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TRANSPORT OF UREIDES AND AMINO ACIDS IN NODULATED SOYBEAN INFECTED BY *MELOIDOGYNE INCOGNITA* AND *M. JAVANICA*

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Summary. Amino acid and ureide composition was investigated in xylem sap of two soybean cultivars inoculated with *Rhizobium japonicum* and infected with *Meloidogyne incognita* and *M. javanica*. Although reduction of nodule number and nodule mass was observed only in plants inoculated with *M. javanica*, the amino acid profile changed with both nematodes. There was a relative increase of aspartic acid and a decrease of asparagine and glutamine. However, when the concentration of amino acids in the sap was calculated only plants infected with *M. javanica* showed a reduction. The same was observed with ureides.

The interaction between *Rhizobium* nodulation in legumes and plant parasitic nematodes has been the subject of several studies because nematode infection can compromise the N fixation process. Infection of nodulated roots by nematodes causes N deficiency symptom (Barker and McGawley, 1998). Several reports have shown morphological alterations of nodules infected by *Meloidogyne incognita* and *Heterodera glycines* (Baldwin *et al.*, 1979; Ko *et al.*, 1991) and the detrimental effects of nematode infection on legume nodulation (Robinson, 1961; Balasubramanian, 1971). Others have shown that depending on the parasitism strategy of the nematode (Hussey and Barker, 1976) or the developmental stage of the nodulating plants, N fixation may even be stimulated (Baldwin *et al.*, 1979).

Little is known about the effect of nematodes on the metabolism of nitrogen compounds in nodulated roots. This would be expected to occur since *H. glycines* caused a reduction in the synthesis of leghemoglobin in *Rhizobium* nodules as well as alterations of its four components (Huang, 1987). Lynd and Ansman (1989) used microplots in the field to study the effect of nematodes on the nodulation of siratro (*Macroptilium atropurpureum*). High inoculum levels of *M. incognita acrita, M. hapla, M. javanica* and *M. arenaria* were detected in the soil. It was observed that glutamate dehydrogenase, aspartate aminotransferase, glutamine synthetase and glutamate synthase, all enzymes of the amino acid metabolism, did not show altered activities by nematode infection. On the other hand, uricase, allantoinase, allantoate ureidoglicolate, malate synthetase and isocitrate glyoxylate synthetase, all involved in the biosynthesis of ureides, showed reduced activity. Nitrate, glutamic (GLU) and aspartic (ASP) acids, and the amides glutamine (GLN) and asparagine (ASN) increased in the nodules of infected plants and there was an increase of ureides and pyruvate.

It has been shown that nematodes may indeed cause significant alterations in the N metabolism in nodulating plants, although to the best of our knowledge no studies have been carried out on the composition of the xylem sap of such plants. Such information would indicate whether the observed alterations in the nodules are reflected by N transport to the shoots. In the present study this subject was addressed by analyzing the amino acid and ureide composition in the xylem sap of nodulating soybean, where nematode inoculation with two root-knot species was carried out with the same root segments inoculated with *Rhizobium*.

MATERIALS AND METHODS

Two Brazilian soybean [*Glycine max* (L.) Merr.] cultivars were used in this study, Ocepar 4 - Iguaçu and BR-16. Ocepar has been rated as tolerant to *Meloidogyne incognita* (Kofoid *et* White) Chitw. race 3 and moderately susceptible to *M. javanica* (Treub) Chitw., and BR-16 as susceptible to both nematodes (EMBRAPA, 1996). Seeds of these cultivars were supplied by Centro Nacional de Pesquisa de Soja, Embrapa, Londrina-PR, Brazil. Nematodes were cultured in tomato plants (*Lycopersicon esculentum* Mill. cv. Santa Cruz). Eggs were extracted from the infected roots according to Hussey and Barker (1973), except that the galled roots were cut into pieces and placed in a mechanical blender containing 0.5% NaOCl solution for 20 seconds (Boneti and Ferraz, 1981). A *Rhizobium japonicum* strain, recently reclassified as *Bradyrhizobium elkanii* (SEMIA 5019 - Hungria *et al.*, 1994), was supplied by Seção de Microbiologia do Instituto Agronômico de Campinas, Campinas-SP, Brazil. The inoculum was obtained growing the bacteria in liquid medium (Norris and Date, 1976) for four days, until intense turbidity was observed. There was no con-

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trol of the number of cells per volume of liquid culture.

Nematode reproduction data were obtained in a separate experiment, by inoculating seedlings of both cultivars growing in a mixture of soil and sand (1:1) with 9,000 eggs. The evaluation of the number of eggs per root system was carried out 50 days after inoculation. Eggs were extracted as indicated above.

In a second experiment, to study the effect of nematodes on nodulation, soybean seeds were germinated in 400 ml plastic pots containing vermiculite and after being screened for homogeneity in height, one seedling was maintained in each pot. When the primary leaf pair had appeared, each seedling was inoculated with *Rhizobium* by dispensing 15 ml of bacteria culture around the seedling stem. Subsequently, 5 ml of a water suspension containing 27,000 nematode eggs were inoculated per seedling, also by dispensing around the stem. As controls, seedlings were inoculated only with *Rhizobium*. Four replicates were used in each treatment.

Twice a week the seedlings received 50 ml of a modified Hoagland solution without N (Hoagland and Arnon, 1950). The plants inoculated or not with *Rhizobium* and nematodes are designated as +R or –R, and +N or –N, respectively.

After 40 days from inoculation the plants were thoroughly watered and the next morning the xylem sap was collected (McClure and Israel, 1979). The shoot was removed by cutting the stem below the cotyledonary node with a razor blade, washed with distilled water, quickly blotted with filter paper and the sap collected with a glass capillary. During the collection, which proceeded for approximately 40 minutes, the sap was stored on ice and later at –20°C. After collection of the sap the roots were washed in running tap water and the nodules collected, counted and weighed. Some of the nodules were stored at –20°C for extraction of leghemoglobins and electrophoretic analysis. Although the roots were not extracted for estimation of nematode eggs, intense galling was observed with both nematodes.

Ureides in the xylem sap were analyzed according to Vogels and van der Drift (1970). Amino acids were analyzed by reversed-phase high performance liquid chromatography and fluorometric detection, after derivation with o-phtalaldehyde (Jarret et al., 1986).

Leghemoglobins were extracted according to Becana and Sprent (1989) and proteins separated in a discontinuous non-denaturing PAGE system using Tris-glycine buffer, pH 8.3, with 10% of polyacrilamide in the resolving gel. Electrophoresis was carried out in a Mini-Protean Electrophoresis System (BioRad) at 4°C, with constant current (15 mA per gel). Proteins were stained with Coomassie Blue GR250.

RESULTS AND DISCUSSION

To estimate the nematode reproduction, both cultivars were inoculated with 9,000 eggs in a separate exper-
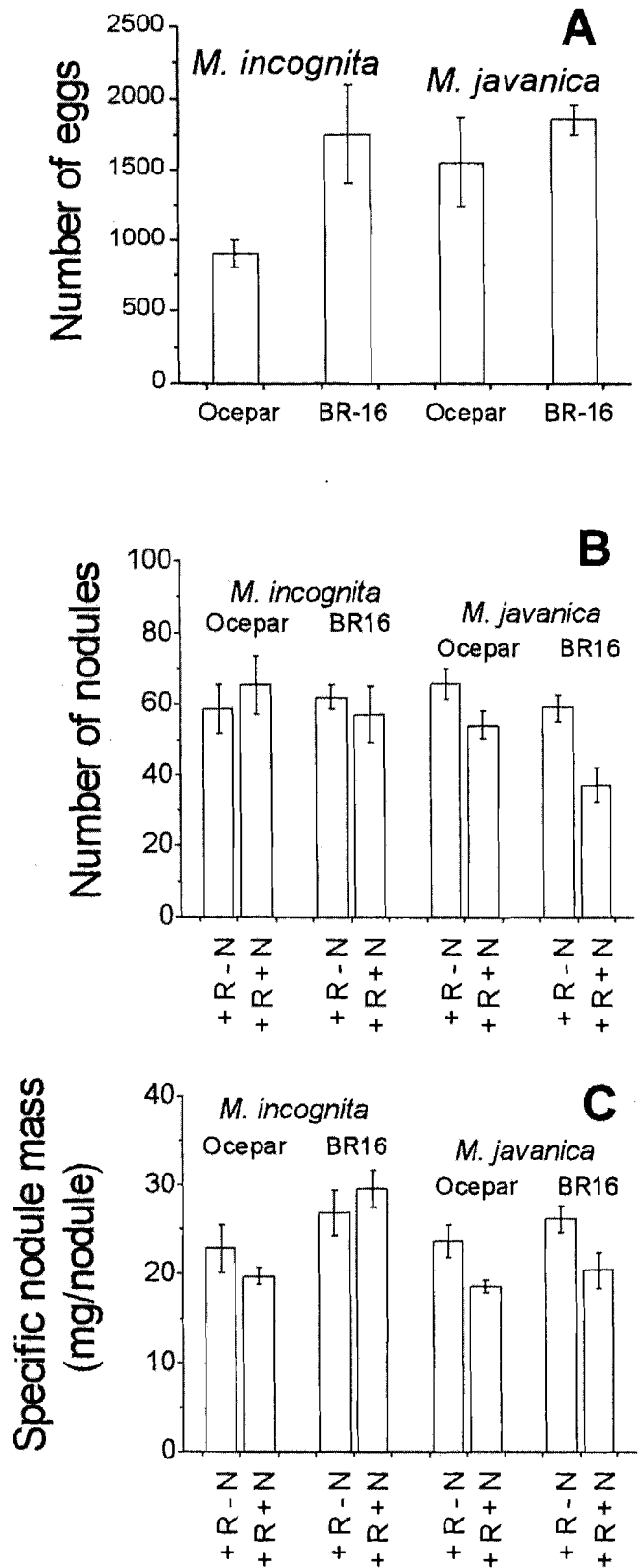
Fig. 1. Nematodes reproduction in soybean plants inoculated with 9,000 eggs in the first experiment (A) and number of nodules (B) and nodule mass (C) of nodulating soybean plants infested with *Meloidogyne incognita* and *M. javanica* in the second experiment. Bars indicate standard deviation. Plants inoculated or not with *Rhizobium* and infested with nematodes are indicated as +R or –R, and +N or –N, respectively.

iment. Nematodes reproduced in the roots of both cultivars, although fewer eggs were found in the combination Ocepar - *M. incognita* (Fig. 1A). For all combinations, the predicted reproduction factor (Pf/Pi) values lower than 1 indicate that the nematode inoculum level (Pi) used was apparently high and that a strong competition among individuals for feeding sites/food had occurred. The presence of egg-mass producing females confirmed that the life cycle had been successfully completed.

In the subsequent experiment where nematodes (27,000 eggs per plant) and bacteria were simultaneously inoculated the number of nematode eggs was not determined since nodules were separated for electrophoretic analysis and this procedure would lead to an underestimation of the eggs in the roots. However, from visual observation we would rate the infection as four to five, according to Taylor and Sasser (1978). No visual differences were observed in plant growth, probably due to the short period between inoculation and evaluation.

Fewer (Fig. 1B) and smaller nodules (Fig. 1C) were recovered from the roots of plants of the treatment +R+N,

inoculated with *M. javanica*. This probably indicates an effect of nematode infection on *Rhizobium* nodulation and nodule growth. However, the same was not observed with *M. javanica*. Reports in the literature show that nodulating plants infected by nematodes may have the number and weight of nodules changed or not according to the nematode species (Hussey and Barker, 1976) and with the susceptibility of the plants to nematodes (Baldwin *et al.*, 1979). In addition, our data were obtained after 40 days of simultaneous inoculation and perhaps more contrasting differences might have been detected if our observations were made over a longer period.

In non-nodulating legumes, ASN is usually the most abundant amino acid in the xylem sap. Depending on the legume, GLN is observed in similar levels of ASP (Shelp and Da Silva, 1990; Kim *et al.*, 1993). On the other hand, in nodulated plants there is a reduction of ASN, increase of GLN, and ureides are present in significant amounts (Vance, 1990). GLN is the first amino acid biosynthesized during incorporation of NH_4 in the nodules, and the evidence is that it diffuses directly
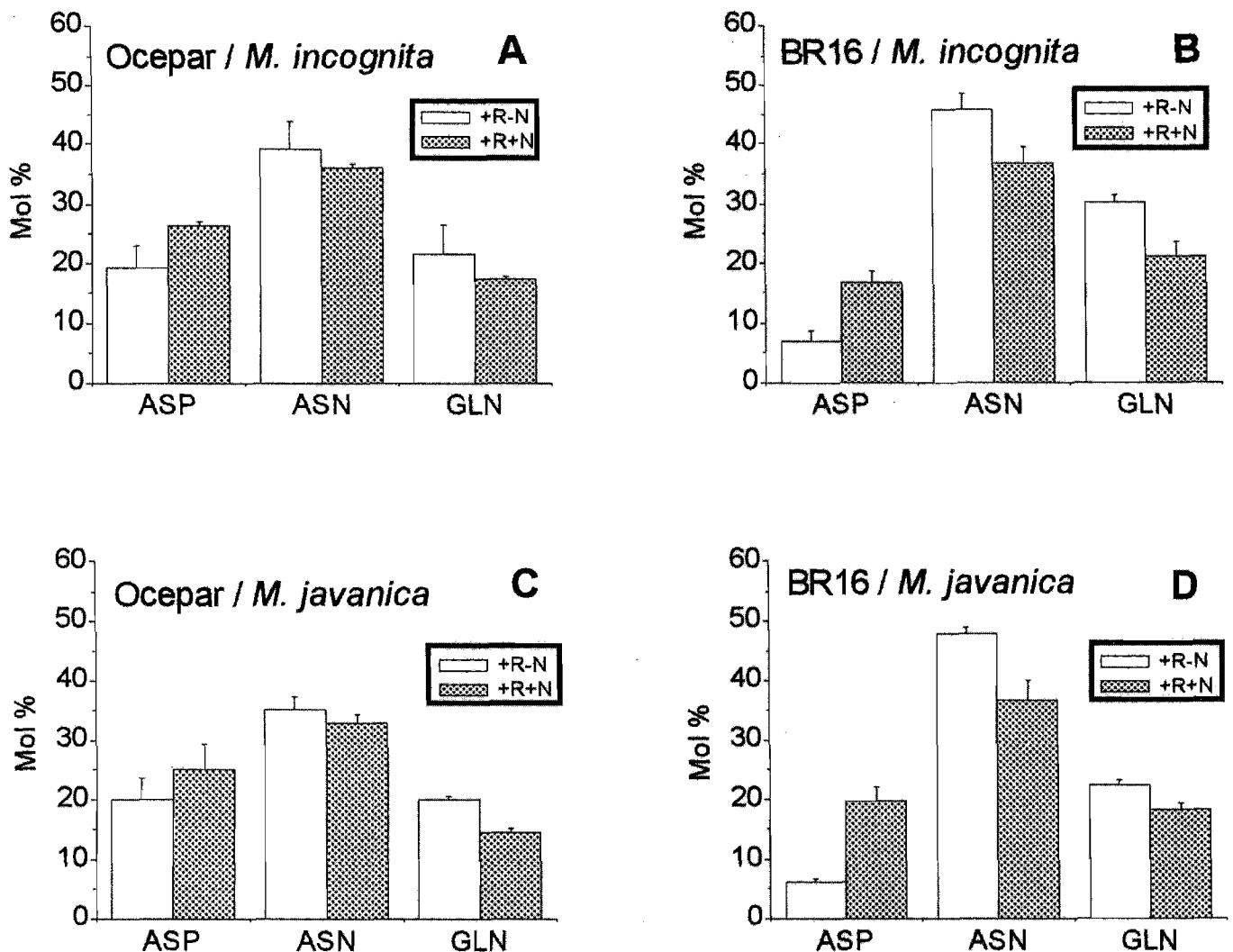

Fig. 2. Amino acid profile of nodulating soybean plants infested with *M. incognita* (A and B) and *M. javanica* (C and D) in the second experiment. Bars indicate standard deviation. Plants inoculated or not with *Rhizobium* and infested with nematodes are indicated as +R or –R, and +N or –N, respectively. ASP = aspartic acid, ASN = asparagine, GLN = glutamine.

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from the nodules to the xylem vessels.

ASN, ASP and GLN represented approximately 65 to 90% of the amino acids detected in the xylem sap of the soybean plants (Figs. 2A-D). Only exceptionally did other amino acids present values larger than 3%. As expected, ASN concentration in the sap was higher than ASP and GLN even in plants infected with nematodes. With nematode infection there was a reduction of ASN and GLN but an increase of ASP, this being more evident with BR16. Stresses imposed by water deficiency (Gonzalez et al., 1995; Serraj et al., 1998), hypoxia (Puiatti and Sodek, 1999) and P deficiency (L. Sodek, personal communication) induce ASP increase in nodulated soybean. Therefore, nematodes would also be considered as stress factors causing an increase of ASP.

The concentration of ASP, ASP and GLN in the xylem sap was also calculated and the data are presented in Fig. 3A, with ureide concentration (Fig. 3B). Nodulated plants infected with M. javanica showed a reduction of amino acid concentration and a tendency for the reduction of ureides.

Ko et al. (1984) studied the impact of H. glycines on the nodulation of soybean and suggested that nematodes would compete for photoassimilates coming from the shoot, and thus inhibiting nodulation. The flow of sucrose from the shoot to the nodule has a decisive role in the biosynthesis of ureides (Gonzalez et al., 1995). Therefore, the lower concentration of amino acids and ureides in plants infected with M. javanica could reflect competition for photoassimilates. Carneiro et al. (2000) observed that M. javanica produced a stronger sink than M. incognita when infecting soybean.

Considering that ureides have four N per molecule and that one or two N are present in ASP, ASN or GLN, the amount of N transported by ureides in nodulated plants (+R-N) was at least four times higher than in amino acids. However, even though nematode (M. javanica) presence caused a more pronounced decrease in amino acid concentration the small decrease in ureides represented a larger decrease of transported N due to the higher proportion of this element in the molecules.

The electrophoresis of nodule extracts with or without nematode infection did not show any difference with respect to leghemoglobin components (data not shown). Huang and Baker (1983) observed that there was a relative decrease of the constituent a of leghemoglobin in soybean nodules infected by H. glycines. This constituent has been suggested as having a higher affinity for oxygen than the other constituents (Uheda and Syono, 1982) and its proportional decrease in relation to the other leghemoglobin constituents appears to be evidence of impaired nodule development or accelered senescence (Huang, 1987). As discussed above, despite the period between inoculation and evaluation being sufficient to detect small variations in ureides, it was not sufficient for the detection of eletrophoretic changes in the leghemoglobin constitution.

In conclusion, our data show that nematodes may

change not only the amino acid composition of nodulated plants but also the concentration. In addition, the N fixation process is affected with consequent reduction of ureides.

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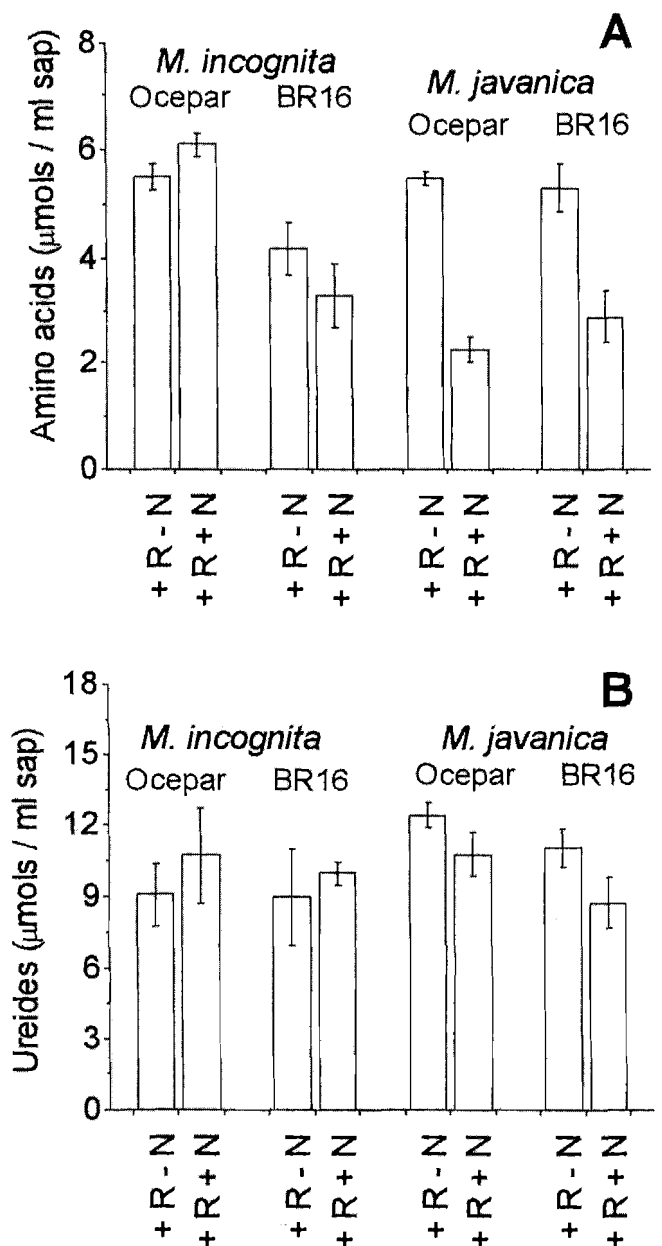
Fig. 3. Amino acid (A) and ureide (B) concentrations in the xylem sap of nodulating soybean plants infested with M. incognita and M. javanica in the second experiment. Bars indicate standard deviation. Plants inoculated or not with Rhizobium and infested with nematodes are indicated as +R or –R, and +N or –N, respectively.

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