Partitioning of nitrate reductase activity in *Coffea arabica* L. and its relation to carbon assimilation under different irradiance regimes

Maria Luiza C. Carelli* **and Joel I. Fahl**

Centro de Pesquisa e Desenvolvimento de Ecofisiologia e Biofisica, Instituto Agronômico, CP 28, 13012-970 Campinas, SP, Brazil.
**Corresponding author: carelli@iac.sp.gov.br*

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The distribution of *in vivo* nitrate reductase (EC 1.6.6.1) activity (NRA) between leaves and roots was studied in young coffee plants (*Coffea arabica* L.) grown in pots with watered sand in a glasshouse and irrigated with nutrient solution. The influence of irradiance regimes on the partitioning of NRA, and its relation with CO_2 assimilation, was also evaluated in plants grown under approximately 20, 50 and 100% of full sunlight. Time-course of nitrate accumulation in nitrogen-starved plants showed a similar pattern in leaves and roots after supplying 15 mmol L^{-1} nitrate, indicating efficient ability of the roots to export nitrate to the shoot. At the same time, NRA was rapidly induced in both tissues. In shoots, NRA partitioning was synchronized among the various leaf pairs. The initial increase in NRA, as each leaf pair emerged, coincided with the optimum NRA values of the next older leaf pair. However, the average shoot NRA remained relatively constant for each sampling date. During the first 23 weeks of vegetative growth, the mean NRA was 32% higher in leaves than in roots. The irradiance regimes influenced the partitioning of NRA between leaves and roots. The NRA leaf /root ratio was 0.72, 1.21 and 1.05, respectively, for plants grown under 20%, 50% and 100% of full sunlight. Leaf NRA was positively correlated with CO_2 assimilation, in response to irradiance regimes. Under favorable CO_2 assimilation conditions, higher NRA was observed in leaves than in roots, and the contrary trend occurred under limiting CO_2 assimilation conditions. Under moderate irradiance regime the leaves were the main site of nitrate reduction, contributing with 70% of the whole plant nitrate assimilation.
Key words: coffee**,** growth, leaf/root ratio, nitrate assimilation, photosynthesis, shading

Partição da atividade da redutase de nitrato em *Coffea arabica* L. e sua relação com a assimilação do carbono em diferentes níveis de irradiância: Estudou-se a distribuição da atividade da redutase de nitrato (ARN) *in vivo* entre folhas e raízes de plantas jovens de café (*Coffea arabica* L.) cultivadas em vasos contendo areia lavada, em casa de vegetação, e irrigadas com solução nutritiva. Também foi avaliada a influência de regimes de irradiância na partição da ARN e na assimilação do CO_2, em plantas cultivadas aproximadamente sob 20, 50 e 100% da luz solar. Após o fornecimento de 15 mmol L^{-1} nitrato, para plantas deficientes em nitrogênio, o acúmulo de nitrato nas folhas e raízes apresentou padrão semelhante, indicando capacidade eficiente das raízes em exportar nitrato para a parte aérea. Ao mesmo tempo, ARN foi rapidamente induzida nos dois tecidos. Na parte aérea, houve um sincronismo na partição da ARN entre os diversos pares de folhas. O aumento da ARN, quando cada par de folhas emerge, coincidiu com as maiores ARN no par de folhas imediatamente mais velho. Contudo, a média da ARN na parte aérea permaneceu relativamente constante, para cada data de amostragem. Durante as primeiras 25 semanas de crescimento vegetativo, a ARN média foi 32% maior nas folhas do que nas raízes. Os regimes de irradiância influenciaram a partição da ARN entre as folhas e as raízes. A relação ARN folha/raiz foi 0,72, 1,21 e 1,05, respectivamente, para as plantas cultivadas a 20%, 50% e 100% da luz solar. A ARN nas folhas foi positivamente correlacionada com a assimilação de CO_2, em resposta aos regimes de irradiância. Em condições favoráveis para a assimilação de CO_2, maior ARN foi observada nas folhas do que nas raízes, ocorrendo o inverso em condições limitantes para a assimilação de CO_2. Sob 50% de irradiância, as folhas foram o principal sítio de redução de nitrato, contribuindo com 70% da assimilação total de nitrato da planta.
Palavras-chave: assimilação de nitrato, café, crescimento, fotossíntese, relação folha/raiz, sombreamento

INTRODUCTION

Nitrate is the predominant form of available nitrogen to most cultivated plants grown under normal field conditions (Beevers and Hageman, 1980). Nitrate absorbed by plants is reduced to nitrite by nitrate reductase (NR, EC 1.6.6.1) in the cytosol of cells. Nitrite is subsequently reduced to ammonium by nitrite reductase in the plastids, and ammonium is then incorporated into amino acids. Probably, NR represents the rate-limiting step in nitrate assimilation, and can be used as a marker for the capacity of roots and shoots of immature and mature plants to assimilate external nitrogen (Beevers and Hageman, 1980; Oaks, 1994). Nitrate is easily transported in the plant and can be reduced both in leaves and roots.

Nitrate reductase activity (NRA) can be measured *in vitro* and *in vivo*. The *in vitro* procedure allows the measurement of the maximum NRA in crude extracts under non-limiting concentrations of substrate and reductant. Although this assay has been widely employed with herbaceous plants, its use has been seriously hampered with woody plants by the presence in the tissues of various inhibitors inactivating the enzyme after extraction (Gojon et al., 1994). The in *vivo* assay is carried out under endogenous tissue concentrations of substrate and reductant and is closely dependent on other simultaneous physiological processes. Therefore, the *in vivo* NRA assay is more adequate for estimating the partitioning of nitrate reduction in woody species such as coffee.

The partitioning of nitrate reduction between roots and shoots may vary with plant species, age and environmental factors. In most herbaceous plants the main site of nitrate reduction are the leaves (Andrews et al., 1986; Gojon et al., 1994). Studies on both nitrate concentration in xylem sap exudates and *in vivo* and in *vitro* NRA showed that woody plants reduce their nitrate almost exclusively in the roots (Cruz et al., 1991, 1993; Gojon et al., 1991; Lee and Titus, 1992; Thomas and Hilker, 2000). However, under high exogenous nitrate availability, NRA has also been detected in the leaves of woody plants (Klepper and Hageman, 1969; Leece et al., 1972; Smirnoff et al., 1984; Lee and Titus, 1992; Olsson and Falkengreen-Grerup, 2003). In addition to environmental factors, nitrate reduction also shows changes during organ and plant development (Andrews, 1986; Kenis et al., 1992).

The site of nitrate assimilation is an important factor to consider in terms of energy cost at the whole plant level. In leaves, the reductant and ATP for nitrate reduction and for the subsequent ammonium assimilation are generated directly or indirectly by light reactions of photosynthesis (Beevers and Hageman, 1980). When nitrate assimilation occurs in the roots, high amounts of photosynthates must be translocated to the roots and oxidized to provide the required reductants, energy and carbon skeletons. Consequently, the energy cost of leaf nitrate assimilation is about half to a quarter of the root assimilation cost (Schrader and Thomas, 1981; Smirnoff et al., 1984). The energetic advantage of leaf nitrate assimilation assumes that light intensities are high enough to saturate photosynthesis. When photosynthesis is light-limited, as in shade plants, nitrate and carbon dioxide assimilation will compete for photochemical energy and no advantage will be gained (Smirnoff and Stewart, 1985). Therefore, irradiance level is an important environmental factor to consider regarding the distribution of nitrate assimilation between roots and leaves, mainly in shade species. However, there are few reports, if any, on this subject.

Coffea arabica evolved as an evergreen understory tree in the African tropical forests, exhibiting typical features of shade-adapted plants. Coffee cultivation in agroforestry systems is a common practice in Latin America, but in Brazil the cultivation under full sunlight is the prevailing system. This latter agricultural system has been highly successful due to the high acclimation capacity of coffee plants to different irradiance regimes, involving changes in physiological, anatomical and ultrastructural characteristics (Voltan et al., 1992; Fahl et al., 1994; Ramalho et al., 1997, 1999, 2000). Several reports have indicated that high nitrogen availability is a decisive factor in the protection against photosynthesis photoinhibition, when coffee plants are exposed to high irradiances (Nunes et al., 1993; Fahl et al., 1994; Ramalho et al., 1999, 2000).

Coffee plants present high potential for nitrate assimilation in leaves as well as in roots. Nevertheless, this is a contentious matter, e.g. some reports have shown higher NRA in leaves (Carelli et al., 1990a; Da Matta et al., 1999), whereas others in roots (Carelli and Fahl, 1991; Queiroz et al., 1993a). Such variations in NRA leaf /root ratio are probably due to differences in plant growth stage and environmental conditions. In spite of this, there is little information concerning the factors influencing the partitioning of nitrate reduction in coffee plants.

This paper reports the results on the effect of irradiance levels and plant development on the partitioning of nitrate reductase activity between leaves and roots, and the relative contribution of each organ to the whole plant nitrate reduction. Carbon assimilation was also evaluated in order to investigate the relationship between these two pathways in young coffee plants grown under three irradiance regimes.

MATERIAL AND METHODS

Plant material and growth conditions: Seeds of *C. arabica* cv. Catuaí Vermelho (Instituto Agronômico de Campinas, Brazil) were germinated in free-draining 5L plastic pots containing watered sand in a glasshouse at Campinas, State of São Paulo, Brazil (22°54'S, 47°05'W, 674 m a.s.l.). After emergence each pot was left with one plant. Three experiments were carried out: (1) the first experiment aimed at evaluating the nitrate accumulation and NR induction in leaves and roots in nitrogen-starved coffee seedlings after supplying nitrate to the root medium; (2) the second was set up to evaluate the partitioning of NRA between roots and leaves during plant development and the contribution of each tissue to the total nitrate reduction of plant; (3) the third experiment was carried out to study the effect of irradiance levels on the distribution of NRA between leaves and roots and on the net carbon assimilation rate *(A)*. In the second and third experiments, after emergence, the plants were irrigated alternately with water and Hoagland's solution (Hoagland and Arnon, 1950) with the following composition: (mmol L^{-1}) KNO_3 (5), $Ca(NO_3)_2$ (5), $MgSO_4$ (2), KH_2PO_4 (1), and (µmol L^{-1}) H_3BO_3 (46), $CuSO_4.5H_2O$ (0.3), $ZnSO_4.7H_2O$ (0.8), $MnCl_2.4H_2O$ (9), $H_2MoO_4.H_2O$ (0.1), Fe-EDTA (89).

Accumulation and reduction of nitrate: Nine-week-old seedlings, previously grown in a glasshouse and irrigated alternately with water and Hoagland's solution, were used in this experiment. Nitrogen-free solution (Hoagland and Arnon, 1950) was supplied to seedlings for an additional period of eight weeks. In nitrogen-free solution, KNO_3 and $Ca(NO_3)_2$ were substituted by 5 mmol L^{-1} KCl and $CaCl_2$, respectively. One day prior measurements, plants were transferred to a growth-chamber under a 12-h daylength, with a photosynthetic photon flux density (PPFD) of 190 µmol m^{-2} s^{-1}, and a temperature of 29°C and 20°C, under light and darkness, respectively. Assays were initiated 2 h after exposing plants to light and abundant irrigation with 15mmol L^{-1} NO_3^- solution. Nitrate concentration was determined by the method of Gallo and Lott (1965) developed specifically for coffee leaves, where the yellow color formed in the nitrophenol-disulfonic acid reaction is measured.

Partitioning of NRA during plant development: In this experiment plants were kept in the glasshouse under natural temperature-irradiance regimes. The irradiance levels inside the glasshouse corresponded to 50% of full sunlight and the average temperature ranged from 19°C to 28°C during the experiment. Activity of NR in each individual leaf pair and in the youngest roots were measured weekly during plant growth beginning at the ninth week after planting (WAP) until the 23rd WAP. At the same time, growth variables leaf area and dry weight for each leaf pair, and root and shoot dry weight were recorded. Leaf area was evaluated with an area meter (LI-3100, Li-Cor, Lincoln, USA). For each sampling date, only plants presenting the same size of a lot of 10 plants were selected, which were kept as standards until the end of the experiment. The contribution of leaves and roots to the whole plant nitrate reduction was performed at the 23rd WAP. After harvest, plants were subdivided into each individual leaf pair, pivoting root, lateral roots and fine roots. Fresh weight and NRA were measured in each plant tissue. The contribution of each tissue or organ to the total nitrate reduction of plant was estimated by multiplying NRA, expressed as µmol NO_2^- h^{-1} g^{-1} FW, by the respective fresh weight of each tissue. Total NRA of shoot, roots and whole plant was obtained by the sum of each tissue or organ.

Effect of irradiance regime on NRA partitioning and carbon assimilation: Three groups of 15-week-old plants were selected and placed outside the glasshouse under the following light treatments: full sunlight, approximately 50% of full sunlight (shaded by a black plastic net screen) and approximately 20% of full sunlight (shaded by two black plastic net screens). Plants were maintained under these conditions during eight weeks before measurements. The youngest fully expanded leaves and the fine roots (~1mm diameter) were taken for NRA assay. Carbon assimilation and PPFD were determined under the

imposed irradiance regimes with a portable photosynthetic gas exchange system (LI-6200, Li-Cor., Lincoln, USA) between 0900 and 1100 h. Carbon assimilation was evaluated in similar leaves taken for NRA assay. At the pot level, PPFD at the time of measurements was 290, 720 and 1540 µmol m^{-2} s^{-1}, respectively, for plants grown under 20%, 50% and 100% of full sunlight.

Nitrate reductase assay: Coffee plants were taken out the pots between 0900 and 1100 h and the belowground organs were rinsed in tap water to remove sand. Roots were carefully detached, immediately blotted dry on paper towels and weighed. The in vivo NRA was measured according to Jaworski (1971), modified by Harper and Hageman (1972). Root and leaf samples (0.2 g) were placed in glass vials with 5mL of incubation mixture containing 0.1 mol L^{-1} potassium buffer (pH 8.0), 0.05 mol L^{-1} KNO_3 and 1% *n*-propanol. Tissues were vacuum infiltrated twice for 2 min and dark incubated in a water-bath with shaking at 30°C for 60 min, with continuous N_2 bubbling. After 15 and 45 min of incubation, aliquots (0.2-0.5 mL) were taken and analyzed for nitrite by a colorimetric method involving the reaction with 1 mL of 1% sulphanilamide in 1.5 mol L^{-1} hydrochloric acid and 1 mL of 0.2% N-(1-naphthyl)-ethylene-diamine. Absorbance readings were made at 540 nm. Activity of NR was calculated from the difference between the values measured at 15 and 45 min, ant their values were expressed as µmol NO_2^- h^{-1} g^{-1} FW.

RESULTS

Accumulation and reduction of nitrate: Figure 1 shows the time-course of nitrate accumulation in leaves and roots in nitrogen-starved coffee seedlings, after supplying 15 mmol L^{-1} nitrate, and the NRA induction in both tissues. Nitrate accumulation in leaves and roots increased linearly showing a similar pattern (Figure 1A). At the end of the 8-h experimental period, nitrate content in leaves and roots increased, respectively, from 4.9 to 11.8 and from 4.4 to 12.4 µmol NO_2^- h^{-1} g^{-1} FW, showing efficient nitrate translocation to the shoots and a similar partitioning of absorbed nitrate between leaves and roots (Figure 1A). At the same time, NRA was induced in both tissues (Figure 1B). Almost no NRA was found in leaves and roots before nitrate exposure, but 8 h after supplying nitrate NRA values reached 1.1 and 1.7 µmol NO_2^- h^{-1} g^{-1} FW, respectively. During the first 2 h of supplying nitrate, leaves

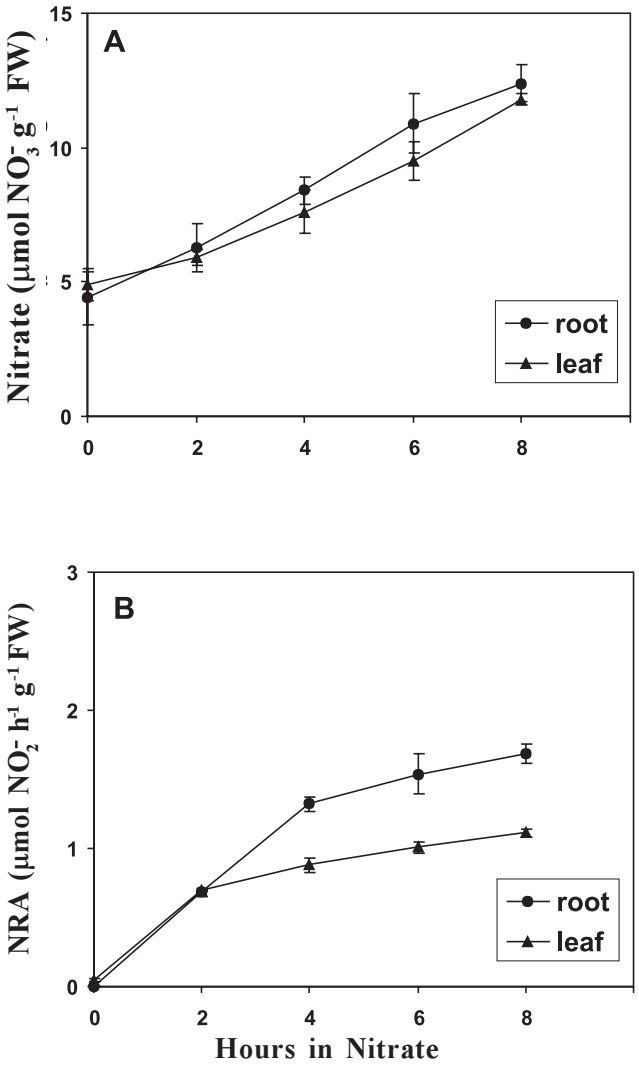

Figure 1. Time course of nitrate concentration (**A**) and *in vivo* nitrate reductase activity (NRA) (**B**) in leaves and roots in nitrogen-starved coffee seedlings, after supplying Hoagland solution (15 mmol L^{-1} NO_3) to the root medium. Values are means ± SE for five plants.

and roots exhibited similar values, but after this time, roots showed enhanced NRA compared to leaves. In both tissues, NRA evolution was not parallel to nitrate accumulation. Nitrate content in leaves and roots increased linearly during the experimental period, whereas NRA in leaves and roots increased linearly up to 2 h and 4 h, respectively, and then began to level off, showing a classical saturation pattern.

Growth and biomass partitioning: Changes in leaf area and dry weight of leaves and roots were evaluated during

early plant development (Figures 2 and 3). The onset of shoot development, represented by cotyledon leaf expansion, occurred nine weeks after sowing. Maximum leaf expansion was observed 7 d before maximum leaf dry weight accumulation (Figures 2 and 3). Before leaf pair development was completed, a new leaf pair began to expand, which occurred at 2-week intervals. Although fully expanded, the first two leaf pairs (after the cotyledon leaves) presented a low leaf area and dry weight, which is usual for coffee seedlings since they only present a typical leaf with the third leaf pair and thereafter.

Production of leaf and root biomass, expressed on a dry weight basis, increased slowly until the 13th WAP and more significantly thereafter (Figure 3B). At the end of experiment (23rd WAP), leaf biomass accumulation was 2.7 times higher than roots, representing 58% of whole-plant dry weight (including stem). However, the relative growth rate was similar for leaves, roots and whole plant (~29 mg g^{-1} d^{-1}; data not shown).

Partitioning of NRA during plant development: During early plant growth, the initial increase of NRA in the first leaf pair coincided with the maximum enzyme activity in the cotyledon leaves (Figure 4). The same pattern was detected in the subsequent growth stages, where decreased activity in the first leaf pair was accompanied by an increase of NRA in the second pair, and thus successively. Activity of NR was low in emerging leaves, increased to a maximum in young fully expanded leaves, but then declined to nearly 75% of maximum values and thereafter remained relatively constant in mature leaves. In fine roots NRA changed periodically during plant development from a maximum of 2.71 to a minimum of 1.21 µmol NO_2^- h^{-1} g^{-1} FW (Figure 4B).

Figure 5 shows the NRA values for the recently expanded leaf and fine roots for each specific age. In most samplings, root NRA was lower than leaf NRA, but at some stages root values exceeded those from leaves. The average data over all samplings made during plant growth were 2.27 and 1.72 µmol NO_2^- h^{-1} g^{-1} FW for leaf and root NRA, respectively, which means the root nitrate reduction capacity was 76% of that obtained in leaves. The NRA leaf/root ratio ranged from 0.8 (15 WAP) to 2.1 (21 WAP), but the changes in this ratio were unrelated to the development stages.

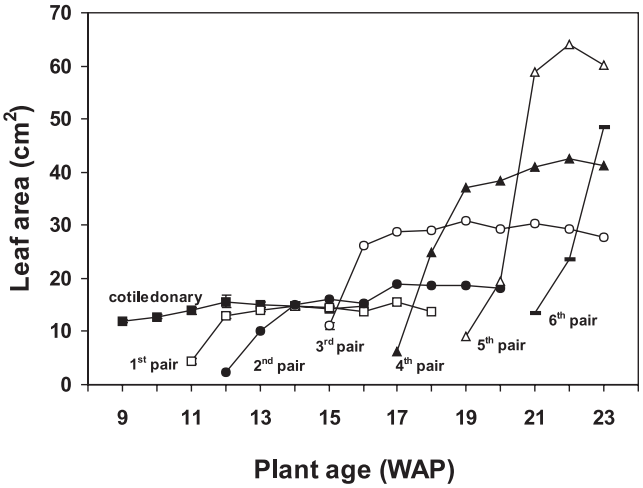

Figure 2. Leaf area of each leaf pair during early vegetative growth of coffee plants. Values are means ± SE for 25 plants.

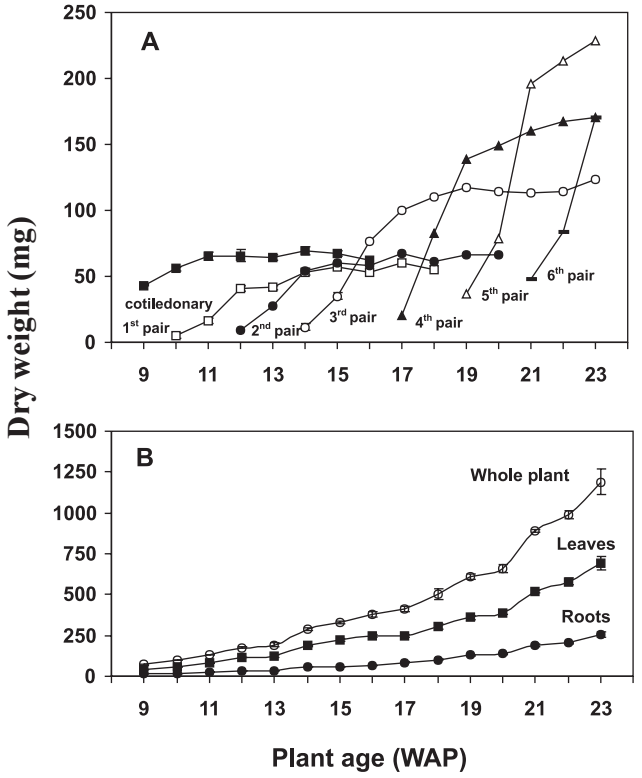

Figure 3. Dry weight of each leaf pair (**A**), and dry weight of total leaves, roots and whole plant (**B**) during early vegetative growth of coffee plants. Values are means ± SE for 25 plants.

Leaf and root contributions for NRA activity of whole plant: The specific contribution of each leaf pair and each root type of 23-week-old plants is presented in Figure 6. It

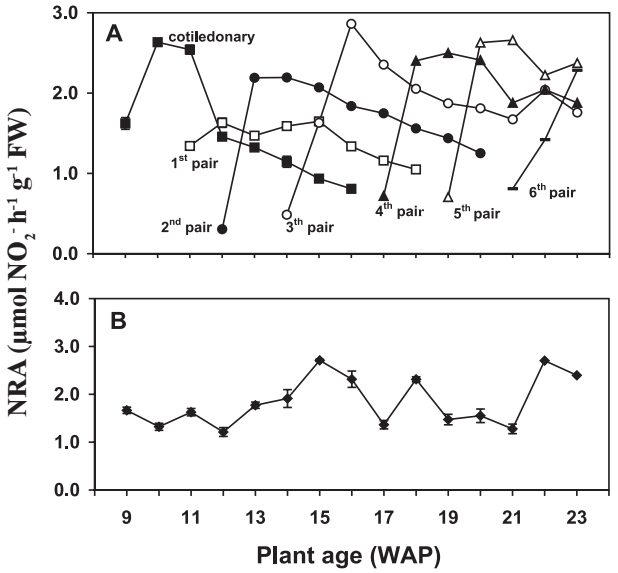

Figure 4. Time course of *in vivo* nitrate reductase activity (NRA) in each leaf pair (**A**) and in fine roots (**B**), during the early vegetative growth of coffee plants. Values are means ± SE of five plants.

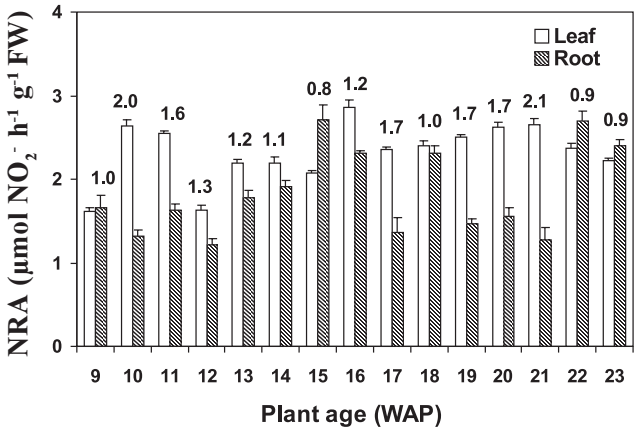

Figure 5. Partitioning of nitrate reductase activity (NRA) between the recently expanded leaf and fine roots during the early vegetative growth of coffee plants. Numbers above bars indicates the NRA leaf/root ratio. Values are means ± SE for five plants.

was observed that the fifth leaf pair (from the base to the top), corresponding to the youngest fully expanded leaves, showed the highest enzymatic activity contributing 34% of the total shoot nitrate reduction and 24% of total plant nitrate reduction. The fine roots, the lateral roots and the pivoting root contributed to 44, 45 and 11%, respectively, of the total root activity. The leaves represented 70% of

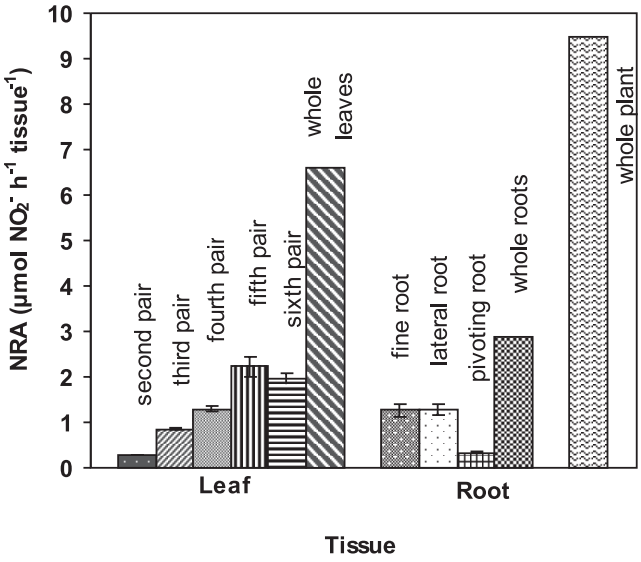

Figure 6. Distribution of nitrate reductase activity (NRA) in leaves and roots of 23-week-old coffee plants. Data are expressed as total NRA per each specific tissue or organ. Values are means ± SE for five plants.

total plant NRA, whereas the roots, only 30%. Under the environmental conditions of this experiment, maximum NRA on a fresh weight basis was obtained in leaves.

Influence of irradiance on partitioning of NRA and carbon assimilation: Leaf NRA was highest in plants grown under 50% of full sunlight, decreasing in the treatments with either 20% or 100% of full sunlight. On the other hand, root NRA was higher in plants grown under 20% of full sunlight, decreasing under higher irradiance regimes (Figure 7A). The NRA leaf/root ratio was 0.72, 1.21 and 1.05 for plants grown under 20%, 50% and 100% of full sunlight, respectively.

Carbon assimilation was similar in leaves under 50% and 100% of full sunlight, as has already been reported by Carelli et al (1999) (Figure7 B). As observed for leaf NRA, intense shading (20% of full sunlight) did decrease carbon assimilation as compared with the other irradiance regimes. Plants grown under 20% of full sunlight showed a similar decrease in NRA (26%) and carbon assimilation (24%) in relation to the plants grown under 50% of full sunlight. The changes in carbon assimilation with increasing irradiance regimes were positively correlated with leaf NRA ($r = 0.78$, $P < 0.01$).

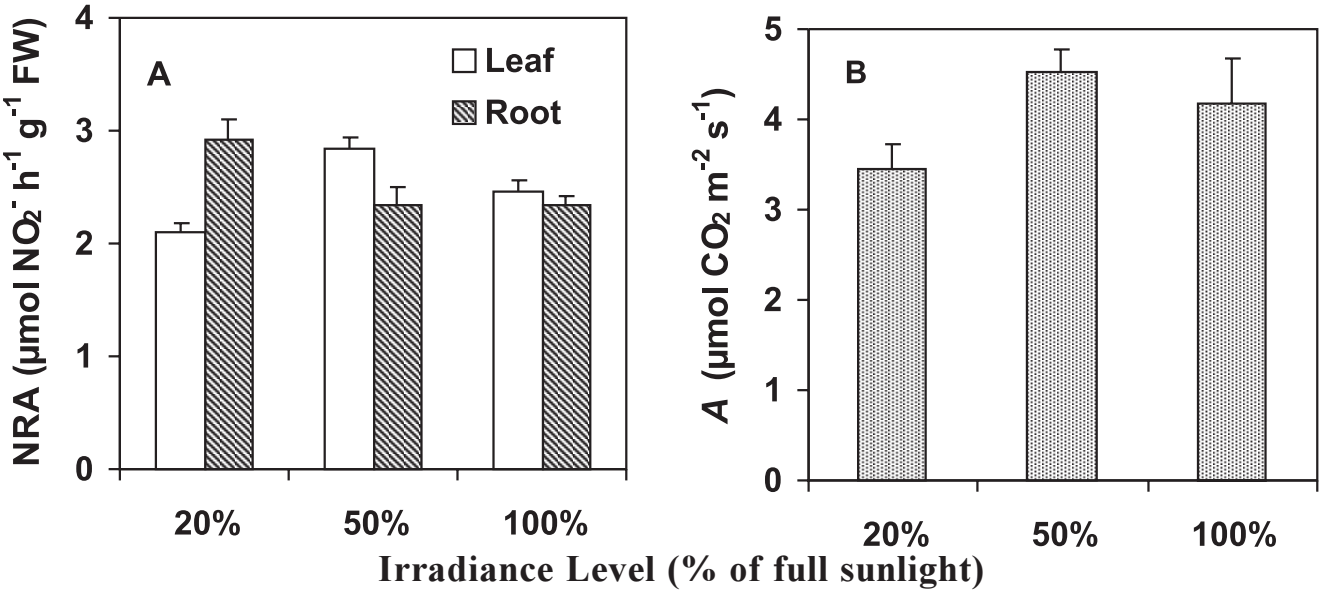

Figure 7. Influence of irradiance level on the nitrate reductase activity (NRA) partitioning between leaves and roots (**A**) and rate of net carbon assimilation (*A*) (**B**) in coffee plants grown under 20%, 50% and 100% of full sunlight.

DISCUSSION

It is generally accepted that distribution of nitrate reduction between roots and shoots is mainly dependent on the root's ability to export nitrate to the shoots (Radin, 1978; Smirnoff and Stewart, 1985; Gojon et al., 1994). In this experiment, under high nitrate availability, roots of young coffee plants showed high capacity to export nitrate to the shoots (Figure 1). After supplying 15 mmol L^{-1} NO_3^- to nitrogen-starved intact plants, both roots and leaves accumulated similar nitrate levels. At the same time, NRA was rapidly induced in roots and leaves in a similar way. These evidences are consistent with the concentrations of nitrogen compounds found in the xylem sap exudates of coffee seedlings reported by Mazzafera and Gonçalves (1999). These authors observed that seedlings fed with Hoagland's solution presented nitrate-N as the most abundant N-form in the sap, representing 52% of the total xylem sap nitrogen, whereas amino acids and ureides accounted for 41% and 6.6%, respectively. The question arising from these findings is whether, under low nitrate concentrations, coffee leaves would exhibit relative high NRA and nitrate concentration. It was previously shown that an increase in the nutrient solution nitrate concentration from 3.75 to 15 mmol L^{-1} increased mainly the NRA in roots (Carelli and Fahl, 1991). Furthermore, during the vegetative development of coffee trees in the field, the overall pattern of nitrate concentration and leaf NRA was unaltered by nitrogen fertilization, as compared to non-fertilized plants (Da Matta et al., 1999). Hence, regardless of the external nitrate concentration, it might be suggested that coffee plants could have high ability to partition the absorbed nitrate and NRA between leaves and roots. In this sense, coffee trees differ from some other woody species, in which the nitrate influx into the leaf may be a major factor limiting the leaf NRA (Smirnoff and Stewart, 1985; Lee and Titus, 1992; Cruz et al., 1993; Gojon et al., 1994).

In the plant shoot, the partitioning of NRA among the various leaf pairs was synchronized during plant development (Figure 4). It should be noted that, although NRA changes during the development of each leaf, the average shoot NRA, for each sampling date, remained nearly constant.

The partitioning of nitrate reduction between root and shoot is not necessarily constant for every species and may vary with plant growth conditions and plant development (Andrews, 1986). During coffee plant development the average NRA was higher in leaves than roots, however, the NRA leaf/root ratio ranged from 0.8 to

2.1 (Figure 5). The observed variations on NRA leaf /root ratio were not due to changes in external nitrate availability, since during the whole period studied the plants received the same nitrate supply. The changes in NRA leaf/root ratio were also unrelated to plant development stages, as observed for other species (Andrews, 1986). Nevertheless, it was observed that the highest root NRA coincided with sampling performed on a completely cloudy day (zero hours of sunshine; data not shown). Unfortunately, the irradiance level at the time of sampling was not recorded. Thus, an experiment to evaluate the influence of irradiance regimes on the partitioning of NRA between leaves and roots was carried out. In such an experiment, results showed that irradiance influenced the partitioning of NRA between leaves and roots (Figure 7). These findings support the results presented in Figure 1, i.e. NRA attained a higher value in roots than in leaves under an irradiance of 190 μmol m^{-2} s^{-1}. Furthermore, this could also explain, at least in part, the disagreement in the literature concerning the partitioning of NRA in coffee plants. In this regard, higher NRA was obtained in roots than in leaves when experiments were conducted under low irradiance (Carelli et al., 1991; Queiroz et al., 1993a), and *vice-versa* under higher irradiances (Carelli et al., 1990a; Da Matta et al., 1999).

Carbon and N assimilation in leaves apparently compete for energy and carbon skeletons in higher plants, but very little evidence is available to support this view (Robinson, 1988; Foyer et al., 1994). In addition, it has been suggested that when photosynthesis is light-limited, the increased nitrate assimilation in roots may allow greater control over the use of limited energy between nitrogen and carbon assimilation (Smirnoff and Stewart, 1985). In fact, nitrate reduction in roots was highest when plants were grown below the photosynthetic light saturation for coffee leaves, which range from 300 μmol m^{-2} s^{-1} for shade-adapted plants to 600 μmol m^{-2} s^{-1} for sun-adapted plants (Kumar and Tieszen, 1980; Fahl et al., 1994; Ramalho et al., 2000). Under intense shading carbon and leaf nitrogen assimilation were limited in a similar way (Figure 7), in relation to plants growing under moderate irradiance (720 μmol m^{-2} s^{-1}).

These evidences suggested that under low irradiance regime, carbon and nitrate assimilation in coffee leaves apparently did not compete for photochemical energy. Rather, the present study emphasizes that nitrate and carbon assimilation may be coordinated in coffee leaves in response to irradiance regimes, as previously documented for the ageing leaf (Fahl et al., 1992). Therefore, it might be suggested that under conditions that maximize carbon assimilation, a higher proportion of nitrate would be assimilated in coffee leaves. In contrast, under limiting conditions for the photosynthetic process and consequently for the leaf nitrate assimilation, higher NRA would occur in roots, compensating the leaf NRA decreases. In fact, it has been documented that nitrate reduction is regulated by concurrent photosynthesis (Pace et al., 1990; Foyer et al., 1994).

During the light period, NRA was generally higher in leaves than in roots (Figure 5). However, nitrate assimilation in roots may continue throughout the night thus making the daily root contribution larger than would be forecasted by NR assays taken at a given time during the photoperiod (Deane-Drummond et al., 1980). In fact, during the dark period, coffee plants exhibit substantial NRA in leaves and roots, NRA being significantly higher in roots than in leaves (Queiroz et al., 1993b). On the other hand, the contribution of shoot and roots to the whole plant nitrate reduction depends on the relative size of each organ and varies with the age of each specific tissue. Taking into account these factors, during the light period the leaves are the main site of nitrate assimilation in young coffee plants grown under a moderate irradiance regime. Even under intense shading (Figure 7) or in the dark period, when root NRA is higher than leaf NRA, the shoot contribution probably would still be higher, since the young coffee plants showed 2.7-fold higher leaf fresh biomass compared to roots. The conclusion that nitrate is predominantly reduced in the leaves of young coffee plants is in good agreement with the general proposal of Andrews (1986) that tropical woody species would carry out a substantial proportion of their nitrate assimilation in the shoots.

In conclusion, young coffee plants exhibited high ability to partition the absorbed nitrate between leaves and roots. Consequently, both tissues showed great potential to reduce nitrate. Irradiance regimes influenced the partitioning of NRA between leaves and roots. Under high irradiance a higher proportion of nitrate was reduced in leaves than roots, and *vice-versa* under low irradiance. Nitrate and CO_2 assimilation were positively correlated in

coffee leaves in response to irradiance regimes. Thus, it may be proposed that under adequate conditions for carbon assimilation, higher nitrate assimilation would occur in coffee leaves, and under limiting conditions for carbon assimilation higher nitrate assimilation would occur in roots. Under moderate irradiance regime, the leaves are the main site of nitrate reduction during the photoperiod, contributing with 70% of the whole plant nitrate assimilation.

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