Growth, photosynthate partitioning and fructan accumulation in plants of V*ernonia herbacea* (Vell.) Rusby under two nitrogen levels

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The effect of two nitrogen concentrations on fructan accumulation and plant growth was analysed in plants of *Vernonia herbacea* during a year period. Plants of this species accumulate inulin-type fructans in the underground reserve organs (rhizophores). The plants were cultivated in glasshouse conditions and received weekly nutrient solutions containing 1.3 mmol.L^{-1} NO_3^- (N-limited) or 10.7 mmol.L^{-1} NO_3^- (N-sufficient). Plants treated with N-sufficient solution presented an increase in total growth with higher biomass, more but smaller leaves resulting in higher total leaf area, higher net assimilation rate, specific leaf mass and higher biomass allocation to aerial organs. In addition, these plants presented higher contents of reducing sugars and lower fructan contents. In contrast, N-limited plants showed reduced total growth with lower biomass, higher biomass allocation to underground organs and higher fructan contents. Therefore, although N-limited plants presented a higher fructan concentration, the amount of fructan produced per plant was similar in both N treatments.

Key words: Asteraceae, cerrado, fructans, underground reserve organs.

Crescimento, partição de fotossintatos e acúmulo de frutanos em plantas de *Vernonia herbacea* (Vell.) Rusby tratadas com dois níveis de nitrogênio. O efeito de duas concentrações de nitrogênio sobre o acúmulo de frutanos e seu crescimento foi analisado durante um ano em plantas de *Vernonia herbacea*. As plantas foram cultivadas em casa de vegetação e receberam semanalmente solução nutritiva contendo 1,3 mmol.L^{-1} NO_3^- (N-limitado) ou 10,7 mmol.L^{-1} NO_3^- (N-suficiente). As plantas tratadas com a solução N-suficiente apresentaram maior crescimento, com maior biomassa, número maior de folhas, porém com menor área, resultando em área foliar total mais elevada, taxa de assimilação líquida e massa foliar específica mais altas, maior alocação de biomassa para os órgãos aéreos. Além disso, revelaram teores mais altos de açúcares redutores e mais baixos de frutanos. Já as plantas que receberam solução N-limitada apresentaram menor crescimento com menor biomassa, maior alocação de biomassa para os órgãos subterrâneos e maior conteúdo de frutanos. Portanto, embora as plantas sob N-limitado tenham apresentado maior concentração de frutanos, sua produção por planta foi semelhante nos dois tratamentos de nitrogênio.

Palavras-chave: Asteraceae, cerrado, frutanos, órgãos subterrâneos de reserva.

INTRODUCTION

The availability of mineral nutrients is one of the key factors that regulate plant growth. Like other environmental constituents, essential inorganic minerals are capable of modifying the morphology of the plant, biomass partitioning and allocation, growth rate and the content of reserve carbohydrates (Lambers and Poorter, 1992; Gerdoc et al., 1996).

The responses of wild species to nutrient availability are similar to those described for cultivated plants. Both respond to nutritional stress by changing reserve allocation

and reducing photosynthesis and growth (Chapin, 1988; Marschner, 1995; Marschner et al., 1996). Chapin (1980, 1988) considered other effects of nutrient restriction, such as increases in leaf longevity and in the accumulation of nonstructural carbohydrates, and inhibition of reproduction. In comparison to rich soil species, plants of oligotrophic environments exhibit low root absorption rates that can be improved in response to increasing soil fertility, although with lower absorption capacity (Marschner, 1995). In this respect, wild species growing in poor soils show low growth rates and lower shoot:root ratios, even under high nutrient availability.

Nitrogen is the main mineral element contributing to plant growth and its deficiency has been associated to a reduction in cell division, cell extension, leaf area and photosynthesis (Chapin, 1980). In addition, nitrogen deficiency may cause a reduction in total biomass and in the shoot: root ratio (Rufty et al., 1984).

Most plants accumulate starch or sucrose as reserve carbohydrates. However, about 15 % of all flowering plants, including the economically important and highly evolved families Asteraceae and Poaceae, store fructose polymers, named fructans (Vijn and Smeekens, 1999). Fructans have been associated with mechanisms of drought and frost resistance (Hendry and Wallace, 1993) and nutritional stress (Améziane et al., 1995, 1997a). These compounds are economically important due to their use in pharmaceutical and food industries as substitute for fat and sugar (Niness, 1999). Therefore, treatments that increase sink strength towards fructan accumulating organs are useful, if one considers their commercial value.

Sink strength is genetically determined, although it can be affected by environmental changes (Pollock, 1986). Among the external factors already studied, changes in nitrogen supply are the most recommended strategy to modify sink strength (Rufty et al., 1984; Farrar, 1993; Améziane et al., 1995, 1997a). The few studies related to this subject showed that low nitrogen supply caused an increase in source and sink strength in *Hordeum vulgare* (Wang and Tilberg, 1996, 1997) and in *Cichorium intybus* (Améziane et al., 1997a) as well as an increase in fructan content in tuberous roots of the latter species (Van den Ende et al., 1999).

Vernonia herbacea is a perennial Asteraceae native to the Brazilian cerrado. It presents underground sink organs, named rhizophores, which accumulate about 80 % of its dry mass in fructans of the inulin type (Carvalho and Dietrich, 1993). These authors carried out a study on the fructan contents and composition during the phenological cycle of *V. herbacea*, and reported that changes in these carbohydrates included a decrease in fructan during sprouting and flowering in spring. This decrease was considered a result of fructan mobilisation to provide substrate for growth. A period of intensive vegetative growth and a concomitant increase in fructan contents occurred during summer, followed by senescence of aerial organs in autumn and dormancy in winter, with rhizophores presenting high fructan contents.

Although plants of *V. herbacea* show high fructan contents when growing naturally in the poor cerrado soil, studies on growth conditions that favour biomass increase and allocation in the reserve organ must be undertaken in order to increase fructan production. The relation between nitrogen supply to plant growth and fructan accumulation was not conclusively demonstrated in earlier studies (Teixeira et al., 1997; Carvalho et al., 1998). However, more recently Cuzzuol et al. (2003) showed that in a narrow range of low concentrations, nitrogen promoted growth and inhibited fructan accumulation and plant productivity in field grown plants, whereas the opposite was found when higher nitrogen concentration was supplied. The chemical composition of leaves of *V. herbacea* allowed us to define nitrogen requirement of these plants (Cuzzuol et al., 2005).

Based on the hypothesis that a nutritional stress can lead to an increase in fructan accumulation, the aim of the present study was to evaluate the effect of a lower nitrogen supply on growth, biomass allocation, and fructan production.

MATERIAL AND METHODS

Plant material, growth conditions and sampling: Plants of *Vernonia herbacea* (Vell.) Rusby were obtained from rhizophores as described in Carvalho et al. (1997). Fifty-day-old plants of about 5 cm high were selected and transferred to plastic pots (3 L) containing washed sand. Plants were grown in a glasshouse during one phenological cycle, under natural photoperiod and temperature (mean minimum and maximum temperatures of 18 and 29°C). The experiments were conducted at the Instituto de Botânica (23º38'25" S, 46º37'19" W), São Paulo, Brazil.

The chemical composition of leaves of *V. herbacea* was determined in the Laboratório do Centro de Solos e Recursos Agroambientais do Instituto Agronômico de Campinas (IAC) and a specific nutrient solution was elaborated to supply adequate nitrogen requirement as previously determined by Cuzzuol et al. (2005): 10.7 mmol.L^{-1} N-NO_3^- (denominated N-sufficient solution), containing 2.5 mmol.L^{-1} $Ca(NO_3)_2$.4

H_2O, 2.3 mmol.L^{-1} KNO_3, 0.52 mmol.L^{-1} KH_2PO_4, 1.7 mmol.L^{-1} $Mg(NO_3)_2.6H_2O$ and 1.3 mmol.L^{-1} Na_2SO_4. Another nutrient solution containing approximately a 10 % lower nitrogen concentration (1.3 mmol.L^{-1} N-NO_3^-, denominated N-limited solution) was prepared as follows: 1.3 mmol.L^{-1} KNO_3, 0.52 mmol.L^{-1} KH_2PO_4, 0.9 mmol.L^{-1} KCl, 2.17 mmol.L^{-1} $CaCl_2.2H_2O$ and 1.5 mmol.L^{-1} $MgSO_4.7H_2O$. Both solutions were supplemented with the micronutrients of Hoagland solution (Hoagland and Arnon, 1938) and the pH was adjusted to 5.5-6.0 with 1N KOH. Plants were separated in two groups that received 100 mL of one of the above solutions each week. Whenever necessary the plants were irrigated with distilled water. Six plants (1 plant = 1 replicate) were collected from each treatment at three-month intervals for growth evaluation and carbohydrate analyses. For carbohydrate analyses of shoots and rhizophores, portions taken from the midregion of these organs were used. In the case of leaves, the first apical and completely expanded leaf was utilized.

Growth analysis: The concept of biomass allocation was used as defined by Poorter and Nagel (2000): the amount of biomass located in a particular organ in relation to the total plant mass. Growth measurements included shoot height, fresh and dry masses of the aerial and underground organs, leaf number and leaf area. Dry mass was determined by drying the tissues at 60 ºC, to constant weight. Leaf area was calculated using the software Leaf Area & Analysis (Skye Instruments Ltd.). Relative growth rate (RGR) and leaf area ratio (LAR) were calculated according to Hunt (1982) and net assimilation rate (NAR) was calculated using the formula $NAR=[(Ln A_2 - Ln A_1) / (A_2 - A_1)] \times [(DM_2 - DM_1) / t_2 - t_1]$, as described by Williams (1946). Photosynthetic rate (PR) was calculated using the formula PR=NAR x K/H (Beadle, 1986), where K is the conversion factor of the amount of dry mass in terms of absorbed carbon (=1.65) as described in Salisbury and Ross (1992), and H is the photoperiod, here considered as 12h, the mean year value for São Paulo (Vianello and Alves, 1991). Specific leaf mass (SLM) and specific leaf area (SLA) were calculated by leaf mass/leaf area and 1/SLM, respectively. Sink strength was quantified as the rate of dry mass increase of rhizophores, whereas sink activity (capacity to store fructans) was calculated by the rate of fructan accumulation (Schubert and Feuerle, 1997).

Extraction and analysis of soluble carbohydrates: Soluble carbohydrates were extracted as described by Carvalho et al. (1998) from fresh rhizophores (2 g), stems (1 g) and leaves (1 g). The crude extract from rhizophores was submitted to ethanol precipitation and fructo-oligosaccharide (considered here as molecules with DP ≤ 20) and fructo-polysaccharide fractions were separated by centrifugation (Asega and Carvalho, 2004). Free and combined fructose were measured in the two fractions and in the crude extracts from stems and leaves by a ketose-specific modification of the anthrone reaction (Jermyn, 1956), using inulin from *Helianthus tuberosus* as standard. Reducing sugars were determined (Somogyi, 1945), using fructose as standard. For quantification of glucose, fructose and sucrose, samples were de-ionized (Carvalho & Dietrich,1993) and analyzed by high performance anion exchange chromatography with a pulse amperometric detector (HPAEC/PAD), using external standards, on a 4 x 250 mm CarboPac PA-1 column, in a Dionex System Mod. DX-300. The gradient was established by mixing eluant A (150 mM NaOH) and eluant B (500 mM sodium acetate in 150 mM NaOH) as follows: 0-2 min, 25 mM; 2-8 min 25-150 mM; 8-28 min, 150-500 mM; 28-30 min, 25 mM with a flow rate of 1.0 ml.min^{-1}. The applied potentials for E1 (480 ms), E2 (120 ms) and E3 (60 ms) were 0.05, 0.060 and –0.60, respectively.

RESULTS

Biomass allocation and plant growth: Biomass increase was detected in rhizophores and aerial organs from December until June in plants of both nitrogen treatments, although in N-sufficient plants the biomass gain was higher (figure 1). From June through September, N-sufficient plants showed a reduction of biomass. This period comprises senescence of aerial organs in plants of both treatments. Thus, biomass measurements of these organs in September refer to newly sprouted shoots. From September on, increases in the biomass of rhizophores, aerial organs and in the ratio shoot: rhizophores were observed.

Most of the biomass allocation was concentrated in the rhizophores, independently of the N treatment, except after 360 days of cultivation, when N-sufficient plants presented higher biomass allocation to the shoots. A decrease in the ratio shoot: rhizophores from March to June in N-sufficient plants is due to the increase in the rhizophore biomass occurring in this period (figures 1 and 2).

The plants presented different growth responses to nitrogen (table 1). N-sufficient treatment affected growth positively, mainly after 360 days (December). At this stage these plants presented 4.3 times more leaves, 2.7 times

higher total leaf area, 2.8 times more shoots and 1.2 time higher shoots than N-limited plants. During the second growth cycle (September to December), plants of both treatments showed a more pronounced growth, reflected in leaf number, shoot height and leaf area. In N-sufficient plants, although the individual leaf area doubled in size, leaf number increased 4 times, leading to a significant increase in total leaf area. Flowering occurred in 50 % of the plants under this treatment.

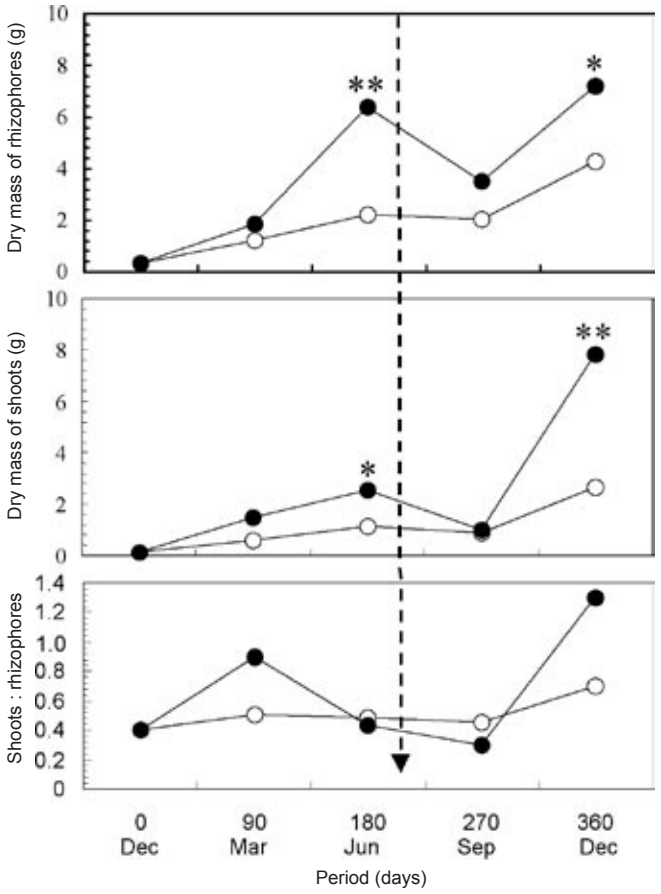

Figure 1. Effect of nitrate on dry mass partitioning and on the ratio shoot:rhizophore in plants of *V. herbacea* treated with 1.3 mmol.L^{-1} (○) and 10.7 mmol.L^{-1} (●) N-NO_3^-. Arrow indicates senescence of shoots. Each value is the average for six samples from separate plants. * $P < 0.05$, ** $P < 0.01$.

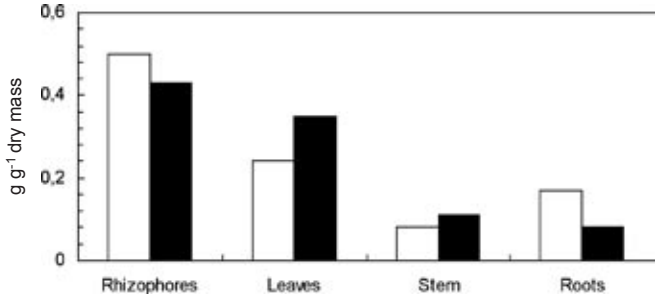

Figure 2. Effect of nitrate on dry mass allocation to rhizophores, leaves, stems and roots in plants of *V. herbacea* treated with 1.3 mmol.L^{-1} (white columns) and 10.7 mmol.L^{-1} (black columns) N-NO_3^- for 360 days. Each value is the average for six samples from separate plants. Differences were non significant between treatments.

Values of RGR, NAR and PR were higher between 0 – 90 days in both treatments and when the two treatments are compared values were higher in N-sufficient plants (table 2).

A decrease in photosynthetic efficiency occurred in N-sufficient plants after 360 days, as demonstrated by the significantly higher LAR value. In September (270 days), N-limited plants presented higher SLA. SLM values were always higher in N-sufficient plants, although statistical significance was found only after 180 and 270 days (table 3).

Soluble carbohydrates: A significant proportion of the plant biomass consisted of soluble sugars (figure 3). Moreover, most of the total soluble sugars in the rhizophores are comprised of fructans, as revealed by the close values of total fructose and total carbohydrates. The concentration of both total fructose (fructans) and total carbohydrates in the rhizophores increased throughout the experimental period, in both nitrogen treatments, except for a clear decrease in September, during the sprouting phase. However fructan concentration was higher in N-limited plants. On the other hand, with respect to fructan production, these plants accumulated 3.78 g.plant^{-1} while in N-sufficient plants this value was 4.03 g.plant^{-1}. Contents of total fructose found in stems and leaves were lower than in the rhizophores and no significant differences were detected between the two treatments. Total fructose concentration reached a maximum of 1 % of the leaf dry mass and 2.5 % of the stem dry mass, while in the rhizophores this value was over 80 % in N-limited and 50 % in N-sufficient plants.

After 360 days, when the major differences in total fructose concentration in the rhizophores were observed between the two treatments (figure 3), carbohydrate fractioning showed that the contents of both fructo-oligo and -polysaccharides were significantly higher in N-limited plants (figure 4).

The concentration of reducing sugars showed an outstanding increase in September, in N-sufficient plants (figure 5), while a less pronounced increase was found in N-limited plants. More glucose and fructose were found in leaves of N-sufficient plants, while sucrose predominated in the rhizophores in both treatments. In the stems, no significant differences between the two treatments were detected (figure 6).

Sink strength, as indicated by the increase in the rhizophore dry mass, was higher in N-sufficient plants, except between 180 and 270 days. This reduction was more pronounced in N-sufficient plants. A similar effect was verified for sink activity (figure 7).

DISCUSSION

Compared to N-limited plants, plants of *V. herbacea* growing under N-sufficient condition presented higher shoots, leaf number, leaf area and shoot and rhizophore dry mass, expressed mainly in the second growth phase (September through December). Plants treated with N-sufficient solution also showed higher biomass allocation to the shoots (table 1 and figures 1 and 2), as reported for cultivated species growing in N-enriched conditions (Lambers et al., 1998). Changes in the ratio shoot:root obtained under different nutrient treatments are of particular interest in plants bearing underground reserve organs. In general, plants allocate more biomass to the roots when growing under nitrogen deficiency (Lambers et al., 1998). In *Cichorium intybus* a low shoot: root ratio is induced by nitrogen deficiency in order to produce fructan rich tuberous roots (Améziane et al., 1997b; Schittenhelm, 1999). In the present study a similar effect was observed (figure 2).

Although rhizophore dry mass was lower in N-limited plants (figure 1), the lower shoot:rhizophore ratio found in these

Table 1. Effect of nitrate on growth and flowering in plants of *V. herbacea* treated with 1.3 mmol.L^{-1} and 10.7 mmol.L^{-1} N-NO_3^-. * P<0.05 and ** P < 0.01 between treatments.

Days	Shoot number		Height (cm)		Flowering (%)		Leaf number		Leaf area (cm^2)			
									Total		Individual	
	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}
0 (Dec)	1.0	1.0	13.4	13.4	0	0	5.3	5.3	0.78	0.78	0.14	0.14
90 (Mar)	1.0	1.0	13.5*	19.8	0	0	6.8*	11.0	6.38**	14.88	0.93*	1.35
180 (Jun)	1.0	1.0	20.1*	26.3	0	0	10.8*	14.6	10.83**	21.25	1.00*	1.45
270 (Sep)	1.5	2.3	21.3	18.3	0**	50	8.0*	12.0	10.10*	7.39	1.26**	0.62
360 (Dec)	1.4**	4.0	27.5*	32.8	0	0	11.0**	47.6	22.30**	60.86	2.03*	1.27

Table 2. Effect of nitrate on relative growth rate (RGR), net assimilation rate (NAR) and photosynthesis rate (PR) in plants of *V. herbacea* treated with 1.3 mmol.L^{-1} and 10.7 mmol.L^{-1} N-NO_3^-. * indicates P < 0.05 between treatments.

Days	RGR (g.g^{-1}.d^{-1})		NAR (g.cm^{-2}. d^{-1})		PR (mg CO_2. cm^{-2}.h^{-1})	
	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}
0-90	0.013*	0.024	0.0038*	0.0073	0.60	1.00
90-180	0.011	0.011	0.0028	0.0035	0.50	0.60
180-270	0.005*	- 0.007	0.0002*	- 0.0036	0.03*	-0.04
270-360	0.010	0.013	0.0031	0.0047	0.40	0.70

Table 3. Effect of nitrate on leaf area ratio (LAR), specific leaf area (SLA) and specific leaf mass (SLM) in plants of *V. herbacea* treated with 1.3 mmol.L^{-1} and 10.7 mmol.L^{-1} N-NO_3^-. * P < 0.05 between treatments.

Days	LAR (cm^2.g^{-1})		SLA (cm^2.g^{-1})		SLM (g.cm^{-2})	
	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}	1.3 mmol.L^{-1}	10.7 mmol.L^{-1}
0 (Dec)	1.84	1.84	12.4	12.4	0.080	0.080
90 (Mar)	4.79	4.17	14.1	12.4	0.072	0.079
180 (Jun)	3.14	2.31	12.4	10.6	0.075*	0.102
270 (Sep)	2.92*	1.51	15.1*	11.0	0.061*	0.090
360 (Dec)	2.87*	3.87	12.2	10.3	0.084	0.096

plants suggests their higher capacity to export assimilates to the sink tissues. In fact, Poorter and Nagel (2000) reported that a decline in biomass accumulation in a particular organ is not necessarily accompanied by a decline in allocation to this organ.

The mechanism controlling biomass partitioning between shoots and roots was named functional equilibrium (Brower, 1983). According to the author, root growth is under the control of photoassimilates while shoot growth is limited by the nutrient availability to the roots. An additional fact to be considered in biomass partitioning is how the photosynthates are metabolized in the sink organ. In *C. intybus*, for example, photosynthate translocation to tuberous roots is under the control of the enzymes SST and FFT whose activities are stimulated by low nitrogen availability (Améziane et al., 1995, 1997a; Van den Ende et al., 1999).

Concerning the growth parameters analyzed, the low values in RGR and NAR indicate that *V. herbacea* presents a low RGR potential as described for plants native to poor soils (Lambers and Poorter, 1992; Paulilo and Felippe, 1995; Sassaki and Felippe, 1998). Based on their adaptive characteristics to nutrient levels in the soil, Chapin (1980, 1988) classified wild species in two groups: in Type I, growth is always slow even when the plants are cultivated under high nutrient supply, whereas in Type II, a low nutrient offer leads to a marked decrease in growth and to clear symptoms of nutrient deficiency. In the present study, a higher supply of nitrogen did not promote increases in these rates, suggesting that *V. herbacea* is a Type I species.

Although Lambers et al. (1998) reported that NAR and RGR are correlated only in monocots, a high correlation (r^2

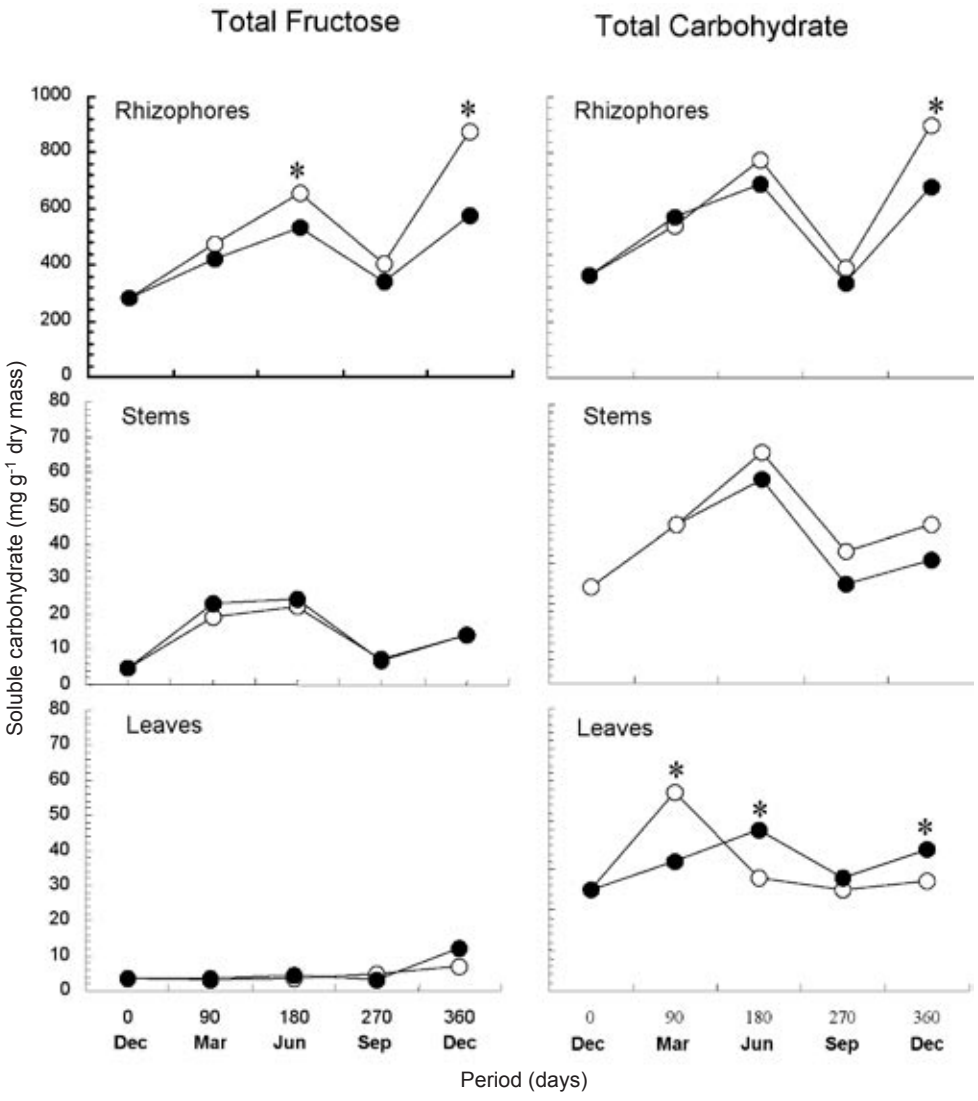

Figure 3. Effect of nitrate on contents of soluble carbohydrates in rhizophores, stems and leaves of plants of *V. herbacea* treated with 1.3 mmol.L^{-1} (○) and 10.7 mmol.L^{-1} (●) N-NO_3^-. Each value is the average for six samples from separate plants. *$P < 0.05$.

= 0.973) between these two rates was found for *V. herbacea*. NAR is related to the balance between carbon assimilated during photosynthesis and carbon consumed in respiration. Higher values obtained for NAR (table 2) during intensive plant growth, in spring and summer (0-90 and 270-360 days), are consistent with this growth phase, when plants are photosynthesizing at a higher rate.

Maximum RGR (0.024 $g.g^{-1}.d^{-1}$) and LAR values (1.5 – 4.8 $cm^2.g^{-1}$) determined for *V. herbacea* are remarkably lower than those reported for other herbs: 0.30 $g.g^{-1}.d^{-1}$ for RGR (Grime and Hunt, 1975) and 75 – 280 $cm^2.g^{-1}$ for LAR (Lambers et al., 1998). Low RGR values are considered by Grime and Hunt (1975) a strategy for plant survival in low fertility soils, a condition found in the cerrado, and reinforces the classification of *V. herbacea* as a Type I species.

Plants of *V. herbacea* presented higher fructan concentration in the rhizophores in June, just before shoot senescence, and in December, when the plants were growing intensively (figure 3). Concentration of this carbohydrate was significantly higher in N-limited plants. According to Münch's hypothesis, phloem loading and unloading is dependent on the turgor gradient between source and sink (Farrar, 1996). Therefore, high contents of photosynthates in leaves (source strength) would exert the necessary pressure for carbohydrate export towards the sink. Comparing the two N-treatments, the source strength of plants growing under N-sufficient solution was higher in June and in December, as indicated by the values of total sugars in leaves (figure 3), while fructan concentration was lower in the sink organs, the rhizophores. This can be explained by the fact that the rate of photosynthate export can be controlled by the source strength, although the mass flow may change independently (Améziane et al., 1995).

In fructan accumulating plants, the capacity of the sink tissues to attract and accumulate photosynthates is under the control of SST and FFT (Améziane et al., 1997a,b). Therefore, although SST and FFT activities were not evaluated in the present study, the higher fructan concentration found in N-limited plants could be associated with increases in the activity of these enzymes, as reported by Wang et al. (2000) for 6-SFT in barley leaves. Since sucrose is the precursor molecule in the biosynthesis of fructans, the metabolism of these two compounds is closely related. Indeed, high sucrose concentration promotes both synthesis and activity of SST, and consequently increases sink capacity to attract and accumulate photosynthates (Pollock and Cairns, 1991; Van den Ende et al., 1999). In N-limited plants higher fructan concentration in the rhizophores (figure 3) occurred parallel to lower sucrose content in these tissues (figure 6). This lower sucrose concentration suggests a more active synthesis and accumulation of fructans in these plants, assuring the necessary gradient of sucrose between phloem and the sink tissue, as discussed by Améziane et al. (1995) for chicory plants.

As a consequence of the more pronounced growth, sink strength and sink activity were generally higher under N-sufficient treatment (figure 7). Since the major proportion of

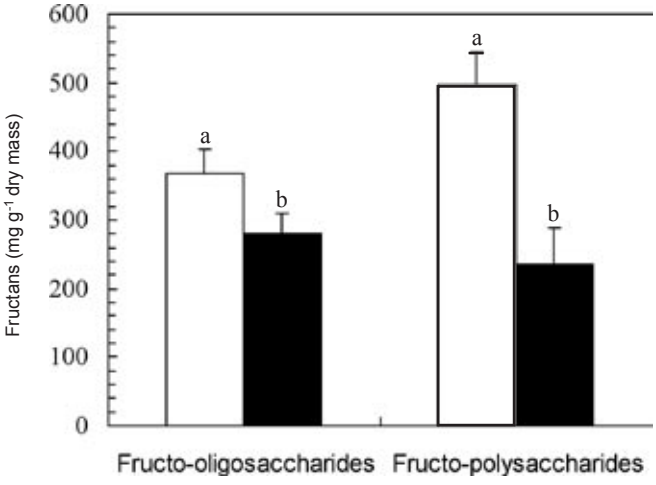

Figure 4. Effect of nitrate on the concentration of fructo-oligo and fructo-polysaccharides in rhizophores of plants of *V. herbacea* treated with 1.3 $mmol.L^{-1}$ (white columns) and 10.7 $mmol.L^{-1}$ (black columns) $N\text{-}NO_3^-$ for 360 days. Each value is the average for six samples from separate plants. Bars refer to SE. Letters compare different treatments (*$P < 0.05$).

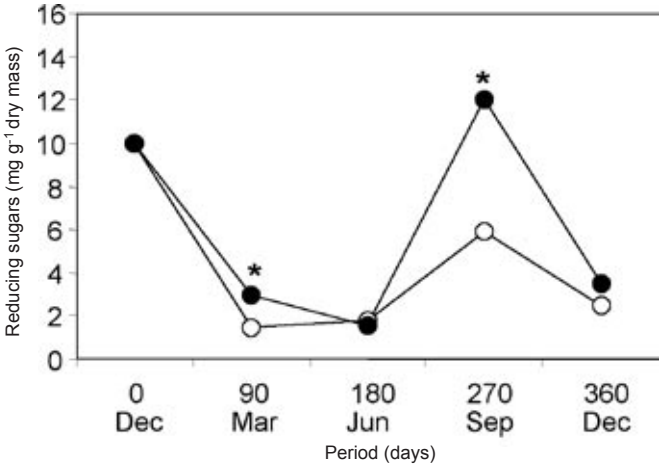

Figure 5. Effect of nitrate on the concentration of reducing sugars in rhizophores of plants of *V. herbacea* treated with 1.3 $mmol.L^{-1}$ (○) and 10.7 $mmol.L^{-1}$ (●) $N\text{-}NO_3^-$ for 360 days. Each value is the average for six samples from separate plants. *$P < 0.05$.

the rhizophore dry mass is comprised of fructans, the variation in sink activity and sink strength in both treatments showed similar profiles throughout the period of investigation.

N-sufficient plants showed a lower proportion of fructo-polysaccharides in the rhizophores (figure 4), confirming previous results reported for *V. herbacea* by Teixeira et al. (1997) and reinforcing the statement that external factors such as nutrients can lead to changes in the proportion of fructan chain sizes (Pollock, 1986). In *C. intybus*, Van den Ende et al. (1999) reported decreases in all fructan chain size classes concomitant with increases in fructan exohydrolase (FEH) after transfer of plants from N-poor to N-rich medium.

The decrease in fructan content in the rhizophores (figure 3) in September was concomitant with the growth of new shoots and occurred possibly as a result of FEH activity. In fact, an increase in FEH activity was demonstrated in *V. herbacea* by Asega and Carvalho (2004) during the growth of induced new shoots. In September we also observed an increase in reducing sugars (figure 5), probably representing the free fructose released during fructan mobilization. This increase in reducing sugars was more evident in N-sufficient plants exhibiting a more pronounced shoot growth when compared to N-limited plants. An increase in reducing sugars in sprouting and flowering phases was shown by Carvalho and Dietrich (1993). The authors reported a reduction of 45 % of fructans from the rhizophores at these stages, while in the present study a reduction of 54 % was detected at sprouting, in September (figure 3). The requirement for carbohydrates during reproductive development was extensively discussed by Bernier et al. (1993).

N-limited treatment (1.3 mmol.L^{-1} N-NO_3^-) caused a general reduction in growth and increases in biomass allocation towards the rhizophores and in fructan concentration when compared to N-sufficient treatment (10.7 mmol.L^{-1} N-NO_3^-). Regardless of the increment in fructan concentration and biomass allocation, fructan overall

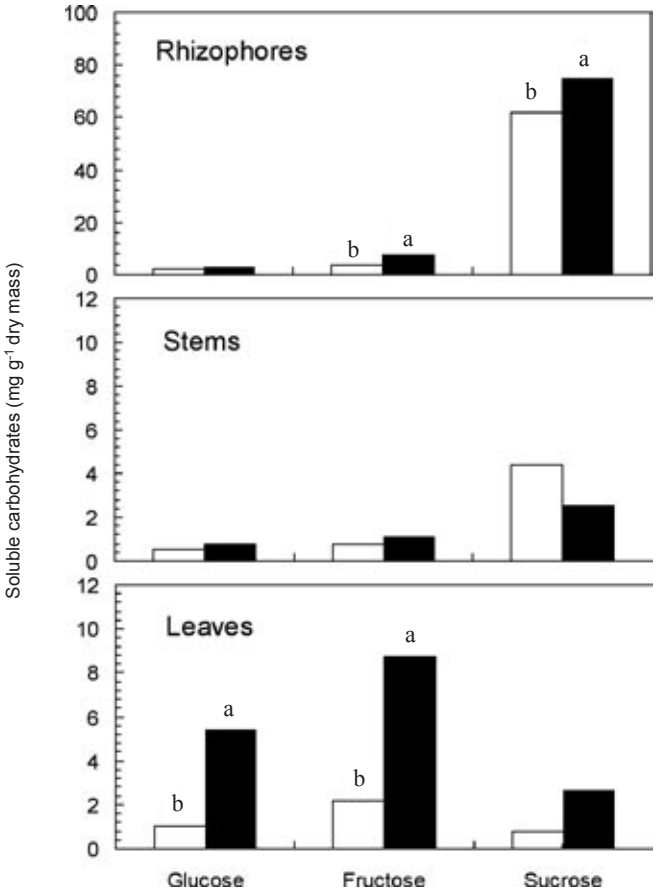

Figure 6. Effect of nitrate on the concentration of glucose, fructose and sucrose in rhizophores, stems and leaves of plants of *V. herbacea* treated with 1.3 mmol.L^{-1} (white columns) and 10.7 mmol.L^{-1} (black columns) N-NO_3^- for 360 days. Each value is the average for six samples from separate plants. Letters compare different treatments (*$P < 0.05$).

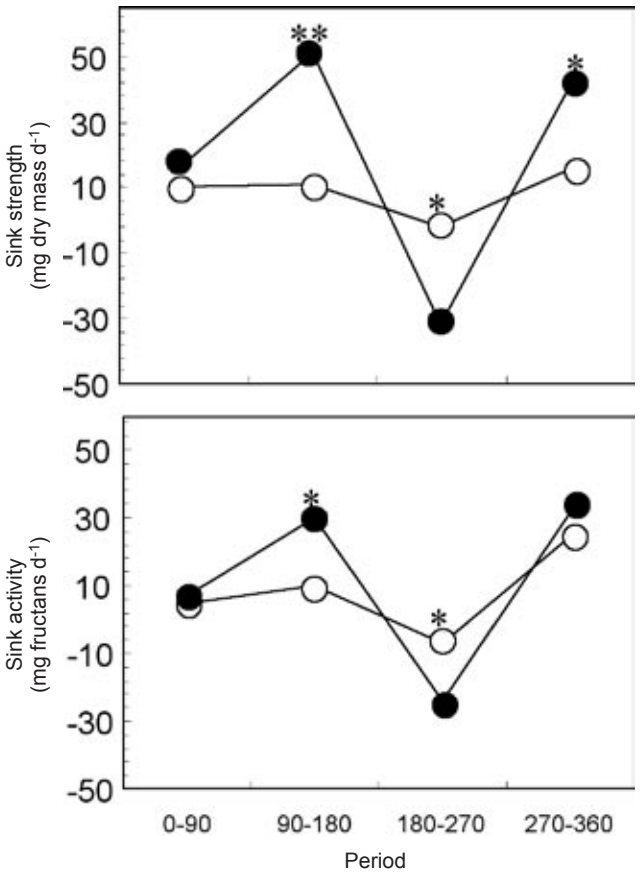

Figure 7. Effect of nitrate on sink strength and sink activity in rhizophores of plants of *V. herbacea* treated with 1.3 mmol.L^{-1} (○) and 10.7 mmol.L^{-1} (●) N-NO_3^- for 360 days. Each value is the average for six samples from separate plants. * $P < 0.05$, ** $P < 0.01$.

accumulation in N-limited plants (3.78 g.plant^{-1}) was very similar to that obtained under N-sufficient treatment (4.03 g.plant^{-1}). Therefore, a nitrogen concentration 8 times lower than the nitrogen requirement of *V. herbacea* affected plant growth but not fructan production.

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