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# Nitrogen Effects on Growth and Fructan Production in *Vernonia herbacea* (Vell.) Rusby, an Asteraceae from the Brazilian Cerrado

**Lilian B. P. Zaidan*** **• Maria Angela M. Carvalho**

Instituto de Botânica, Núcleo de Pesquisa em Fisiologia e Bioquímica, CP 3005, 01031-970, São Paulo, SP, Brazil

*Corresponding author*: * **lilianzaidan@uol.com.br**

## ABSTRACT

The Cerrado, second largest biome in Brazil, hosts a high biodiversity and is listed as one of the most endangered ecosystems (hotspots) of the world. The cerrado soils are old, deep, with low water retention, poor in nutrients and with high levels of aluminum. Although plants native to the cerrado are well adapted to oligotrophic soils, growth and productivity can be increased by addition of mineral nutrients. *Vernonia herbacea* is a perennial herb accumulating about 80% of inulin-type fructans in the underground organs, thus being considered an alternative source for inulin production. The present review is focused on the role of nitrogen in growth, biomass allocation and inulin production in plants of *V. herbacea* grown in the field and under glasshouse conditions. The studies here reported summarize the efforts towards the understanding of the physiology and biochemistry of this promising species of the Cerrado and could contribute to the sustainable use of this biome.

**Keywords:** biomass allocation, inulin, reserve carbohydrate, underground organ, vegetative growth

## CONTENTS

## INTRODUCTION

The cerrado vegetation covers about 2,000,000 $km^2$ and accounts for 21% of the Brazilian land area, being outsized only by the Amazon Rain Forest (Eiten 1972; Coutinho 2002). The central continuous Cerrado region comprises a core area in the Central Plateau, extending in great peninsulas and disjunctive areas in the northeastern and towards southern areas of Brazil (**Fig. 1**).

Approximately 50% of the Cerrado is located between 300 and 600 m above sea level and only 5.5% is over 900 m. The cerrado soil is deep, porous, permeable, a mixture of sand and clay in different proportions with overall low water retention, poor in nutrients and with a low cation exchange capacity. The soil is acid, the pH varies between 4 and 5, due partly to high levels of $Al^{3+}$ (Eiten 1972).The predominant climate is seasonal tropical, with dry winters and wet summers. The mean annual temperature is 22-23°C and the mean monthly temperatures show only slight seasonality. The absolute maximum monthly temperatures may reach 40°C and do not vary much throughout the year, while the absolute minimum monthly temperatures present large variation, reaching values close to or below 0°C in May, June and July. Frost may occur during this period, especially in the southern area of the Cerrado. Mean annual rainfall is between 1200 and 1800 mm and in contrast with the temperature, it is markedly seasonal and concentrated in spring and summer (from October to March). From May to September the monthly rainfall is markedly reduced and may reach zero, resulting in a dry season that lasts from 3 to 5 months (Coutinho 2002). These conditions contributed altogether to the formation of a xeromorphic vegetation with a high diversity of plant species. The vascular flora comprises about 7,000 species, according to Castro *et al.* (1999). The cerrado vegetation is physiognomically diverse, from open fields to woodlands. Among these two extremes a range of intermediate forms such as “campo sujo”, scrub, “campo cerrado”, cerrado *sensu stricto* and “cerradão” compose a mosaic determined by soil fertility and the occurrence and intensity of fire.

The cerrado vegetation is constituted basically by two distinct components, a wood/shrub and an herbaceous/undershrub stratum, this latter one comprising more than double the number of wood species. Some authors claim that species in the herbaceous/undershrub layer may account for approximately 80% of the flora (e.g. Mendonça *et al*. 1998; Figueiredo-Ribeiro *et al*. 2007). The wood component presents root systems that allow the plants to reach deep soil layers with permanent water availability while the herbaceous component is formed by perennial species with

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**Invited Mini-Review**

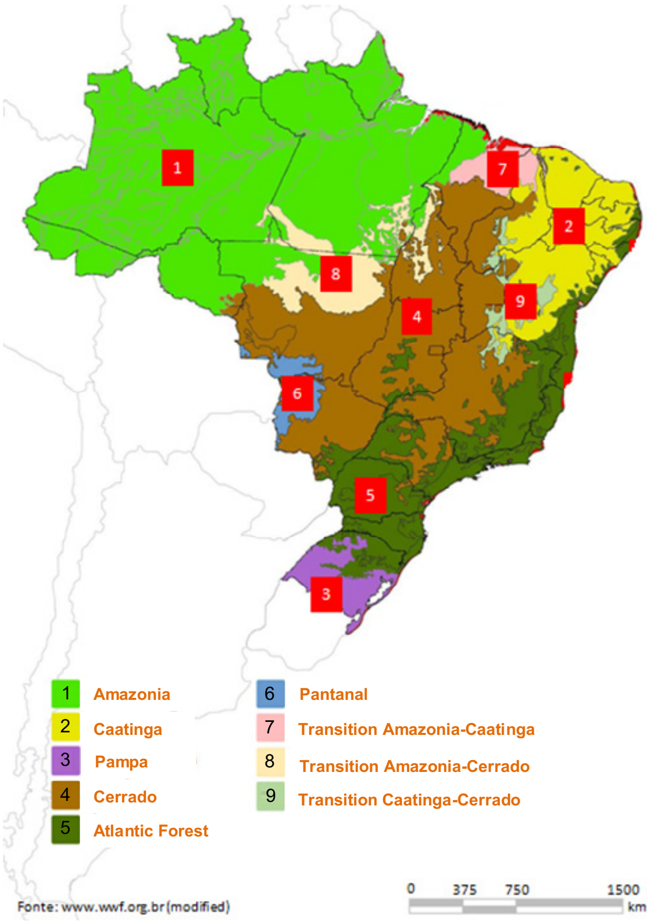

**Fig. 1 Localization of the biomes in Brazil and the area occupied by the Cerrado.**

well developed underground organs, such as bulbs, xylopodia, soboles and tuberous roots that guarantee their survival during the dry period and occasional fire events.

Fire is considered an important feature of the Cerrado and from the earliest times Man has been the main responsible for the burn-offs, due to the use of the open forms of the Cerrado for cattle pastures. Fire in the Cerrado also occurs by natural causes, such as lightning in the rain season. Casual fires may also occur in the dry season, attaining a maximum in August. At this period most of the epigeous phytomass of the herbaceous/undershrub stratum is dry, and in addition to the senescent leaves from trees and shrubs accumulated on the soil, form an easy and highly combustible material (**Fig. 2**). With the arrival of the wet season in September, sprouting and flowering occur and the incidence of casual fires drops markedly (França *et al*. 2007). One of the most interesting effects of fire in the cerrado is the synchronization of flowering of several herbs (Coutinho 1990), among them *Vernonia herbacea* (**Fig. 3**).

Mantovani and Martins (1988) carried out a phenological study of vascular plants of a preserved cerrado area in the state of São Paulo, at the Reserva Biológica e Estação Experimental de Moji Guaçu (RBEE Moji Guaçu, 22° 35′ S and 47° 44′ W). This study showed that the phenological behavior of the vegetation is related to climate stimuli and is a result of a series of adaptive strategies that optimize pollination and seed dispersal, and minimize thermal, water and nutritional stresses.

The association of the flowering and leaf exchange periods with environmental changes has been mentioned since the early studies of Warming in 1908 and throughout most studies concerning the cerrado vegetation. Monasterio and Sarmiento (1976) observed for the savannas in Venezuela that the seasonal changes affect differently the species and layers of this vegetation. On a later study, those authors pointed out the possibility to establish plant groups based on carbon assimilation, vegetative growth and flowering to describe the phenological behavior of species from the neotropical savannas, among them, the Brazilian cerrados (Sarmiento and Monasterio 1983). Concerning carbon assimilation, the authors defined two groups, i) plants that assimilate carbon continuously and ii) plants that assimilate carbon seasonally. The first group includes plants with continuous growth, producing leaves throughout the year. The second one includes annual or perennial plants with vegetative growth and flowering during the rain period undergoing the dry season restricted to their reserve and propagation structures (underground organs and seeds).

**Fig. 2 (Top row)** Fire in the cerrado (**A**); a cerrado area after a fire event (**B**); recovery of the vegetation after two months (**C**).
**Fig. 3 (Bottom row) Flowering of Asteraceae species in the cerrado, among them *Vernonia herbacea* (right), two months after a burn-off.**

## *Vernonia herbacea*

The Asteraceae are very well represented in the Cerrado and account for 17% of the angiosperm flora in the RBEE Moji-Guaçu (Mantovani and Martins 1988). There, Figueiredo-Ribeiro *et al*. (1986) reported the predominance of herbaceous species with underground reserve organs and later, Tertuliano and Figueiredo-Ribeiro (1993) demonstrated the predominance of inulin-type fructans as reserve carbohydrates in underground organs of 60% of the Asteraceae. The genus *Vernonia* comprises 5% of the Asteraceae species. *Vernonia herbacea* (Vell.) Rusby stands out for accumulating about 80% fructan on a dry mass basis (Carvalho and Dietrich 1993) in the reserve organ, known as rhizophore (Menezes *et al*. 1979; Hayashi and Appezzato-da-Glória 2005).

*V. herbacea* (**Fig. 4**) is a perennial herb that shows a marked phenological behavior consistent with the seasonality of the cerrado vegetation and the climate characteristics of this habitat. The seasonal growth pattern exhibited by these plants involves sprouting of buds from the rhizophores at the end of the dry season followed by a period of rapid aerial growth and flowering. Vegetative growth continues throughout the rain season until early autumn. Growth is then arrested and leaves start to show signs of senescence, leading to shoot abscission. Plants enter dormancy at the beginning of the dry season and undergo this unfavorable period restricted to their rhizophores (Carvalho and Dietrich 1993). These phenological phases are associated to variations in the contents and composition of fructans. Fructans are accumulated mainly during the period of intensive plant growth during summer and early dormancy, at the beginning of autumn, as a result of translocation from the aerial organs prior to senescence. A decrease in fructan contents is observed during sprouting and flowering in spring (Carvalho and Dietrich 1993). Variations in the activities of the fructan metabolizing enzymes in the rhizophores (Asega and Carvalho 2004; Portes and Carvalho 2006) are consistent with these changes and with changes in temperature (Asega *et al*. 2011). Besides their role of reserve, fructans have been recognized as protective compounds against drought, as shown in *Vernonia herbacea*, which undergoes seasonal drought in the Cerrado (Garcia *et al*. 2011).

## Fructan utility

Fructans are more than plant reserve carbohydrates. Since the mid 1930s, fructans have been used in tests for human kidney function and since then the interest in medical uses for inulin-type fructans and inulin derivatives is increasing (Vijn and Smeekens 1999; Roberfroid 2007). Inulin has also been recognized as a beneficial food ingredient, since it is a soluble fiber that cannot be digested by humans. In fact, fructans promote the growth of *Lactobacilli* and *Bifidobacteria* species in the colon and causing the decline of pathogenic bacteria in the intestines (Roberfroid 2005). Small fructans with degree of polymerization (DP) of 3 to 6 are sweet tasting and therefore constitute natural low-caloric sweeteners, while high-DP fructans are being used in alimentary products as substitute for fats, due to their organoleptic properties. High-DP fructans also hold great promise for a variety of nonfood applications (Fuchs 1993). Inulin and other fructans are naturally present in many edible plants, including onion, garlic, leek and artichoke.

Due to the growing interest in these compounds, new methods of producing fructose syrup or fructo-oligosaccharides (FOS) include now the use of microbial Β-fructofuranosidases. Therefore, results obtained in cultures of *Glyocladium virens* (Pessoni *et al*. 2009), a filamentous fungi isolated from the rhizosphere of *V. herbacea* in the Cerrado and capable of producing FOS in sucrose-containing media, indicated that the Brazilian Cerrado represents a profitable environment to search for these microbes.

**Fig. 4 Adult plant of *Vernonia herbacea* showing the inulin accumulating underground organs (rhizophores).**

The inulin found in roots of chicory (*Cichorium intybus*) is the primary fructan currently used as food additive. According to Van den Ende *et al*. (2002), the annual production of inulin from chicory increased from 1,000 to 100,000 tons in the last decade due to the growing interest in this compound by pharmaceutical and food industries.

## *Vernonia herbacea* as a potential source of inulin

On a comparative study to evaluate the rate of glomerular filtration in male Wistar rats, inulin from *V. herbacea* showed to be similar to the commonly used commercial inulin from *Dahlia* (Sigma) (Dias-Tagliacozzo *et al*. 1996). Thus, *V. herbacea* constitutes a potential source for inulin production, although it lacks a history as vegetable crop. Therefore, the industrial use of inulin from this source is still dependent on investigations concerning plant growth in the field and fructan production, as well as its application as food additive and for pharmaceutical purposes. Aiming at the increase in rhizophore biomass and inulin production, several studies (Teixeira *et al*. 1997; Carvalho *et al*. 1998; Cuzzuol *et al*. 2003, 2005, 2008) were conducted focused on mineral nutrition, plant growth and biomass allocation, as discussed later.

## Mineral nutrition and plant growth

Mineral nutrients are key factors that regulate plant growth. Essential inorganic minerals can modify 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). Both wild and cultivated plant species respond similarly to nutrient availability. However, in comparison to plants growing in rich soils, plants of oligotrophic environments exhibit low root absorption rates that can be improved in response to increasing soil fertility (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). Consequently, nitrogen deficiency causes a reduction in total biomass and in the shoot:root ratio (Rufty *et al.* 1984).

Nutrient solutions are widely used by plant physiologists to investigate the mechanisms by which mineral elements control plant growth. Many nutrient solutions have been established according to the plant species and the aim of the study. The most significant differences among the various formulations are due to differences in the concentration of macronutrients. In fact, most of them are modifications of the original and widely used Hoagland nutrient solution, formulated for tomato plants (Hoagland and Arnon 1938). According to these authors, a nutrient composition is not truly better than another, since plants have a high capacity to adapt to different nutritional conditions.

In early studies, the nutrient solutions were established according to the chemical composition of the soil in which plants were successfully cultivated. More recently, the chemical composition of the leaves is considered, since these are the organs that better reflect the nutritional status of the plant (Parker and Norvell 1999). The most significant differences between these two types of solutions lay on the concentrations of N, P and K, higher in the solutions based on the leaf chemical composition.

In oligotrophic environments, such as “restinga” and cerrado, wild plants accumulate fewer nutrients in comparison to crops, reflecting the nutritional condition of the soil. Few species from the cerrado have been investigated with this purpose. Studies on tree species showed that the addition of 100% Hoagland solution was not effective on the early growth and could lead to leaf abscission or early leaf senescence as reported for *Dalbergia miscolobium*. When 50% of the ionic strength was used, a better growth response was observed (Sassaki and Felippe 1980). In *Qualea grandiflora*, another tree species from the cerrado, the best growth response of all vegetative organs and of the root: shoot ratio was reached when Hoagland solution was diluted 10 times (Paulilo and Felippe 1995).

In relation to herbaceous species from the cerrado, it was shown that the addition of nutrient solution, in general promoted shoot growth. In *Bidens gardneri* (Asteraceae), for example, growth was directly proportional to the ionic strength of the nutrient solution applied (Klein *et al.* 1996). In *V. herbacea* the use of complete Hoagland’s solution promoted shoot growth but not rhizophore biomass (Teixeira *et al.* 1997). Since the interest in this species lies on the rhizophore, studies were conducted to establish a nutrient solution to improve rhizophore growth and inulin production. Compared to Hoagland, the *Vernonia* solution, formulated based on the nutrient contents of the leaves, contains less macronutrients (**Table 1**). Different ionic strengths were assayed and the 50% *Vernonia* solution was the most effective for rhizophore biomass increase and fructan production (Cuzzuol *et al.* 2005a), confirming that plants adapted to the oligotrophic soils of the cerrado demand less mineral nutrients to achieve better growth and productivity.

Treatments that increase sink strength towards fructan accumulating organs are useful, if one considers inulin industrial application. Sink strength is genetically determined, although it can be affected by environmental changes (Pollock 1986). Among the external factors already studied, change in nitrogen supply is the most recommended strategy to modify sink strength (Farrar 1993; Améziane *et al.* 1995, 1997). This was clearly shown for *Hordeum vulgare* in which low nitrogen supply caused an increase in source and sink strength (Wang and Tillberg 1997) and for *Cichorium intybus* (Améziane *et al.* 1997). In this latter species an increase in fructan content in tuberous roots also occurred under low nitrogen (Van den Ende *et al.* 1999).

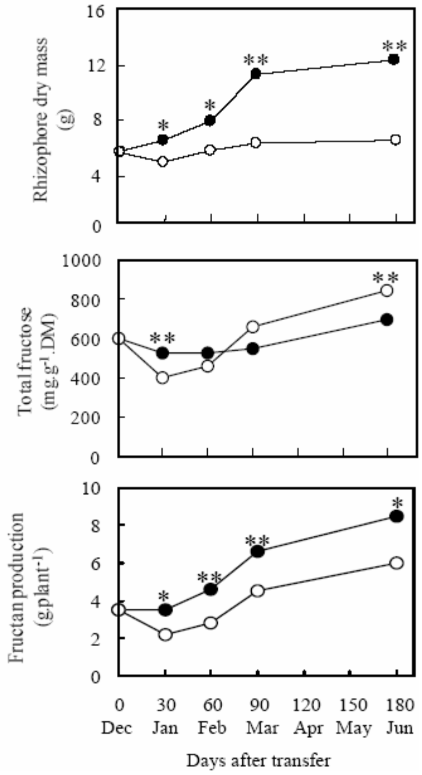

**Fig. 5 Rhizophore dry mass, total fructose and fructan production in plants of *Vernonia herbacea* transferred from N-sufficient (●) to N-limited (○) solution.** Modified from Cuzzuol *et al.* (2008).

In the case of *V. herbacea*, the analysis of the chemical composition of leaves provided information regarding plant nitrogen requirement (Cuzzuol *et al.* 2005a) and the basis for studies on the effects of low nitrogen on growth, biomass allocation and fructan production. Nitrogen in the *Vernonia* nutrient solution is supplied as nitrate. When other nitrogen sources, ammonium and a mixture of nitrate and ammonium, were used in the same final concentration of 10 mM nitrogen, no differences in growth and productivity were observed (Carvalho *et al* 2006). Plants of *V. herbacea* growing for one year under a limited nitrogen supply presented reduced growth and higher fructan content than plants treated with sufficient nitrogen. However, the total fructan production was similar in both plant groups due to the higher rhizophore biomass in N-sufficient plants (Cuzzuol *et al* 2005b). When plants were cultivated for one year under N-sufficient supply and subsequently transferred to N-limited condition for six months, fructan concentration was significantly higher and growth was reduced in comparison to plants receiving N-sufficient solution throughout, confirming the inverse relationship between nitrogen concentration and fructan content (**Fig. 5**). Nevertheless, the higher gain in rhizophore biomass after 18 months of cultivation in N-sufficient solution led to a fructan production of 8.3 g plant$^{-1}$, surpassing in 38% the 6.0 g plant$^{-1}$ fructan production of the N-limited plants (Cuzzuol *et al* 2008).

## Field experiments

In addition to the studies performed in glasshouse conditions, field trials were conducted nearby a preserved area of cerrado where *V. herbacea* grows naturally aiming to evaluate the potential of this species as inulin-producing crop in

**Table 1** Concentration of macro and micronutrient in Vernonia and Hoagland solution.

| Nutrient solution | N-$NO_3^-$ | P-$H_2PO_4^-$ | $K^+$ | $Ca^{+2}$ | $Mg^{+2}$ | S-$SO_4$ | B | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | mmol·L$^{-1}$ | | | | | µmol·L$^{-1}$ | | |
| Vernonia | 10.7 | 0.5 | 2.6 | 2.2 | 1.7 | 1.3 | 46 | 0.03 | 90 | 12.6 | 1.3 |
| Hoagland | 15.0 | 1.0 | 6.0 | 5.0 | 2.0 | 2.0 | 46 | 0.03 | 90 | 12.6 | 1.3 |

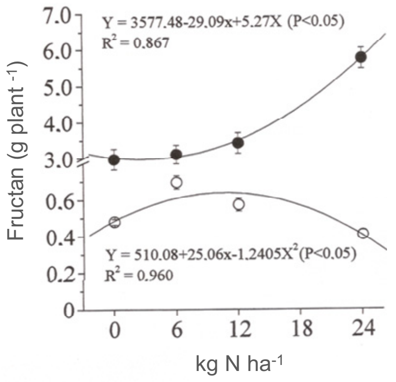

**Fig. 6 Effect of different nitrogen concentrations on fructan production of *Vernonia herbacea* cultivated in a cerrado area for 6 (○) and 12 (●) months.** Values are means of six replicates ± s.e. Modified from Cuzzuol *et al.* (2003).

alternative to the widely used *Cichorium intybus*, *Helianthus tuberosus* and *Dahlia* sp. (Franck and De Leenheer 2005). The combined effect of mineral fertilization and period of cultivation on the production of inulin was evaluated in plants of *V. herbacea*. At six months of cultivation, a high correlation between shoot growth and nitrogen concentration (up to 24 kg N ha$^{-1}$) was detected, although fructan concentration in the rhizophores declined (650 mg g$^{-1}$ DM in the control plants and 330 mg g$^{-1}$ DM in plants under 24 kg N ha$^{-1}$). At twelve months growth was stimulated in plants receiving between 6 and 12 kg N ha$^{-1}$ while fructan production was promoted only in plants treated with 24 kg N ha$^{-1}$. These plants produced 70% more fructan when compared to control plants (**Fig. 6**), and an estimated fructan yield of 0.5 ton ha$^{-1}$ (Cuzzuol *et al.* 2003). This yield obtained after one year was similar to the two year production observed in a previous study (0.522 ton ha$^{-1}$) (Carvalho *et al.* 1998). Thus, in *V. herbacea*, for a higher fructan yield, the application of 24 kg N ha$^{-1}$ and twelve months of cultivation are recommended.

## PROSPECTIVES AND CONCLUDING REMARKS

The high biodiversity of the cerrado vegetation includes a number of fructan-producing species that represent alternatives to the current inulin-producing crops. Due partly to the presence of fructans, these species are well adapted to the environmental factors prevailing in the cerrado, among them, drought and oligotrophic soils, usually adverse to crops. Fructans are reported to confer tolerance against drought, low temperatures, and low nutrient availability through osmotic regulation and/or membrane protection (Hincha *et al.* 2003). Increasing atmospheric [$CO_2$] can be another stressing factor with consequences on natural and agricultural ecosystems (e.g. Bazzaz 1990; Drake *et al.* 1997; Ainsworth and Long 2005). More recently, the effects of [$CO_2$] on growth and inulin production in *V. herbacea* were analyzed in plants growing in open top chambers (OTC) (Oliveira *et al.* 2010). In plants submitted to elevated $CO_2$ concentration for four months, increases in photosynthesis led to a higher rhizophore biomass and a promotion in fructan productivity (**Fig. 7**). There is a long distance between experiments conducted in OTCs and what is observed in field conditions or in nature. Nevertheless, the experiment here reported indicate that plants of *V. herbacea* can benefit from elevated atmospheric $CO_2$ by increasing growth and carbon allocation for the production of inulin and may contribute to predict a future scenario for the impact of this atmospheric condition on the herbaceous vegetation of the cerrado.

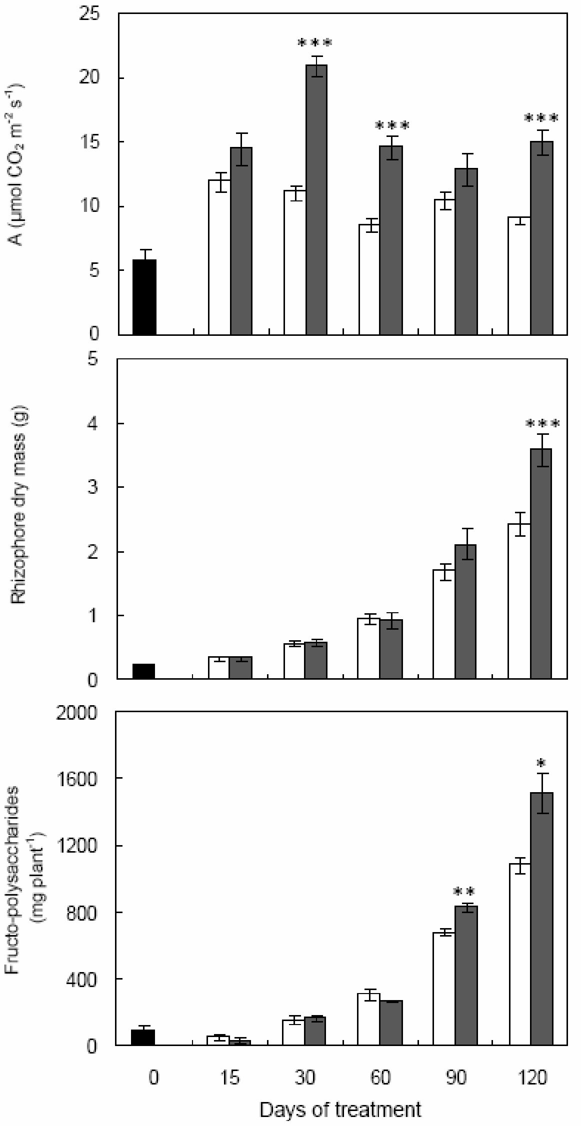

**Fig. 7** Photosynthesis (**A**), rhizophore dry mass and fructan production in plants of *Vernonia herbacea* submitted to 380 μmol. mol$^{-1}$ (open square) and 720 μmol. mol$^{-1}$ (gray square) $CO_2$ for 0 (black square), 15, 30, 60, 90 and 120 days. Values are means ± s.e. (n=3). * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Modified from Oliveira *et al.* (2010).

The information accumulated on various physiological and biochemical aspects of *V. herbacea* points to recognize this species as a model system for studies concerning plant growth, fructan metabolism and inulin productivity, as well as how these are affected by environmental factors. Under this perspective, studies on fructan metabolism focusing on the interactions between drought and high [$CO_2$] and in tissue culture are underway and should contribute to the knowledge of the role of fructans in plants other than reserve compounds.

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