) Filipjev and Schuurmans Stekhoven, 1941 was first reported to infect coffee (*Coffea arabica*) by Monteiro and Lordello (1974). This nematode is a major pest in several countries where this crop is grown (Campos et al., 1990; Inomoto et al., 1998). Poor root growth, leaf shedding and nutritional deficiency are typical symptoms of infested coffee trees (Monteiro and Lordello, 1974). Reduction in photosynthesis also occurs (Kubo et al., 2003).

In view of some variation in terms of host-range variability (Edwards and Wehunt, 1973) and recent morphological and molecular studies showing differences among isolates (Duncan et al., 1999), the taxomony of *Pratylenchus coffeae* has been re-examined. An isolate from *Citrus aurantium* was recently considered as a new species (Inserra et al., 2001). Two other Brazilian isolates, K_5 from *C. arabica* and M_2 from *Aglaonema* spp. are differentiated by their host-range (Silva and Inomoto, 2002). The isolate K_5 is more pathogenic than M_2, causing almost complete destruction of the roots, and inducing chlorosis and leaf fall (Silva and Inomoto, 2002; Kubo et al., 2003).

Compared to *Meloidogyne* spp., very little is known about the damage caused by *Pratylenchus* in terms of growth and development of coffee (Kubo et al., 2003). Therefore, the aim of this work was to study the effect of *P. coffeae* on $^{14}CO_2$ assimilation and radioactivity partitioning in this crop, using isolate K_5 (Silva and Inomoto, 2002). Labelled CO_2 has long been used as a useful technique to study metabolic alterations in plants infested by nematodes (Bird and Loveys, 1975).

Nematodes maintained on alfalfa callus (Riedel et al., 1973) were extracted (juveniles and adults) from 45 to 90 day old cultures after the last transfer to new calli using the Baermann method, with modifications for flat recipient (Southey, 1986).

Five-month-old coffee (*C. arabica* cv. Catuaí Vermelho) seedlings, growing in plastic pots containing 450 ml of soil previously sterilised with methyl bromide (150 ml of CH_4Br/1000 l of soil), were inoculated with nematode suspension adjusted to obtain 1000 and 8000 nematodes/seedling. Control plants were not inoculated. The plants were kept under greenhouse conditions being watered every second day. After 45 days of inoculation, the plants were exposed to $^{14}CO_2$. Each pot containing one seedling was enclosed in a plastic bag together with two Eppendorf tubes inside, one with 0.5 ml 3 M HCl and other with 50 μCi $NaH_3{}^{14}CO_2$ (58 mCi $mmol^{-1}$, Amershan-Pharmacia). The contents of the Eppendorfs were mixed by external handling and the seedlings were maintained in a glasshouse under a net cutting 25% of the natural light, from 8:00 a.m. to 13:00 p.m., when the bags were opened. The next morning (8:00 a.m.), leaves, stems and roots were collected and weighed. Roots were washed under tap water flow and blotted dry before weighing. The material was frozen at −60 °C before being extracted twice with methanol (2 × 10 ml/5 g) in a Polytron homogeniser. The debris was eliminated by filtration followed by centrifugation (10,000 rpm for 15 min) and 1 ml aliquots were used for the determination of radioactivity after addition of scintillation fluid. The same extracts were used for determination of pigments (Lichtenthaler and Wellburn, 1983), total soluble sugars (Dubois et al., 1956) and sucrose (Van Handel, 1968). Fractions were vacuum concentrated (SpeedVac, Savant) and applied on 3MM Whatmann paper for chromatographic separation of the soluble sugars (Chaplin, 1986). Chromatography was developed for 72 h and the sugars visualised by spraying with 5% H_2SO_4 and heating at 100 °C until colour development. Sucrose was identified by comparison with pure sucrose applied laterally on the paper. The identified spots were cut out from the chromatography paper and homogenised in the Polytron with 10 ml ethanol. After centrifugation, aliquots were taken for determination of the radioactivity. It was thereby possible to calculate the specific radioactivity for sucrose. The experiment was repeated twice, separated by a 1-week interval. The first assay was carried out with five replicates and the second with eight. In the first experiment the stems were not collected. Carbohydrate determinations were made only in the second assay.

Both assays showed that infection by the isolate K_5 of *P. coffeae* caused growth reduction in coffee seedlings (Figure 1A and D). Despite the simultaneous inoculation of the seedlings of both assays and a similar fresh mass accumulation (see Figure 1A and D), less radioactivity was detected in the second assay (Figure 1B and E). This probably occurred because in this assay the incubation with $^{14}CO_2$ was carried out on a cloudy day. However, data from both experiments showed a similar trend, that is, seedlings infected with 8000 nematodes assimilate less radioactivity indicating lower photosynthesis. These data are consistent with the radioactivity per mass data (Figure 1C and F) since there was a decrease as the inoculation nematode level was increased. In addition, the low radioactivity per root mass indicated that less sucrose was transported from the shoot to the roots in infested seedlings.

Stem radioactivity in the second assay was similar to the leaves (Figure 1F). A possible explanation is that the stem in young coffee plants has chlorophyll and, therefore, may be active in photosynthesis. A second possibility is the contribution of the radioactivity of the transported compounds in the xylem.

The data shown in Figure 1 demonstrate the contrast in terms of carbon partitioning between plants infected by lesion nematodes and sedentary nematodes. Carneiro et al. (2000) observed that soybean plants infected with *M. incognita* and *M. javanica* had higher total radioactivity and specific mass radioactivity in the roots, as the inoculum level increased. This was probably related with the formation of the feeding sites in the roots, acting as strong metabolic sinks. Since *Pratylenchus* is not sedentary and does not form feeding sites, the destruction of the root system by this nematode seems to be more readily felt by the leaves, leading to a faster decrease in carbon assimilation.

In addition to the decrease of mass accumulation and carbon fixation (Figure 1), chlorophyll in the leaves also decreased with infestation by *P. coffeae* (Figure 2). The inverse relationship between sucrose and total soluble sugars is of special interest (Figure 2B and C). The decrease of sucrose in the leaves with nematode infestation follows the reduction in carbon fixation, seen in Figure 1. On the other hand, the increase in soluble sugars might be well explained by starch hydrolysis due to a higher respiration rate since the

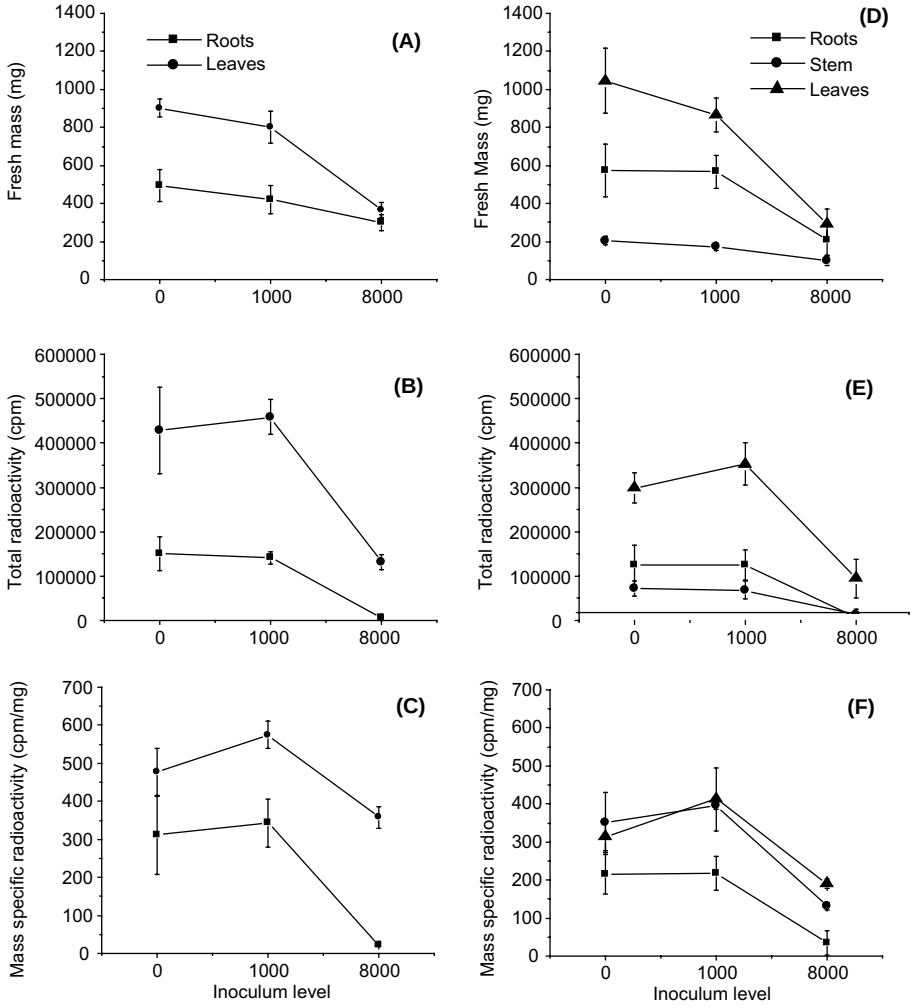

Figure 1. The effect of *P. coffeae* on fresh mass (A, D), total radioactivity distribution (B, E) specific mass radioactivity (C, F) in coffee seedlings of the assays 1 (A–C) and 2 (D–F). Bars indicated standard deviation. Means of five (assay 1) and eight (assay 2) replicates.

specific radioactivity of sucrose in the roots decreased (Figure 2D), clearly indicating reduced transport of sucrose from the leaves. In addition, the discrete reduction of endogenous sucrose in the roots (Figure 2C) might also be due to an increase of invertase activity as observed for diseased plants (Sturm, 1999) channelling the resulting reducing sugars for respiration. An increase in respiration by nematode infestation has been either suggested or reported (Hussey, 1985; Poskuta et al., 1986; Carneiro et al., 2000), although all results were obtained with root knot nematodes. The way lesion nematodes attack plants and the stress they provoke might be comparable to mechanical injuries, which cause a significant rise in the respiratory metabolism (Davies, 1987).

The previous suggestion that photosynthesis and the transport of the photoassimilates to the roots in the coffee seedlings were affected by *P. coffeae* is confirmed by the data in Figure 2D. The decrease of radioactivity in the roots (Figure 1B and F) indicates that less sucrose was transported to the roots from the leaves and consequently there was a decrease in the specific radioactivity of sucrose. Being transported less to the roots and not being diluted by the sucrose

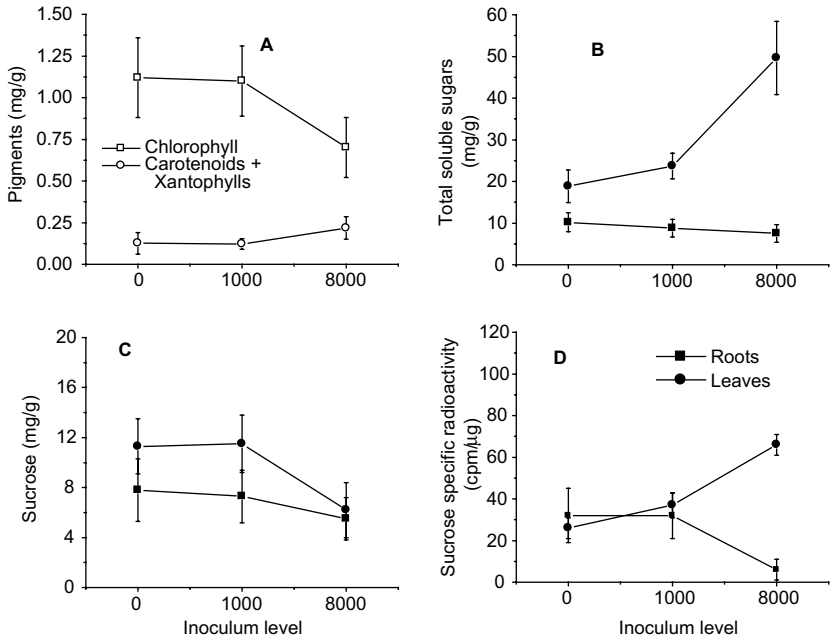

Figure 2. The effect of *P. coffeae* on pigment contents (A), total soluble sugars (B), sucrose (C), and specific radioactivity of sucrose (D) in coffee seedlings of the assay 2. Bars indicated standard deviation. Means of five (assay 1) and eight (assay 2) replicates.

formed after the plastic bags containing $^{14}CO_2$ had been removed, because of low photosynthesis, the specific radioactivity in the leaves can be expected to increase in infested plants.

In both assays, on most occasions, significant alterations were observed only following infestation with 8000 nematodes. This is in agreement with a previous study with coffee seedlings infested with *P. coffeae* where it was demonstrated that photosynthesis was decreased at inoculation levels higher than 2250 (Kubo et al., 2003).

Therefore, we conclude from our data obtained with coffee seedlings that the effects of *P. coffeae* on the carbon assimilation and partition are quite distinct from those with root knot nematodes. In contrast to these nematodes, where the feeding sites are regarded as strong metabolic sinks even leading to increase of photosynthesis in the beginning of infestation, the root lesion nematodes caused a rapid detrimental effect on carbon fixation and photoassimilates distribution in the plant due to direct damage of the roots. The same reason seems to explain the decreased uptake of nitrate and ammonium in coffee infested with *P. coffeae* (Vaast et al., 1998).

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