Seasonal variation of soluble carbohydrates and starch in *Echinolaena inflexa*, a native grass species from the Brazilian savanna, and in the invasive grass *Melinis minutiflora*

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(With 8 figures)

Abstract

Echinolaena inflexa (Poir.) Chase is an abundant C3 grass species with high biomass production in the Brazilian savanna (cerrado); *Melinis minutiflora* Beauv. is an African C4 forage grass widespread in cerrado and probably displacing some native herbaceous species. In the present work, we analysed seasonally the content and composition of soluble carbohydrates, the starch amounts and the above-ground biomass (phytomass) of *E. inflexa* and *M. minutiflora* plants harvested in two transects at 5 and 130 m from the border in a restrict area of cerrado at the Biological Reserve and Experimental Station of Mogi-Guaçu (SP, Brazil). Results showed that water soluble carbohydrates and starch amounts from the shoots of both species varied according to the time of the year, whilst in the underground organs, variations were observed mainly in relation to the transects. Marked differences in the pattern of the above-ground biomass production between these two grasses relative to their location in the Reserve were also observed, with two peaks of the invasive species (July and January) at the Reserve border. The differences in carbohydrate accumulation, partitioning and composition of individual sugars concerning time of the year and location in the Reserve were more related to the annual growth cycle of both grasses and possibly to specific physiological responses of *M. minutiflora* to disturbed environments in the Reserve border.

Keywords: biological invasion, cerrado, starch, sugars, tropical grasses.

Variações sazonais de carboidratos solúveis e amido em *Echinolaena* inflexa, uma espécie nativa do cerrado, e na gramínea invasora *Melinis minutiflora*

Resumo

Echinolaena inflexa (Poir.) Chase é uma gramínea C3 muito abundante em áreas de cerrado e com alta produção de biomassa. *Melinis minutiflora* Beauv. é uma gramínea C4 de origem africana introduzida no Brasil para fins forrageiros, que se espalhou amplamente por áreas de cerrado, provavelmente deslocando espécies nativas. No presente trabalho, o conteúdo e a composição de carboidratos solúveis, o teor de amido e a biomassa aérea foram analisados sazonalmente em plantas de *E. inflexa* e *M. minutiflora* coletadas em dois transectos, a 5 e 130 m da borda da Reserva Biológica e Estação Experimental de Mogi-Guaçu, uma área restrita de cerrado (SP, Brasil). Os resultados mostraram que os carboidratos solúveis e o conteúdo de amido da parte aérea de ambas as espécies variaram de acordo com a época do ano, enquanto que nos órgãos subterrâneos as variações foram observadas principalmente em relação aos transectos. Diferenças marcantes no padrão de distribuição de biomassa aérea das duas espécies em relação à localização na reserva também foram observadas, com dois picos de produção da espécie invasora (em julho e janeiro) na borda da reserva. As diferenças observadas no acúmulo de carboidratos, partição e composição dos açúcares em relação à época do ano e localização na reserva foram mais relacionadas ao ciclo anual de crescimento de ambas as espécies e possivelmente a respostas fisiológicas específicas de *M. minutiflora* a ambientes perturbados na borda da reserva.

Palavras-chave: invasão biológica, cerrado, amido, açúcares, gramíneas tropicais.

1. Introduction

Biological invasion is a serious threat to the biodiversity of natural ecosystems (Vitousek, 1990). Invasion by alien grasses has been shown to modify the physiognomy and structure of neotropical savannas, not only by excluding native species, but also increasing fire frequency and intensity, thereby modifying the ecosystem as a whole (Humphrey and Schupp, 2004; D'Antonio and Vitousek, 1992; Hoffman et al., 2004; Pivello et al., 1999a). Perennial African C4 grasses are very successful invaders in savanna areas, as they have traits that give them a strong competitive advantage compared to the native species, such as tolerance to grazing, fire and low soil nutrient availability (D'Antonio and Vitousek, 1992; Williams and Baruch, 2000; Rosas et al., 2006).

In Brazil, several exotic grass species were introduced as cattle forage and spread widely in open areas of a savanna-like vegetation (cerrado). Among them is the perennial *Melinis minutiflora* Beauv. (Molasses grass), which is native to Africa but nowadays is widespread throughout the tropics (Baruch and Gomez, 1996; D'Antonio and Vitousek, 1992; Hoffmann et al., 2004). This species, as well as other African grasses, holds several traits such as high seed production, efficient vegetative growth and investment in shoot production (Filgueiras, 1990; Klink and Joly, 1989; Simões and Baruch, 1991) that allow them to form dense mats that exclude many other herbaceous native species (Pivello et al., 1999b). Additionally, C4 photosynthetic pathway frequently found in African species may increase their efficiency over the C3 native grasses in occupying open and sunny environments (Klink and Joly, 1989).

Despite the ability of *M. minutiflora* to outcompete native grasses, Pivello et al. (1999a) observed that in the Emas Biological Reserve (SP) the native C3 grass *Echinolaena inflexa* (Poir.) Chase showed phytosociological patterns (absolute density, frequency, vigor) and distribution very similar to those observed in *M. minutiflora*. High values of above-ground biomass and density of both species indicated that the invasive grass was not displacing the native one. The great ability of both species to produce biomass and to grow vegetatively could explain their high density (Klink, 1996; Filgueiras, 2002), which could be directly related to photosynthetic rate and carbohydrate partitioning. As shown for temperate forage grasses, the accumulation of non-structural carbohydrates (NSC) has been considered an efficient strategy to allow growth and survival in periods when photosynthesis becomes reduced (Kingston-Smith et al., 1998).

In spite of differences concerning origin and photosynthetic metabolism, previous studies showed similar composition of individual sugars in vegetative tissues of *M. minutiflora* and *E. inflexa* (Moraes et al., 2002) and suggested they have similar metabolic strategies to overcome the stressing conditions of the cerrado. The diurnal pattern of NSC in both species (Souza et al., 2005) was attributed to possible differences of starch metabolism, the main carbon reserve of tropical grasses (Chatterton et al., 1989). Furthermore, although previous studies demonstrated co-existence of *E. inflexa* and *M. minutiflora* in different cerrado fragments, the invasive species seems to be present in higher density in border areas, which could also be related with particularities concerning carbohydrate metabolism.

Therefore, in the present work, we investigate the contents of water soluble carbohydrate and starch in vegetative organs of *M. minutiflora* and *E. inflexa* harvested from two transects in a restrict cerrado fragment, representing the border and the internal parts of this site, aiming at to correlate carbohydrate accumulation, phytomass production and the distribution of both species in the cerrado.

2. Material and Methods

2.1. Plant material and study area

Vegetative tissues (leaves and culms) and underground organs of *M. minutiflora* and *E. inflexa* were harvested from plants growing in a Brazilian savanna area located in the Biological Reserve of Mogi-Guaçu (22° 15'-22° 16' S and 47° 08'-47° 12' W, Mogi-Guaçu, SP, Brazil) in April, July and October/2001 and January/2002. Plants were harvested in two transects, each 300 m long, one at 5 m (named external transect - ExT) and the other at 130 m (named internal transect - InT) from the Reserve border. Along each transect, 1 × 1 m² plots were randomly selected and plant material for carbohydrate analysis was collected from 6 plots. Above-ground material was also randomly harvested in 10 other plots and measured for total biomass (phytomass) determination at each harvesting time.

2.2. Extraction and analyses of Water Soluble Carbohydrates (WSC)

After harvesting, underground organs (roots for *M. minutiflora* and roots and rhizomes for *E. inflexa*) and aerial vegetative tissues (excluding reproductive structures) were separated. Fresh weight (FW) of each tissue fraction was determined in the field and coarsely chopped. Each fraction from individual plants was thoroughly mixed, and three sub-samples were taken for carbohydrate analyses. WSC was extracted from fresh tissues in boiling 80% ethanol followed by hot water (Pollock and Jones, 1979). The aqueous and ethanolic extracts were pooled and concentrated in a rotary evaporator. Total sugars were determined by the phenol-sulphuric acid procedure (Dubois et al., 1956), using glucose as standard. Aliquots of carbohydrate extracts were deionized using Sep-Pack Accell Plus QMA and CM cartridges, and hydrophobic compounds were removed using Sep-Pack tC$_{18}$ cartridges. All cartridges were supplied by Waters Chromatography Division of Millipore Co. (USA).

Oligosaccharides were analysed by high performance anion exchange chromatography with pulsed amperomet-

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ric detection (HPAEC-PAD), on a Dionex DX-300, in a CarboPac PA-1 (4 × 250 mm), using a linear gradient of sodium acetate in sodium hydroxide (150 mM), according to Itaya et al. (1997). The elution program consisted of a sodium hydroxide gradient (1 cm^3/min) of 12 mM (0-2 minutes), 24 mM (2-5 minutes), 44 mM (5-9 minutes), 54 mM (9-13 minutes), 62 mM (13-16 minutes), 66 mM (16-18), 96 mM (18-25 minutes), 120 mM (25-30), 140 mM (35-40 minutes) and 12 mM (41 minutes). Quantification of individual sugars was performed according to Timmermans et al. (1994), by the external standard method using authentic standards (Sigma).

2.3. Determination of starch content

Dried samples of aerial and underground organs were ground to a fine powder in a ball mill for starch analysis and performed according to the method proposed by Arêas and Lajolo (1980), modified as follows: 10 mg of ground material was weighed in Eppendorf tube and submitted to extraction with 80% ethanol (v.v^{-1}) at 80 °C. After centrifugation at 13,000 g for 5 minutes, the supernatant was discarded. This procedure was repeated twice. The residue was solubilised in 1.5 mL of 50 mM 3-[N-morpholin] propan-sulfonic acid buffer (MOPS), pH 7.0, at 30 °C for 1 hour. To initiate the digestion, 28 U.mL^{-1} of α-amylase (Megazyme) in 50 mM 3-[N-morpholin] propan-sulfonic acid buffer (MOPS), pH 7.0, were added and the extract was incubated for 1 hour at 70 °C. After incubation, 1 mL of 300 mM sodium acetate buffer, pH 4.8, was added, and a new incubation was performed with 28 U.mL^{-1} of amyloglucosidase (Megazyme) in 100 mM sodium acetate buffer, pH 4.8, for 30 minutes at 50 °C. The reaction was stopped by the addition of 10% 0.8 M perchloric acid. Free glucose content was determined using a glucose oxidase/peroxidase (GOD/POD) "kit" appropriated for Elisa (Centerlab). An aliquot of the mixture was transferred to a tube and 150 μL of glucose oxidase was added. After incubation for 15 minutes at 30 °C, free glucose was quantified by spectrophotometry at 490 nm.

2.4. Statistics

A Tukey multiple comparison test (with rejection level > 5%) was applied on mean values of carbohydrate contents. To examine the patterns of carbohydrate composition changes in space and time the data matrix was explored through a Principal Components Analysis (PCA). The PCA was based on the correlation matrix obtained from the original data matrix of carbohydrate composition and total biomass in each location and time. The original values were standardised by ranging. The PC-ORD version 3.0 package for Windows (McCune and Mefford, 1997) was used for the analysis.

A multivariate linear model analysis was also performed to verify if phytomass variation in plants from both transects could be predicted by the accumulation of carbohydrates. The data were analysed by means of a stepwise method, adopting phytomass as a dependent

variable and the accumulation of WSC, starch, glucose, fructose and sucrose as independent variables. The adjustment procedure started from the saturated model (with all the variables present); variables which had lower influence to explain phytomass variation (greatest value of p) were successively removed. At each step, the adjustment and the variable significance were evaluated; only those that significantly contributed to explain the variation remained.

3. Results

Meteorological data for the period of this study were sourced from a weather station located at the Biological Reserve. Monthly mean values of maximum, medium and minimum temperatures and total precipitation, relative air humidity and total radiation are shown in Figure 1. Precipitation was low (less than 80 mm) from April to August/2001, with the lowest value in June (5.5 mm) and high in March, November and December (Figure 1a). Temperature decreased from March to July/2001, with a minimum value of 7.5 °C in July. Temperatures started to increase in August and the highest mean values were recorded in January/2002 (Figure 1a). The lowest values of total radiation occurred from May to September/2001, and the highest ones from October/2001 to January/2002 (Figure 1b). Monthly fluctuations in the meteorological
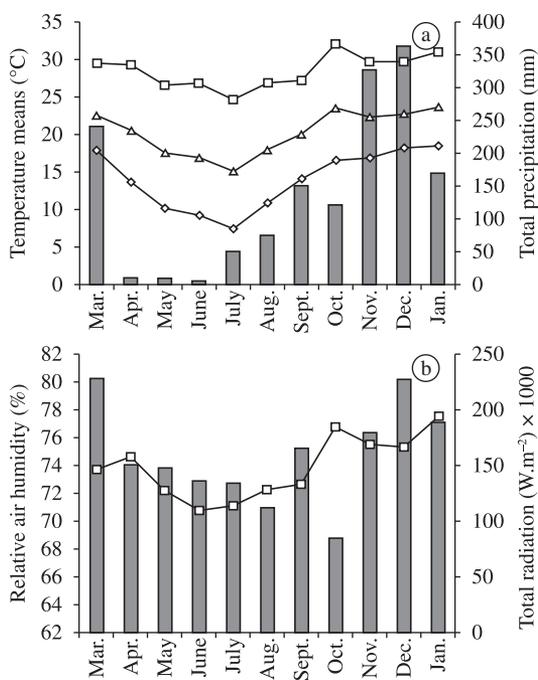
Figure 1. Meteorological data of the Mogi-Guaçu Biological Reserve (SP, Brazil) during the study period (March/2001 to January/2002). a) monthly average values for maximum (□), mean (Δ) and minimum (◊) temperature and total precipitation (bars); b) relative air humidity (bars) and total radiation (□).

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data are typical for a subtropical region, with low levels of precipitation and radiation in April, May, June and July (part of autumn and winter seasons), and high values in November, December and January (part of spring and summer seasons).

Therefore, carbohydrate data evaluated in April and July, corresponded to the driest period, and in October and January, corresponded to wet seasons in this region. Water soluble carbohydrate (WSC) contents in the aerial tissues of *E. inflexa* and *M. minutiflora* harvested in the internal (InT) and external (ExT) transects showed not to be significantly affected by the location in the Biological Reserve (Table 1). However, significant differences were observed among the analysed periods (Figure 2). In the native grass higher values were found in July, while in the invasive species *M. minutiflora* the highest WSC content was observed in April, followed by a decline in July.

With respect to the underground organs, both species showed high levels of WSC in July (Figure 3), except *M. minutiflora* in the internal transect, presenting the highest WSC content in April, similarly to what was observed for the shoots (Figure 2).

The main components of the WSC fraction in the shoots of both species were glucose, fructose and sucrose. Glucose (ranging from 0.06 to 4.62 mg.g^{-1}DW) was the major sugar in the shoots of *E. inflexa* and *M. minutiflora* in the four analysed periods, followed by fructose (ranging from 0.01 to 1.55 mg.g^{-1}DW) and sucrose (ranging from 0.04 to 1.31 mg.g^{-1}DW) - (Figure 4). In spite of the great variation observed in the concentrations of all these sugars in aerial tissues of both species, it seemed to be less affected by the location in the Reserve in the native species *E. inflexa* (Figure 4a, C, E). In contrast, glucose concentration in the alien *M. minutiflora* was significantly different in both transects, especially in July and October (Figure 4b).

In the underground organs of both species sucrose was the main sugar, ranging from 0.33 to 3.33 mg.g^{-1}DW in plants from the internal and external transects; the higher sucrose contents were observed in April and July (Figure 5).

Starch was present in much higher amounts than glucose, fructose and sucrose, ranging from 11 to 55 mg.g^{-1} DW in the shoots of both species during the different analysed periods (Figure 6). In the native grass *E. inflexa* starch content was higher in the shoots in April
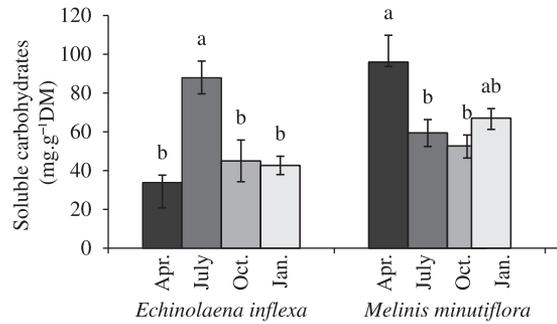

Figure 2. Soluble carbohydrate content (mg.g^{-1}DM) in the shoots of *Echinolaena inflexa* and *Melinis minutiflora* plants growing in the Mogi-Guaçu Biological Reserve (SP, Brazil) in April, July, October and January. Values indicated by different letters are significantly different (P < 0.05).
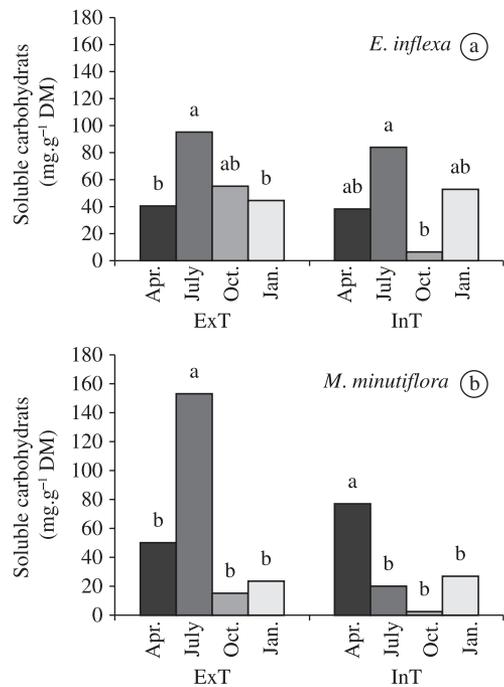
Figure 3. Water soluble carbohydrate content (mg.g^{-1}DM) in the roots of *Echinolaena inflexa* and *Melinis minutiflora* harvested in the internal (InT) and external (ExT) transects at Mogi-Guaçu Biological Reserve (SP, Brazil) in April, July, October and January. Values indicated by different letters are significantly different (P < 0.05).

Table 1. Water soluble carbohydrate (WSC) content (mg.g^{-1}DM) in the shoots of *Echinolaena inflexa* and *Melinis minutiflora* growing in Mogi-Guaçu Biological Reserve (SP, Brazil) during the seasons of the year. ExT – external transect; InT – internal transect. Data are expressed as mean ± SE (n = 6). Letters compare means between transects for each species. Values followed by the same letter do not differ significantly (P < 0.05).

		WSC (mg.g^{-1}DM)			
		April	**July**	**October**	**January**
E. inflexa	Ext	74.9 ± 6.3[a]	84.0 ± 13.9[a]	54.8 ± 20.1[a]	33.8 ± 7.1[a]
	InT	37.8 ± 5.2[a]	91.7 ± 8.3[a]	34.9 ± 6.9[a]	51.4 ± 3.6[a]
M. minutiflora	Ext	29.0 ± 9.8[a]	61.9 ± 13.1[a]	53.2 ± 8.1[a]	68.1 ± 8.0[a]
	InT	116.2 ± 24.5[a]	56.6 ± 7.3[a]	51.6 ± 9.7[a]	79.9 ± 7.2[a]

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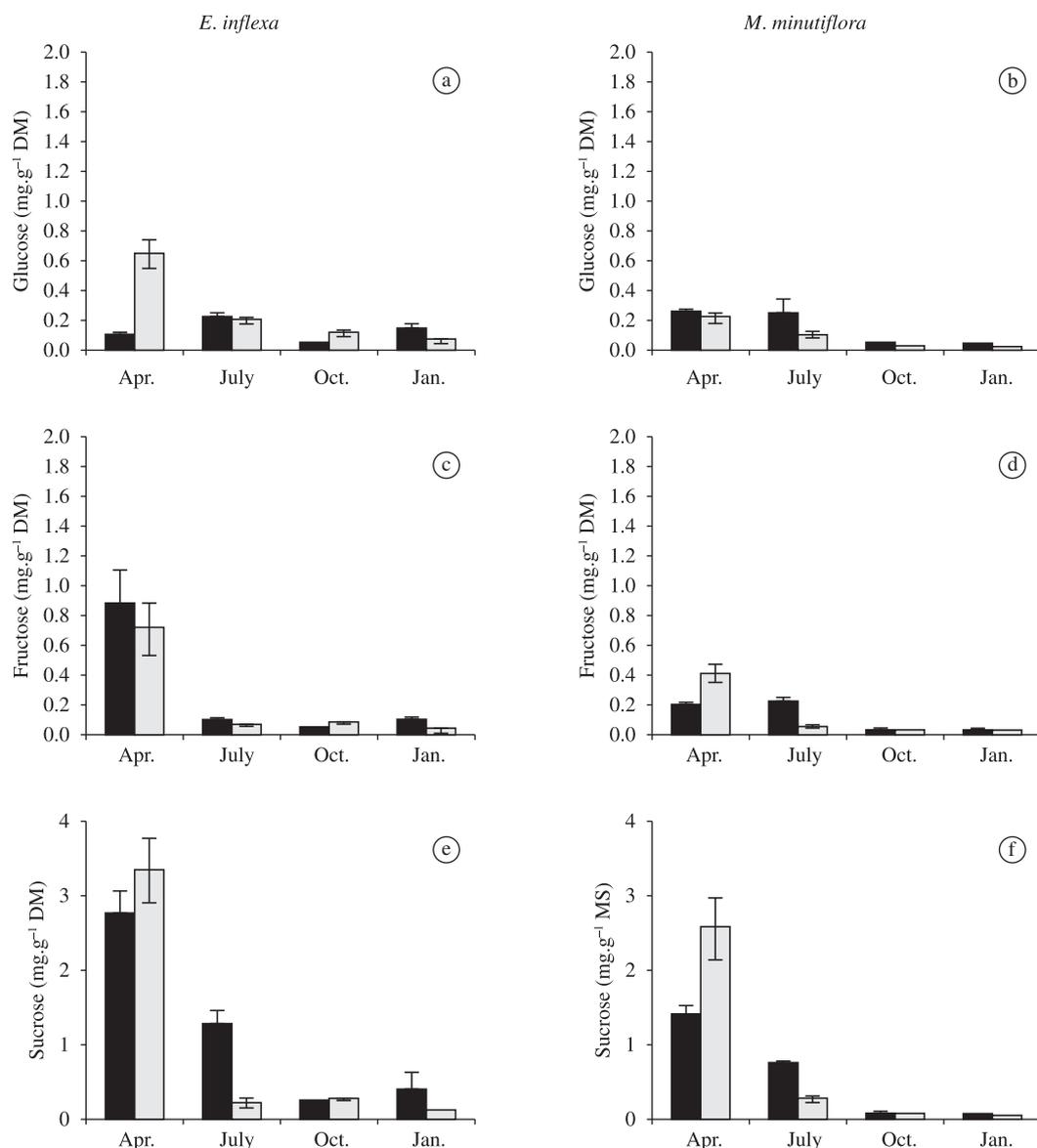*Braz. J. Biol., 2010, vol. 70, no. 2, p. 395-404*
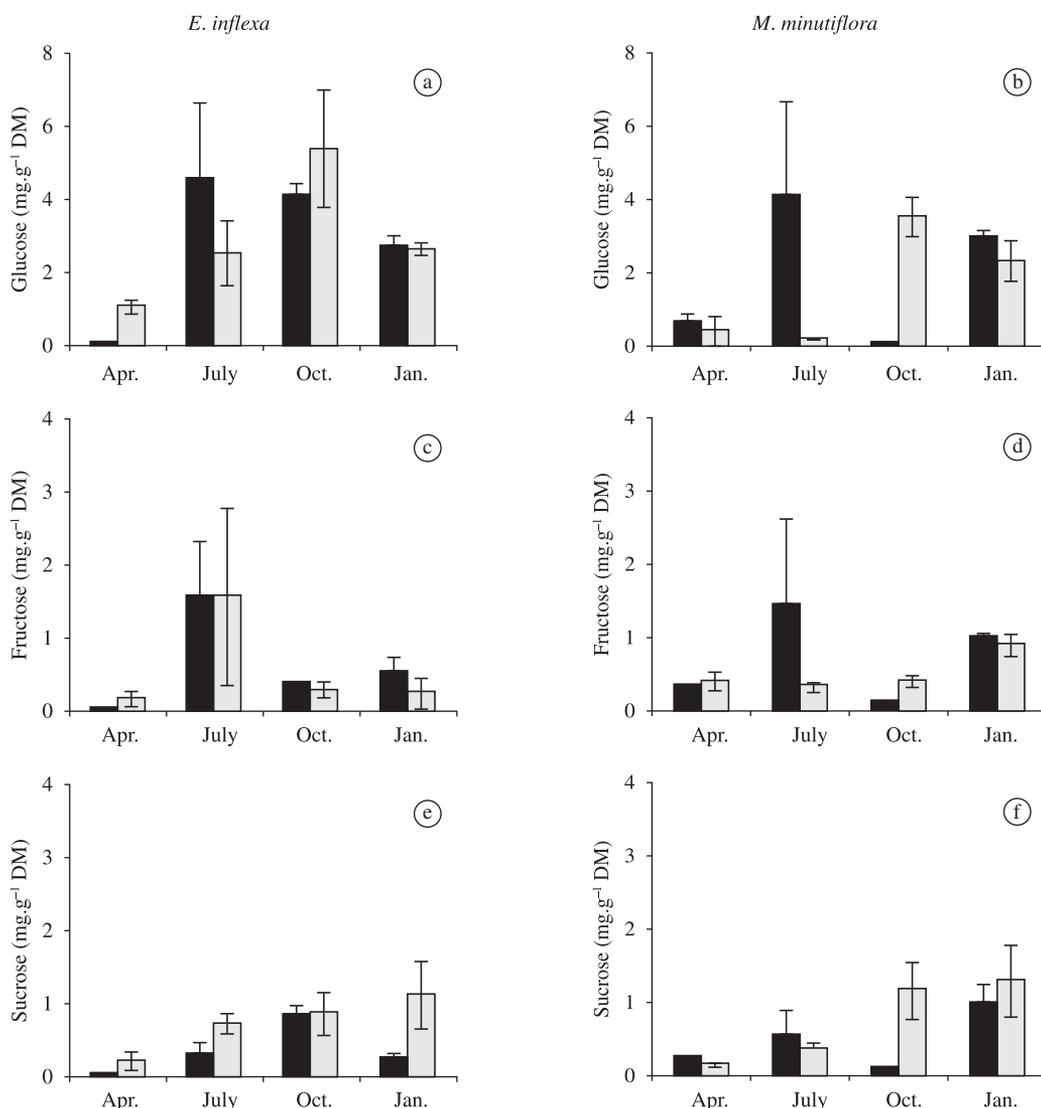
Figure 4. Contents of glucose (a, b), fructose (c, d) and sucrose (e, f) in the shoots of *Echinolaena inflexa* and *Melinis minutiflora* harvested in the external (black bars) and internal (gray bars) transects at Mogi-Guaçu Biological Reserve (SP, Brazil). Bars indicate standard error.

and July (Figure 6a), and the distribution pattern was similar in both transects. In *M. minutiflora,* the pattern of starch variation was similar to that found in *E. inflexa* in ExT, but in InT higher amounts were observed in October and January (Figure 6b). In general, the starch amounts in the underground organs of both species were lower than those of the shoots throughout the analysed period (Figure 6c, d), except in October for *E. inflexa* at the internal transect.

Differences in the above-ground production of phytomass by the native and the invasive species were observed in both transects (Figure 7). Near the Reserve border (ExT) (Figure 7a), *E. inflexa* was not found in July, coinciding with the remarkable phytomass production by *M. minutiflora*. This last species also presented

a peak of phytomass production in January. In the internal transect (InT - Figure 7b), both species were present throughout the analysed period, *E. inflexa* predominating in April. However, in contrast with ExT, the invasive species *M. minutiflora* did not show peaks of phytomass production in the internal transect.

The pattern of carbohydrate (WSC and starch from shoots and underground organs) concentrations related to species, time and space was explored through the interpolation of the two first axes resulting from the Principal Component Analysis (Figure 8). The two first axes explained 78% of the data variance corresponding to 58% and 20% in the first and in the second axis, respectively. The major contribution to the first component was aerial phytomass ($r = –0.999$), while precipitation
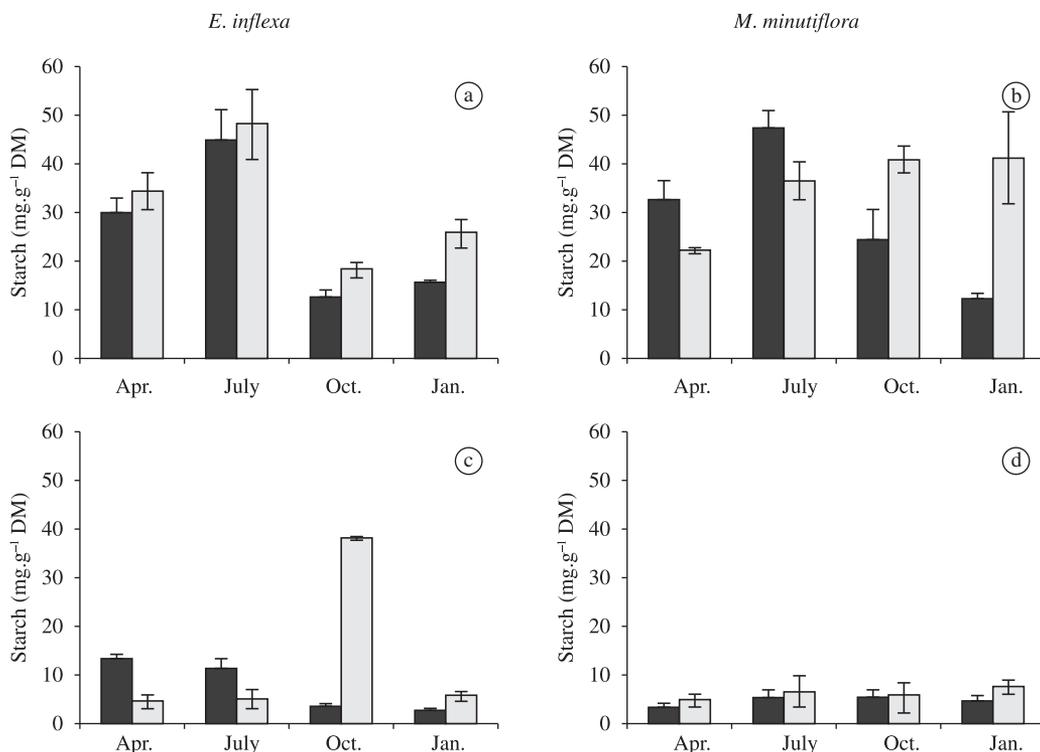
Figure 5. Contents of glucose (a, b), fructose (c, d) and sucrose (e, f) in the roots of *Echinolaena inflexa* and *Melinis minutiflora* harvested in the external (black bars) and internal (gray bars) transects at Mogi-Guaçu Biological Reserve (SP, Brazil). Bars indicate standard error.

(r = –0.979) was the variable that most contributed to the second component (Table 2). Axis 1 seems to reflected a separation between dry (April and July) and wet (October and January) periods, with most of January and October sample units placed at the positive side of the axis, and July and April sample units at the negative side. Sample units of wet months were associated with starch contents and relative air humidity, while sample units of the dry months were related to total WSC, radiation and precipitation levels.

Multivariate stepwise analysis showed that 77% of the variability in the phytomass production by *E. inflexa* located in ExT could be significantly pre-

dicted by a linear combination of WSC and starch accumulation in shoots (Table 3). Results showed that the higher the relative amounts of soluble carbohydrates and starch, the lower was the phytomass. The analysis also showed that 57% of the variability of *E. inflexa* phytomass located in the internal transect could be significantly predicted by lower levels of glucose. Analysis involving *M. minutiflora* showed that 82% and 63% of the variability of phytomass production by this species, respectively in ExT and InT, could be significantly predicted by higher contents of glucose and lower contents of fructose.

E. inflexa

M. minutiflora

Figure 6. Contents of starch (mg.g⁻¹DM) in the shoots (a, b) and roots (c, d) of *Echinolaena inflexa* and *Melinis minutiflora* harvested in the external (black bars) and internal (gray bars) transects at Mogi-Guaçu Biological Reserve (SP, Brazil) in different periods of the year. Bars indicate standard error.
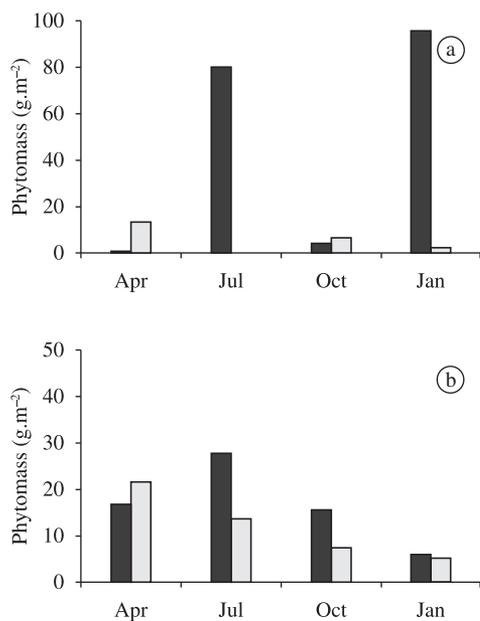

Figure 7. Above-ground phytomass production by *Echinolaena inflexa* (gray bars) and *Melinis minutiflora* (dark bars) in the external (a) and internal (b) transects at Mogi-Guaçu Biological Reserve (SP, Brazil) during the study period.

Table 2. Contribution of the six analysed variables to the first and second axes of a principal component analysis (PCA) considering carbohydrate content and phytomass production by *Echinolaena inflexa* and in two different transects at Mogi-Guaçu Biological Reserve (SP, Brazil). WSC - Water soluble carbohydrates.

Variables	Principal components	
	Axis 1	Axis 2
Phytomass	–0.370	0.111
WSC	–0.390	–0.525
Starch	–0.474	**–0.693**
Glucose	**–0.728**	0.506
Fructose	**–0.871**	–0.349
Sucrose	–0.639	0.579
Variance explained	39%	26%

4. Discussion

The content of the WSC from the shoots of the native *E. inflexa* and the invasive *M. minutiflora* varied significantly according to the period of the year (Figure 2), but was not related with the location at the Biological Reserve, indicating that WSC from the aerial tissues
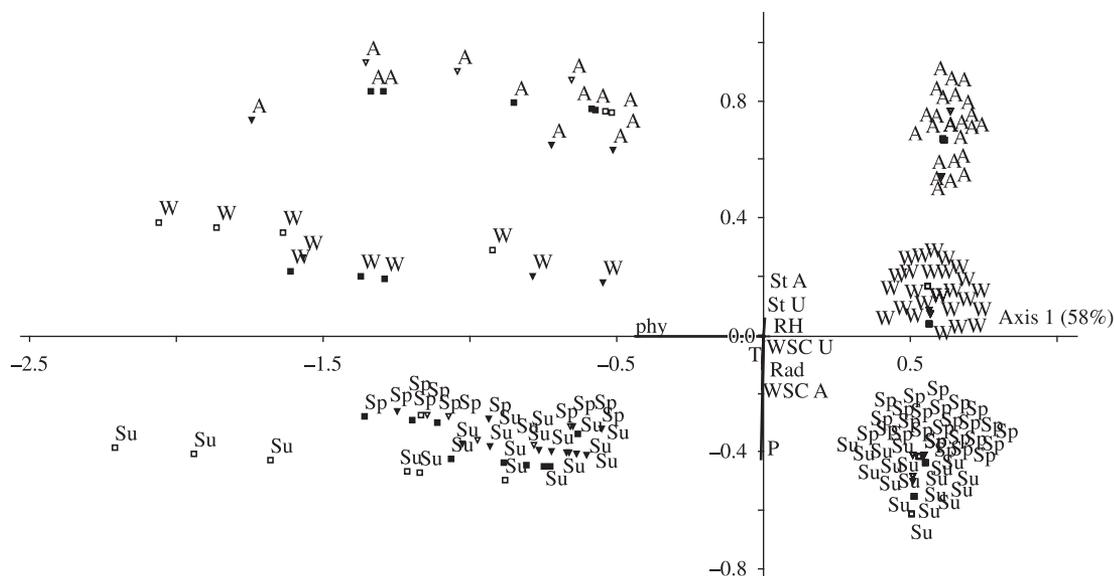
Figure 8. Interpolation of the eigen values obtained for the two first axes resulting from the PCA analysis on the matrix of carbohydrate values of *Melinis minutiflora* and *Echinolaena inflexa* in the external and internal transects at Mogi-Guaçu Biological Reserve (SP, Brazil) in April, July, October and January. (open bars) *M. minutiflora,* ExT; (closed bars) *M. minutiflora*, InT; (open triangles) *E. inflexa*, ExT; (closed triangles), *E. inflexa*, InT. Abbreviations: Phy = phytomass; St A = starch from aerial organs; St U = starch from underground organs; WSC A = water soluble carbohydrates from aerial organs; WSC U = water soluble carbohydrates from underground organs; Rad = radiation; RH = relative air humidity; W = winter; A = autumn; Sp = spring; Su = summer.

Table 3. Results from the predictive linear model of phytomass by *E. inflexa* and *M. minutiflora* growing in two different transects at Mogi-Guaçu Biological Reserve (SP, Brazil). ExT - external transect; InT - internal transect; WSC - Water soluble carbohydrates from shoots.

Species	Transect	Variables	Coefficient	P
E. inflexa ($\log_{10}$)	ExT	WSC	−0.02	0.015
	Constant = 2.79	Starch	−0.05	0.013
	R^2 = 0.77 (p = 0.001)			
	InT	Glucose	−0.26	0.005
	Constant = 1.32			
	R^2 = 0.57 (p = 0.005)			
M. minutiflora ($\log_{10}$)	ExT	Glucose	1.21	0.000
	Constant = 0.66	Fructose	−2.62	0.001
	R^2 = 0.82 (p < 0.001)			
	InT	Glucose	−2.41	0.073
	Constant = 33.58	Fructose	−15.68	0.038
	R^2 = 0.63 (p = 0.012)			

might not be related to phytomass production. Indeed, PCA analysis indicated that both the above-ground phytomass and carbohydrates were more associated with the season (and possibly to their different phenological cycle) than to the location of both species in the Reserve.

Shewmaker et al. (2006) found marked seasonal variations in the concentration of NSC in eight tall fescue (*Festuca arundinacea* Schreb.) cultivars, being inversely related to dry mass yield. Olson and Wallander (1997),

studying the effects of defoliation in other grasses, suggested that the absolute content of NSC alone could not be considered an indicative of aerial biomass production and other factors should contribute to the ability of shoot development, such as the type of carbohydrate accumulated and/or the photoassimilate partitioning. Differences in photoassimilate partitioning have also been found by Kingston-Smith et al. (1998) in 13 grass species, including tropical species such as the starch-accumulating *Zea*

mays and the temperate fructan-accumulating *Hordeum vulgare*. In the present study, differences in the underground reserve organs, carbon partitioning and distinct phenological cycle of *E. inflexa* and *M. minutiflora* (Filgueiras, 2002) could be associated with their above ground production of biomass in the Reserve. The decrease in WSC in the underground organs of *M. minutiflora* in InT in July was coincident with one of the peaks of phytomass production by this species, suggesting that WSC from the roots could supply energy for its survival in the internal transect of the Reserve, in periods when photosynthesis is limited.

The amounts of the individual sugars of the WSC fraction also differed between underground organs and aerial tissues for both species. In the shoots, the main component was glucose, probably as a result of starch hydrolysis during periods of water restriction and high temperatures. Several studies have demonstrated the role of this sugar in osmotic adjustment under water and heat stresses (Vágújfalvi et al., 1999; Yoshida et al., 1998; Liu and Huang, 2000). In the roots, on the other hand, the most important sugar was sucrose, with higher levels during the dry season for both species, suggesting a storage function, as well as a role in the maintenance of osmotic potential in the underground organs among other functions.

The amount of starch was much higher in shoots than in roots for both species, indicating that the reserve of energy to produce aerial biomass rests in transitory starch, accumulated in leaves during the photosynthetic period. In the native species *E. inflexa*, the amount of starch varied in the shoots, being lower during rainy periods (January and October) when this species increases vegetative growth, and higher in dry periods (July), which is consistent with a lower investment in vegetative growth. Results showed that the higher the relative concentration of NSC in the aerial tissues, the lower was the phytomass. In contrast, the higher the concentration of glucose (probably as a consequence of starch hydrolysis), more intense was the vegetative growth and phytomass production by *M. minutiflora* in the external transect. Similar behaviour was reported for the tropical grass species *Paspalum notatum* which showed a reduced growth rate during dry months, followed by a reduction in biomass production, when growing in a subtropical climate (Ribeiro et al., 2006).

The analysis of the phytomass production by the two grasses showed marked differences according to their location in the Reserve. The exotic species *M. minutiflora* showed high values of phytomass in July and January at the Reserve border (ExT), while the native grass *E. inflexa* exhibited a comparatively low biomass production in ExT. *M. minutiflora* may have competitive advantage at the Reserve border thanks to the carbohydrates accumulated in the root system, in addition to the more efficient photosynthetic metabolism characteristic of C_4 grasses. According to Leishman et al. (2007), the potential for rapid growth associated with an efficient carbon fixation in open and sunny areas seems to be a key factor of the success of invasive species in environments not resource limited, as the Reserve border can be. Therefore, our data indicate that the border (ExT) is a better habitat for the alien grass at least in July. Indeed, Hoffmann et al. (2004) have already reported the preference of *M. minutiflora* for habitat borders in a cerrado region.

On the other hand, in the inner part of the Reserve (InT) both grasses showed similar phytomass production throughout the year, confirming previous suggestions by Pivello et al. (1999) that the invasive grass *M. minutiflora* did not displace the native *E. inflexa*. This might be due to their efficient vegetative propagation and ability to use efficiently their reserves of non-structural carbohydrates (mainly starch) in periods when photosynthesis is reduced. The peaks of phytomass of *M. minutiflora* in July and January in the Reserve border could be related to its reproductive behaviour, with abundant flowering in July and large shoot growth in the rainy season (January).

In conclusion, the differences in carbohydrate accumulation, partitioning and composition of individual sugars concerning time of the year and location in the Reserve seemed to be more related to the annual growth cycle of both grasses and possibly to specific physiological responses of *M. minutiflora* to disturbed environments in the Reserve border.

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