

Fire and Drought: Soluble Carbohydrate Storage and Survival Mechanisms in Herbaceous Plants from the Cerrado

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The Cerrado biome covers a large area of central Brazil, containing a mosaic of physiognomies determined by soil fertility, seasonal rainfall, and fire. The ground layer vegetation presents a high eudicot diversity and a high proportion of belowground phytomass, represented mainly by a diversity of underground organs storing carbohydrates. Fructans and other soluble carbohydrates are claimed to be more than reserves, conferring protection against abiotic stresses due to osmoregulation properties and rapid turnover. Inulin- and levan-type fructans are prominent and found respectively in the underground organs of Asteraceae and Amaranthaceae, abundant families in the herbaceous Cerrado flora. Poaceae, mainly Panicoideae, is also abundant and accumulates glucose, sucrose, and malto-oligosaccharides, but not fructans, in contrast with temperate grasses. Here, we review evidence that fructans in the herbaceous flora of the Cerrado play a significant role in tolerance to drought and fire, promoting the rapid recovery of the ground-layer vegetation after environmental disturbances.

Keywords: savanna, seasonality, soluble carbohydrates, stress tolerance, underground storage organs

Brazil has a land area of about 8.5 million square kilometers (km²). In this vast area, the central region is covered by a floristically rich savanna known as Cerrado that covers nearly 23% of Brazil's land surface (figure 1) and is exceeded in area only by the Amazon rainforest (Ratter et al. 1997). The Cerrado is regarded as both the largest and richest savanna in the world—and the most threatened one (Forzza et al. 2012). It is also recognized as a global biodiversity hotspot (Myers et al. 2000).

Cerrado vegetation consists of a complex mosaic of different physiognomies, from open grassland to woodland. Within this diversity of vegetation types, two distinct layers can be recognized: a woody layer composed of trees and large shrubs and a ground layer dominated by C₄ perennial grasses and a diverse assemblage of subshrubs and herbs. These two layers differ not only structurally but can also be considered two distinct functional types, because trees, grasses, and herbaceous eudicots differ in patterns of soil-water extraction (Silva IA and Batalha 2011, Rossatto et al. 2013).

Savanna vegetation varies globally in terms of the extent of tree cover, with neotropical savanna biomes exhibiting

generally greater cover. Among savannas, however, the Cerrado is distinctive for several reasons. The ground layer has the highest eudicot species diversity of any savanna worldwide. This layer also has uncommonly high proportion of belowground phytomass, reaching more than 70% of the aerial phytomass (Haridassan 2000). In addition, the majority of eudicot species with perennial storage organs store fructans, a less common type of storage carbohydrate.

Over the last few decades, large extensions of Cerrado have been transformed into pastures and plantations of sugarcane and soybean to meet economic interests linked to the production of bioenergy, food industry, and agriculture. The rich taxonomic, genetic, and physiological diversity of the Cerrado is at risk of being lost before being understood. Studies of species that still remain in preserved areas of the Cerrado show that the accumulation of soluble carbohydrates, including fructans, as was discussed by Hendry (1993) for other fructan-containing flora, enables them to tolerate seasonal drought and fire and might be an advantage to survive the predicted climatic scenario of rising temperature, carbon dioxide concentration, and restriction of water availability (Oliveira et al. 2013). In this review, we describe

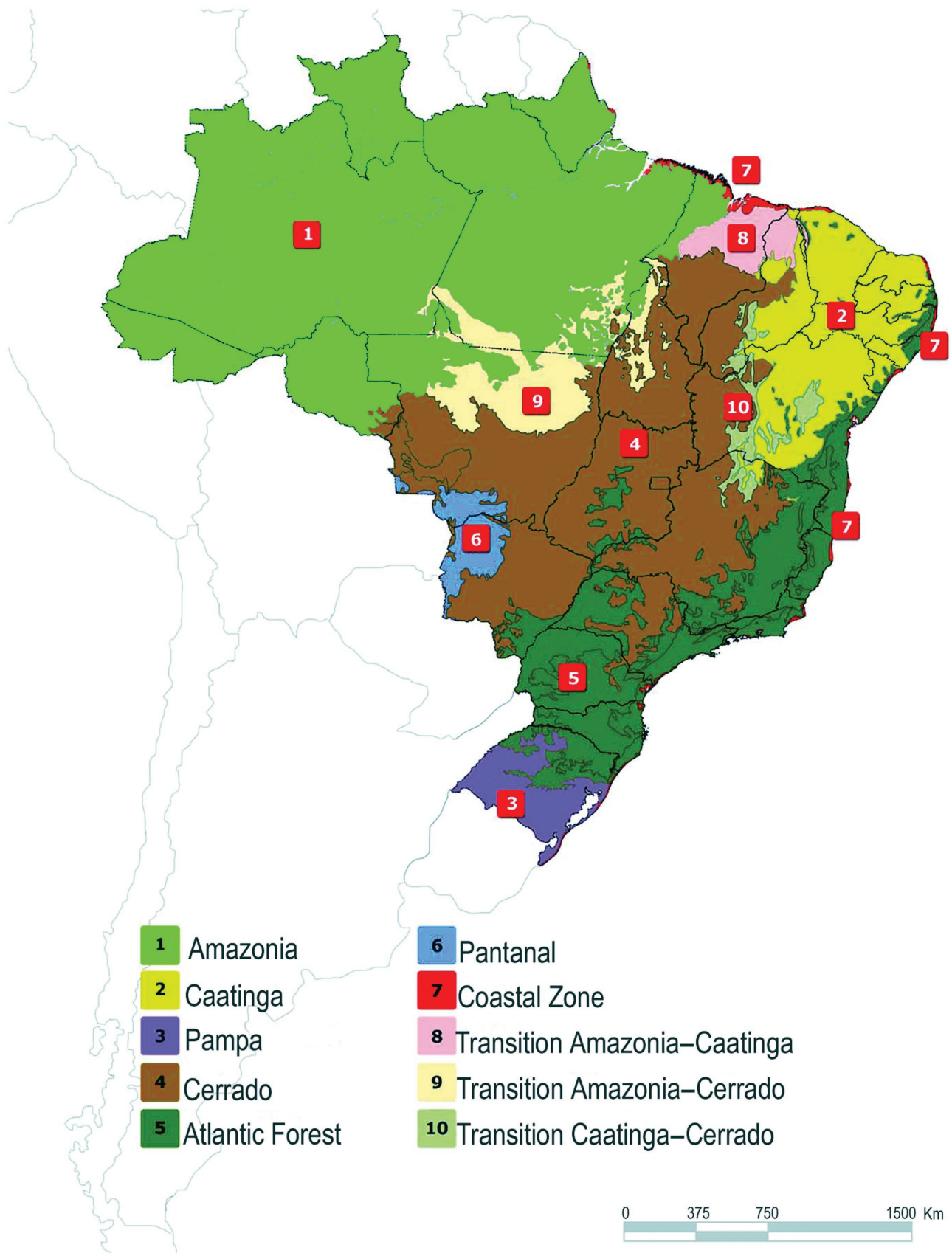


Figure 1. Localization and areas of the Brazilian biomes, including the Cerrado. Adapted with permission from the World Wide Fund for Nature (www.wwf.org.br).

the general abiotic features of the Cerrado, briefly introduce the taxonomic diversity of the herbaceous eudicots in the ground layer, and focus on the occurrence and ecophysiological significance of soluble carbohydrates, particularly fructans, in the belowground storage organs of these plants.

The abiotic characteristics of the Cerrado

The diversity of the Cerrado vegetation is the result of a combination of several current and historical influences, including seasonal precipitation, soil fertility and drainage, fire events, and the alternation of the glacial and interglacial periods of the Quaternary (Oliveira-Filho and Ratter 2002). Cerrado physiognomies occur throughout a wide geographical area and include a range of forms from open fields to woodlands, namely “campo sujo,” scrub, “campo cerrado,” cerrado *sensu stricto*, and “cerradão,” composing a mosaic determined mainly by soil fertility, water table depth, and fire frequency (Franco et al. 2014).

As with most savannas, rainfall seasonality is one of the key environmental determinants of the Cerrado. Average annual precipitation is around 1500 millimeters (mm), varying from 750 mm to 2000 mm, the major part occurring from October to March and the monthly average in the wet season ranging from 150 mm to 500 mm. The dry season occurs in autumn–winter, from May to September, when rainfall is significantly reduced to a range between 0 mm and 50 mm (Silva FAM et al. 2008). This imposes significant soil-water deficits, especially for herb and subshrub species (Rossato et al. 2013), similar to other open-habitat grasslands worldwide (Strömberg 2011). Rainfall seasonality also has direct effects on the relative air humidity (RH). More than 90% of the Cerrado core has average annual RH between 60 and 80%. However, during the dry season, RH decreases significantly, reaching the lowest levels (40%–60%) in August and September. In some areas, RH decreases to levels as low as 10% during the warmest hours of the day (Silva FAM et al. 2008). Temperatures differ significantly between Southern and Northern regions of the Cerrado. Winter average minimum temperature in the south is 8 degrees Celsius (°C), whereas in the north, it reaches 16°C. In summer, the average maximum temperature is 24°C in the south and 36°C in the north (Silva FAM et al. 2008).

Soil diversity strongly influences Cerrado vegetation (Reatto et al. 2008). Edaphic factors such as effective soil depth, proximity of the groundwater to the surface, drainage patterns, and fertility are determinants of phytophysiognomies and species distribution (Haridasan 2000). Generally, open physiognomies tend to be replaced by forests as water availability or soil fertility increases (Oliveira-Filho and Ratter 2002).

The major soil class in the Cerrado, oxisols, occupies almost 50% of the area (Reatto et al. 2008). These soils are deep, dystrophic, well drained, and acidic, with low cation exchange capacity (CEC) and high levels of aluminum saturation (Haridasan 2000). Sandy entisols are also prominent, occurring in approximately 15% of the Cerrado area. These

are deep soils as well, although they are quartz based and have sandy texture. Thirteen other soil types are present in minor proportions, according to the Brazilian soil classification system (Reatto et al. 2008).

Another important feature of the Cerrado is the frequency of fire events, due partly to natural causes and partly to human activity in open areas for cattle pasture and agriculture. The average fire frequency in protected areas of the Cerrado is three to six years, depending on vegetation type. Usually, fire is more frequent in open physiognomies, such as the shrub savanna, and less frequent in woodlands (Pereira-Júnior et al. 2014). Casual fires may be caused by lightning in the rainy season or in the dry season because of the accumulation of high combustible material provided by the dry herbaceous–subshrub stratum. With the arrival of the wet season in spring, sprouting and flowering occur, and the incidence of fires drops markedly. One of the most interesting effects of fire in the Cerrado is the synchronization of the sprouting and flowering of several herbs (Coutinho 1990), mainly of the Asteraceae family.

Herbaceous diversity and the potential for soluble-carbohydrate studies

Several Cerrado physiognomies are characterized by the type of vegetation that predominates on the ground layer, in which the diversity of herbs, subshrubs, and small shrubs is much higher than that of trees (Ratter et al. 1997). Indeed, the ratio between woody and herbaceous species in the Cerrado ranges from 1:2 to 1:4.5 (Filgueiras 2002, Batalha and Martins 2007). The herbaceous component constitutes approximately 65% of the whole flora in areas such as the Cerrado Reserve “Pé-de-Gigante” in São Paulo state (Batalha and Mantovani 2001) and 75% at Emas National Park, in Goiás state (Batalha and Martins 2007). The richest and most frequent families in these floristic inventories were Asteraceae, Fabaceae, Poaceae, Rubiaceae, Bignoniaceae, Malvaceae, Apocynaceae, Euphorbiaceae and Malpighiaceae.

The highest species richness in the Cerrado domain occurs in the Asteraceae, which has 1215 species distributed among 182 genera, most of them herbs, subshrubs, and shrubs (Nakajima et al. 2013). Most species have restricted geographic ranges. Another herb family that deserves attention is the Amaranthaceae, with 145 species occurring in Brazil and 101 in the Cerrado (Marchioretto et al. 2013). More than 50 species of Amaranthaceae are of the genus *Gomphrena* (Siqueira 1985, Vieira and Figueiredo-Ribeiro 1993, Fank-de-Carvalho et al. 2012), distributed mainly in the high-altitude Cerrado.

Another highly abundant family in the herbaceous layer of the Cerrado is the Poaceae. The Poaceae is divided into several subfamilies according to morphological, anatomical, biochemical (photosynthetic pathways), and molecular data, and all but one (Puelioideae) are represented in Brazil (Longhi-Wagner et al. 2012). Filgueiras and colleagues (2015) estimated the Brazilian grass flora as 210 genera and

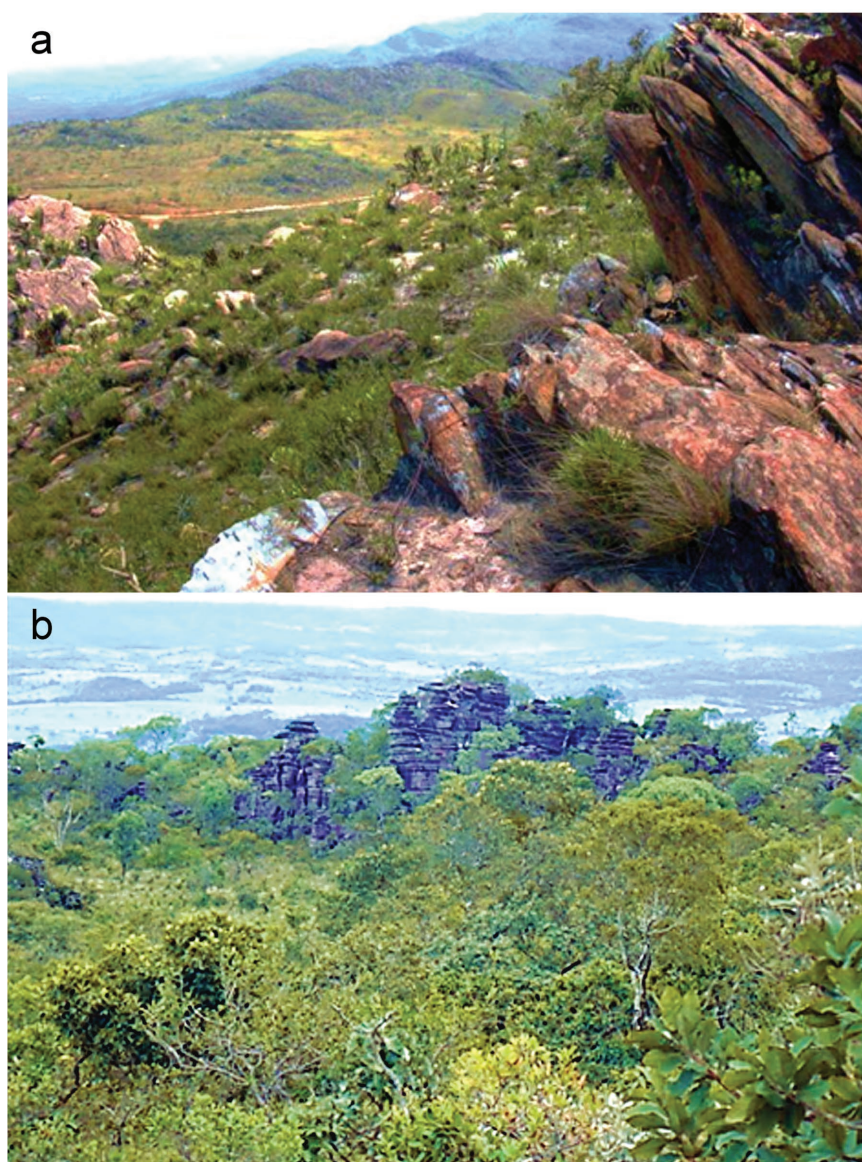


Figure 2. Rocky fields in Cerrado areas featuring fructan accumulating herbs and subshrubs: (a) General view of rupestrian fields in Cerrado at Serra do Cipó, MG, Brazil and (b) Serra dos Pirineus, GO, Brazil. Photographs: Rita de Cássia L. Figueiredo-Ribeiro.

1418 species. A floristic survey in 13 sites of montane grasslands, distributed from northeast to south Brazil (roughly 13°S–28°S), listed 311 species within 72 genera. The genus *Paspalum* includes the highest number of species (49), whereas 25 genera contain only a single species. A relatively high percentage of species (approximately 50%) was found restricted to single study sites, and very few species were widespread. Local grass floras are largely dominated by species of subfamily Panicoideae, except in southernmost sites where larger numbers of Pooideae reflect the southern latitude and much higher altitudinal range (Longhi-Wagner et al. 2012).

Besides high floristic diversity, herbaceous Cerrado plants have a variety of adaptive strategies to overcome

the harsh environmental conditions imposed by the prolonged dry season, nutrient-poor soils, and a fire-prone environment (figure 2). Two of the most important strategies are the phenology—characterized by senescence of aerial organs during the dry season and resprouting in the rainy season—and the presence of thickened and bud-bearing underground storage organs, widely found in families with a predominance of herbaceous species.

The diversity of underground reserve organs

An interesting peculiarity of Cerrado vegetation is the high ratio of below-ground-to-aerial phytomass (Haridasan 2000). Most herbaceous and subshrub Cerrado species are perennial (Figueiras 2002), and a large portion of their biomass is stored in a high diversity of thickened underground organs (figures 3 and 4), such as rhizomes, rhizophores, tubers, bulbs, tuberous roots, and xylopodia (Appezato-da-Glória et al. 2008). Underground storage organs bearing buds can provide not only the regeneration of individual plants after seasonal droughts or other environmental disturbance but also a high rate of vegetative propagation because of the existence of a bud bank (Klimešová and Klimeš 2007, Clarke et al. 2013). Similar types of underground perennial organs have been reported in other savanna systems as well, such as the “underground forests” in African savannas (White 1976, Simon and Pennington 2012, Maurin et al. 2014).

Different types of underground organs have distinct characteristics regarding morphogenesis, the proportion of storage tissues, and the type of stored compounds. Xylopodia, the most frequent underground organ in geophytes of Cerrado, are morphologically complex structures (Appezato-da-Glória et al. 2008) that originate from hypocotyl tuberization, primary roots, or occasionally, lateral roots (Clarke et al. 2013). They are extensively lignified, present viable buds and accumulate soluble carbohydrates frequently of the raffinose series, as is exemplified by *Ocimum nudicaule* (Lamiaceae), essential to enable resprouting after seasonal dormancy or environmental disturbances (Figueiredo-Ribeiro and Dietrich 1983). In xylopodia, tissue storage capacity is generally limited to xylem parenchyma; however, in some species, such as *Viguiera discolor*, the lignified xylopodium functions

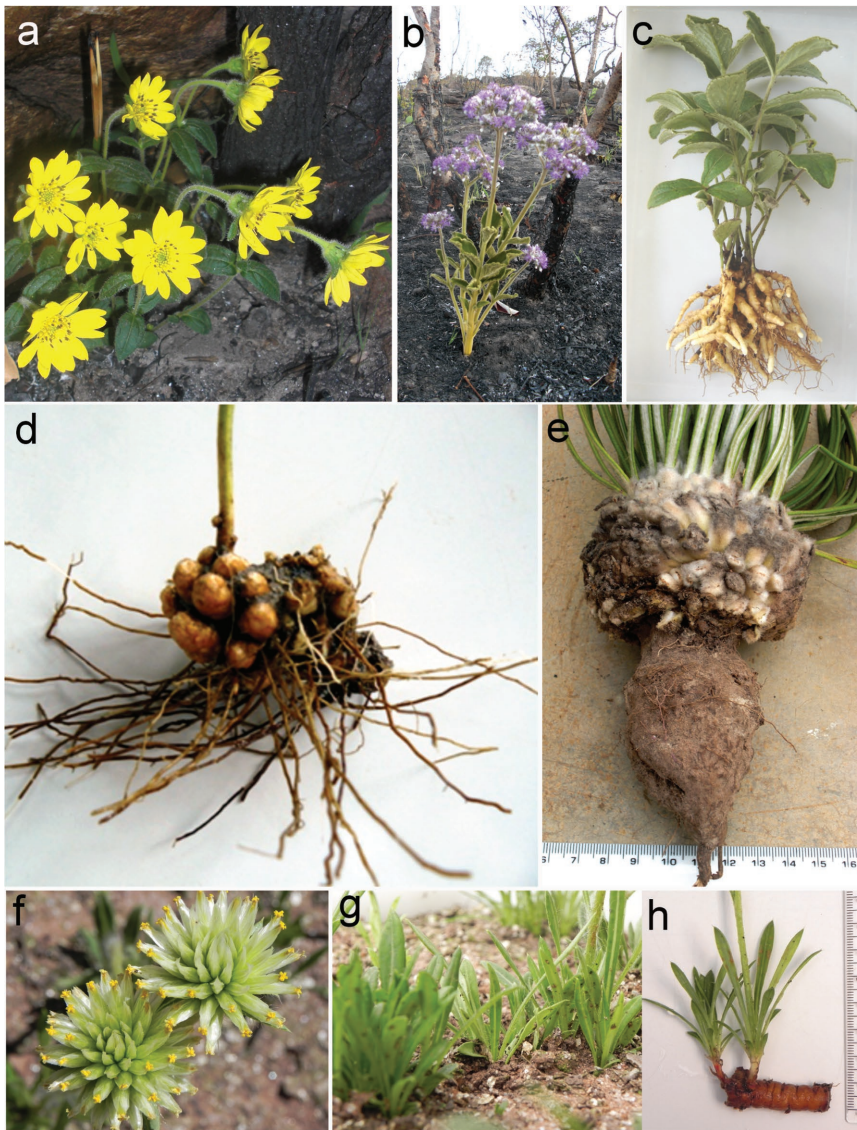


Figure 3. Fructan accumulating species from Cerrado areas: (a) *Aspilia foliacea* and (b) *Chrysolaela obovata* flowering 5 weeks after fire at Serra dos Pirineus, GO, Brazil; (c) rhizophores of *Chrysolaela obovata* and (d) *Chrysolaela simplex*; (e) underground organs of *Prestelia eriopus*; (f) *Gomphrena marginata* inflorescences, (g) sprouts, and (h) sprouting fragment of the underground system. Photographs: Rita de Cássia L. Figueiredo-Ribeiro.

primarily in vegetative propagation, whereas parenchyma-rich tuberous roots are the main site of fructan accumulation (Figueiredo-Ribeiro et al. 1986, Isejima et al. 1991).

Another type of underground organ is rhizophore, a cauline underground system that can initiate both shoots and roots (figure 3). These structures were reported in Angiosperms for some species of Asteraceae, Dioscoreaceae, and Smilacaceae (Hayashi and Appezzato-da-Glória 2005). These fleshy structures store reserves, such as starch and fructans, in parenchyma (Hayashi and Appezzato-da-Glória 2005, Martins and Appezzato-da-Glória 2006, Carvalho

et al. 2007, Appezzato-da-Glória et al. 2008).

Tuberous roots are also found in herbaceous species of the Cerrado (figures 3 and 4), as is exemplified by the fructan-containing Amaranthaceae *Gomphrena macrocephala* (Vieira and Figueiredo-Ribeiro 1993), *G. marginata* (Silva FG et al. 2013), the Asteraceae *Viguiera discolor* (Figueiredo-Ribeiro et al. 1986, Isejima et al. 1991), and the starch-accumulating Bixaceae *Cochlospermum regium* (Dietrich and Figueiredo-Ribeiro 1985, Carvalho and Dietrich 1996), among others. In some cases, these roots develop vegetative buds, which contribute to the reestablishment of plants after aerial senescence.

The establishment of a bud bank is correlated with selective forces such as drought, fire, and seasonality (Klimešová and Klimeš 2007). The resulting interaction between morphological and physiological characteristics determines resource allocation for resprouting, including the degree of meristem protection, the relative proportion of vegetative and reproductive growth, and the requirement for the allocation and location of storage reserves (Clarke et al. 2013).

Soluble storage carbohydrates in herbaceous species of the Cerrado

The regrowth of herbaceous plants following seasonal drought or fire requires carbon. In the absence of current photosynthates, carbon must come from the mobilization of reserves. Underground reserve organs are the primary source of carbohydrates and nitrogen storage compounds, such as proteins in the Fabaceae species *Centrosema bracteosum* and in the Amaranthaceae *G. macrocephala*, among others (Figueiredo-Ribeiro et al. 1986). Starch is the major reserve car-

bohydrate in vascular plants, but in some plants, soluble carbohydrates based on sucrose predominate. Studies on the contents and composition of reserve compounds in herbaceous species of the Cerrado revealed the presence of high amounts of soluble carbohydrates, such as free glucose and fructose, sucrose, oligosaccharides, and fructans (Figueiredo-Ribeiro et al. 1986, Tertuliano and Figueiredo-Ribeiro 1993, Joaquim et al. 2014, Almeida et al. 2015, Silva TM et al. 2015). A list of plant species with their growth form, type of underground organ, and storage carbohydrates is presented in supplemental table S1.

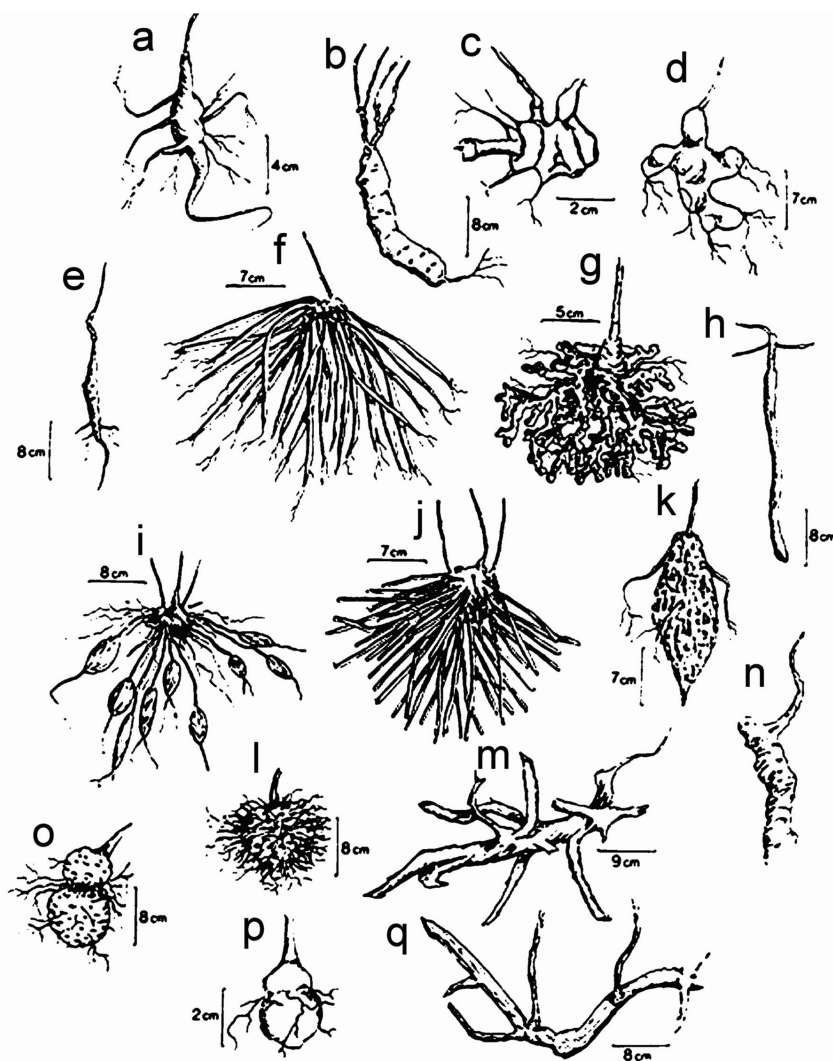


Figure 4. Examples of the diversity of underground organs of Cerrado species: (a) *Eriosema crinitum*, (b) *Froelichia lanata*, (c) *Smilax syringoides*, (d) *Vernonia cognata*, (e) *Eriosema longifolium*, (f) *Ruellia geminiflora*, (g) *Vernonia herbacea* (currently *Chrysoleaena obovata*), (h) *Eriosema heterophyllum*, (i) *Viguiera discolor*, (j) *Aspilia montevidensis*, (k) *Mandevilla velutina*, (l) *Rechsteineria sceptrum*, (m) *Centrosema bracteosum*, (n) *Gomphrena officinalis* (currently *G. macrocephala*), (o) *Rechsteineria spicata*, (p) *Asterostigma lividum*, (q) *Mikania hirsutissima*. Adapted from Figueiredo-Ribeiro and colleagues (1986).

Water-soluble storage carbohydrates that accumulate in the vacuole offer a number of potential advantages over starch accumulation in plastids. First, the vacuole is physically large in comparison with other organelles, facilitating the accumulation of reserves in high concentrations. This accumulation is particularly significant in species in which the catabolism of existing reserves rather than current photosynthates is the main driver for growth (Hendry 1993). Second, the rapid polymerization and depolymerization of water-soluble storage carbohydrates, especially fructans, offer an alternative mechanism for osmoregulation.

Fructans are of particular interest relative to other soluble carbohydrates, because they are known to protect plants against low temperature, freezing, and drought stress (Portes et al. 2008, Valluru and Van den Ende 2008, Garcia et al. 2011), because osmotically inert fructan polymers can rapidly be converted into osmotically active sugars. Fructan turnover is very high, and sucrose is the sole substrate for fructan synthesis, with no subsequent involvement of phosphorylated intermediates (Pollock 1986 and references therein). Indeed, in the presence of a small number of appropriate enzymes, the accumulation and/or hydrolysis of fructans is a function of sucrose abundance and is therefore very responsive to the balance between supply and demand. Fructans have also been shown to protect membranes under stress conditions and are thought to have reactive oxygen species-scavenging capacity (Hincha et al. 2007, Peshev et al. 2013, Tarkowski and Van den Ende 2015).

Fructans are sucrose-based linear or branched polymers of fructose, and evidence of the advantages of fructan metabolism includes the fact that the synthetic enzymes are less sensitive to low temperatures, contrasting with enzymes involved in starch synthesis, which are very sensitive to temperatures below 5°C. On the basis of three trisaccharides—1-kestotriose, 6-kestotriose, and 6^G-kestotriose—five types of fructans can be distinguished and are characteristic of different plant groups: inulin, common in Asterales; levan, in Poales (*Dactylis*, *Lolium*); some Amaranthaceae (*Gomphrena*) and Buxaceae (*Pachysandra*); graminans in Poales (*Triticum*, *Hordeum*); neo-inulin-type in Asparagales (*Asparagus*, *Allium*); and neo-levan-type in Poales (*Avena*). A

general fructan biosynthesis pathway is presented in figure 5.

Fructans are widespread in prokaryotes, fungi, and algae and in about 15% of the flowering plants, particularly in Poales, Asterales, Campanulales, Dipsacales, Polemoniales, Ericales, and Liliales (Hendry 1993). In the Cerrado, the presence of fructans has been especially well documented in Asteraceae species in a preserved area at Mogi-Guaçu (22°35'S and 47°44'W), SP, Brazil (Mantovani and Martins 1988). In this area, Asteraceae represents approximately 17% of the flora, with *Vernonia* and *Eupatorium* being the most abundant genera, constituting 5% and 4.5%, respectively, of the local flora. Figueiredo-Ribeiro and colleagues (1986)

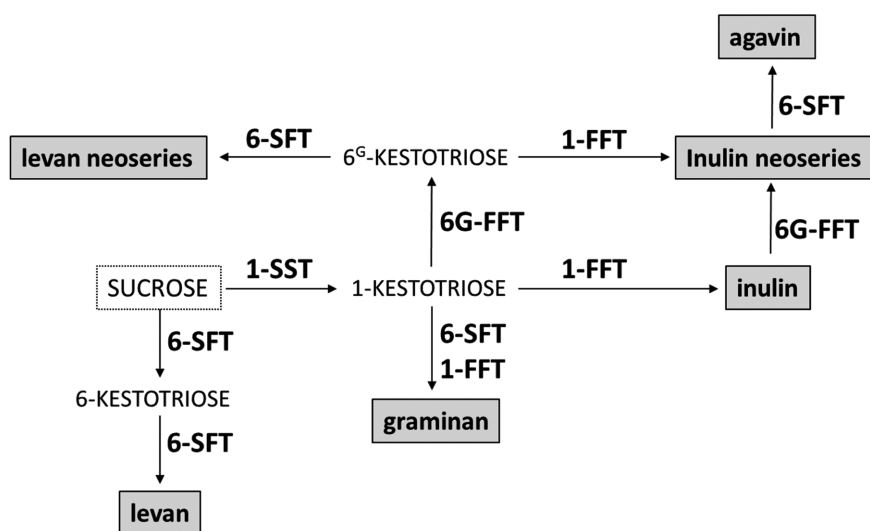


Figure 5. A model of fructan synthesis in plants. Starting from sucrose, and in the absence of sugar nucleotide intermediates, structurally different fructan molecules can be produced by the concerted action of different fructosyltransferases: the S-type enzymes, 1-SST and 6-SFT, which use exclusively sucrose as fructosyl donor, and the F-type enzymes, 1-FFT and 6G-FFT, which use fructans as fructosyl donors. Adapted from Vijn and Smeekens (1999).

reported the predominance of high amounts of soluble carbohydrates in the underground organs of herbaceous Asteraceae species, and a later study by Tertuliano and Figueiredo-Ribeiro (1993) confirmed the predominance of inulin-type fructans as reserve compounds in the underground organs of approximately 60% of the Asteraceae. Thirty-five herbaceous species of Asteraceae were assayed, representing six tribes, and the presence of fructans was detected in 19 species of the Eupatorieae, Heliantheae, and Vernonieae. In two species, *Vernonia herbacea* (Vernonieae) and *Viguiera discolor* (Heliantheae), fructans constituted as much as 80% of the reserve organ on a dry-mass basis. Spherocrystals of inulin were histologically detected in approximately 80% of the examined species. The underground organs varied in the water content, fructan concentration, and tissue localization of inulin spherocrystals, distributed mainly in the reserve parenchyma cells or associated with the vascular tissue. The localization of inulin spherocrystals in different tissues of the storage organ was considered a taxonomic marker within the Asteraceae (Tertuliano and Figueiredo-Ribeiro 1993). The presence of fructans inside and outside the protoplasts of cells from the tissues of tuberous roots of *Campuloclinium chlorolepis* (Vilhalva et al. 2011) and the rhizophores of *Chrysolaena obovata* (Garcia et al. 2015) were visualized by scanning electron microscopy and evidenced globular bodies consistent with typical inulin spherocrystals under polarized light. The localization of fructans associated with cell walls in several tissues of these organs, clearly shown by histochemical and ultrastructural analyses, reinforces the hypothesis

of the interaction of fructose polymers with cell membrane and possibly their role in membrane stabilization in plants subjected to low temperature and water deficit.

Differently from all inulin-containing species of Asteraceae from the Cerrado, the presence of levan-type fructan with a high degree of polymerization (DP) was described for the first time in a member of Amaranthaceae, *Gomphrena macrocephala* (Vieira and Figueiredo-Ribeiro 1993), and the molecular structure was later confirmed (Shiomi et al. 1996). In tuberous roots of *G. macrocephala*, fructans constituted approximately 50% of the organ dry mass, in which they were detected in the parenchyma of the secondary xylem and in vessel elements of the xylem (Vieira and Figueiredo-Ribeiro 1993). Fructan accumulation was also detected in the underground organs of five other *Gomphrena* species from the rupestrian fields at the Espinhaço mountain range. Rupestrian fields, or “campo rupestre,” describe a unique

Cerrado physiognomy at altitudes higher than 900 meters (m) found on shallow, sandy soils and rock outcrops that are maintained by frequent seasonal fires, often set by humans (Longhi-Wagner et al. 2012).

Extending for approximately 1000 km roughly north and south through the states of Minas Gerais and Bahia, the rupestrian fields of the Espinhaço Mountain Range show a relatively high rate of endemism at the species level in several families (Pirani et al. 2003). A screening of reserve compounds accumulated in different organs of the herbaceous species of predominant families was performed recently in the Espinhaço Range at “Serra do Cipó” (Minas Gerais, Brazil), “Serra de Itacambira” (Minas Gerais, Brazil), and “Serra Dourada” (Goiás, Brazil) and included approximately 40 species of Orchidaceae, Eriocaulaceae, Velloziaceae, Iridaceae, Verbenaceae, Lamiaceae, Convolvulaceae, Acanthaceae, Apiaceae, Amaranthaceae, and Asteraceae. The latter presented the largest number of species, corresponding to nearly 70% of the collected species. Fructans were detected in all the Asteraceae and Amaranthaceae species, ranging in concentration from 3% to 35% of the organ dry mass. Of the Asteraceae species analyzed, *Lessingianthus psilophyllus* and *Richterago polymorpha* presented high concentrations of inulin-type fructans (Joaquim et al. 2014), similar to other Asteraceae from Cerrado already studied (Carvalho et al. 2007). In fact, all the Asteraceae presented inulin-type fructans, some of them with a high degree of polymerization. However, *Habenaria caldensis*, *Oncidium hidrophyllyum* (Orchidaceae), and *Klotzschia brasiliensis* (Apiaceae) presented starch, and *Lippia lupulina* (Verbenaceae) and *Justicia*

asclepiadea (Acanthaceae) contained significant amounts of raffinose family oligosaccharides (RFOs) as the main reserve carbohydrate.

Geophytes in general, mainly those with bulbs, can accumulate starch, fructans (e.g., *Allium* species), or both (e.g., *Lachenalia minima*, Hyacinthaceae, and *Galanthus nivalis*, Amaryllidaceae) as the main reserve carbohydrate (Miller 1992, Orthen 2001, Orthen and Wehrmeyer 2004). Glucomanan can also occur, but generally in addition to other storage carbohydrates (Miller 1992). In *Lachenalia minima*, fructans and starch are present in similar amounts, but they seem to have different functions, with starch as the main carbon source and fructans associated with water status adjustments (Orthen 2001). In tulip bulbs, fructans constitute 5%–10 % of the dry matter and—combined with the soluble carbohydrates yielded during starch hydrolysis—they have been associated with changes in water contents throughout the phenological cycle (Kamenetsky et al. 2003).

Most of the Iridaceae are geophytes, with underground organs bearing buds and reserve compounds, consistent with their occurrence in seasonal environments, such as the Cerrado. Considering the great diversity within some tribes of Iridaceae of South America, five species of Cerrado Iridaceae representing the tribes Tigridieae, Sisyrinchieae, and Trimezieae were accessed at Serra Dourada, GO, Brazil, and shown to accumulate carbohydrates as the main reserves in the underground organs. The species of Trimezieae and Tigridieae showed considerable amounts of starch and glucose, fructose, sucrose, and maltose. In contrast, *Sisyrinchium vaginatum*, of the Sisyrinchieae, showed traces of starch and also high contents of soluble carbohydrates, mainly RFOs (Almeida et al. 2015).

The vegetative aerial organs of perennial tropical Cerrado grasses revealed greater diversity of soluble carbohydrates when compared with those of temperate grasses. Carbohydrate composition appears to depend on the phylogenetic relationships and geographic origin of the species. Among the 24 accessions examined by Moraes and colleagues (2013), fructans were not found in any of the analyzed species of the subfamilies Panicoideae and Arundinoideae, even in leaves and stem bases, the main storage sites in fructan-accumulating grasses. Grasses of the Pooideae are well known to accumulate high amounts of fructans in the aerial organs, mainly stem bases (Chatterton et al. 1989); however, up to this date, species of this subfamily have not been reported to occur in the Cerrado core flora. In contrast, species of Panicoideae, well represented in this survey, contained mostly free glucose and starch, distinguishing them from the Pooideae.

Differences in the amounts and composition of storage carbohydrates of the 24 grasses indicate the diversity of their regulatory metabolic pathways, especially starch, in source tissues. The absence of fructans in Cerrado grasses is in agreement with the low levels of free fructose and sucrosyl-oligosaccharides found. Instead, a linear malto-oligosaccharide series was detected in all analyzed organs

of most studied species (Moraes et al. 2013). Generally, in photosynthetic tissues, maltose is the main product of starch degradation by β -amylases, and malto-oligosaccharides are intermediates of starch metabolism generated either by starch debranching enzymes or by β -amylases. The comparative studies of Souza and colleagues (2005) showed that the diurnal pattern of storage carbohydrates in the perennial invasive grass *Melinis minutiflora* (C_4) and in *Echinolaena inflexa* (C_3), native from the Brazilian Cerrado, was attributed to differences in starch metabolism. Therefore, the high levels of free glucose in Cerrado grasses might be a result of transitory starch degradation.

Despite the ecological and economic importance of storage carbohydrates, relatively little is yet known about the nature and regulation of these compounds in grasses. Given the role of grasses in stabilizing ecosystems, there is a particularly urgent need to extend the study of grass carbohydrate metabolism to regions not yet accessed, such as the rupestrian fields of Brazil, which are rich in Pooideae species (Filgueiras et al. 2015).

Experimental evidence for the role of soluble carbohydrates in herbaceous species. Changes in the total amounts and in size distribution of fructan molecules have been observed at different phenological phases or developmental stages (Carvalho et al. 2007). The presence of fructans in Cerrado herbs has been associated with the seasonal growth pattern exhibited by the Cerrado flora as well as with the drought and low temperatures prevailing in winter (Carvalho et al. 2007). In *Vernonia herbacea*, *Viguiera discolor*, and *Gomphrena macrocephala*, seasonal growth and development have been well described and associated with changes in fructan content and composition. In *V. herbacea*, in particular, fructan mobilization occurs during sprouting, whereas fructan biosynthesis occurs in the vegetative phase.

Reserve carbohydrates bridge the temporal gap between resource availability and the demands of growth. This function is similar in plants that accumulate fructans, starch, or other reserve carbohydrates. However, there is also evidence that fructans, as well as other soluble carbohydrates, play the dual role of directly protecting tissues from damage due to drought and low temperatures (Van den Ende 2013). Transgenic tobacco and sugar beet plants transformed to accumulate fructans showed enhanced drought tolerance under laboratory conditions compared with the wild-type plants (Pilon-Smits et al. 1999), and potatoes modified to produce fructans showed a reduction in water stress-induced proline accumulation (Knipp and Honermeier 2006). In terms of tolerance to low temperature, results obtained with rice suggested that transgenic rice lines expressing wheat-derived fructosyltransferase genes accumulated large amounts of fructans and exhibited enhanced chilling tolerance at the seedling stage (Kawakami et al. 2008).

Owing to their high water solubility, fructans can play a role in the osmotic adjustment of cells by varying the degree of polymerization of molecules, in addition to

a membrane-protecting role during dehydration, as was shown experimentally by the interaction of isolated liposomes and fructans (Hincha et al. 2007). However, the role of fructans in abiotic stress tolerance, such as cold or subzero acclimation, is a very complex process that cannot be fully explained by the action of a single molecule or mechanism (Valluru and Van den Ende 2008) and may be related to differences in molecule size and structure, as well as to cell localization (Livingston et al. 2006).

Studies with *V. herbacea* showed that over long periods under low water availability, the rhizophores showed high water retention, related to fructan osmoregulation ability, because water retention was accompanied by changes in fructan composition (Dias-Tagliacozzo et al. 2004, Carvalho et al. 2007). These changes included increases in the oligo-saccharide-to-polysaccharide ratio and in the extractable activity of fructan enzymes (Garcia et al. 2011).

The effect of low temperature on fructan mobilization was also investigated in *V. herbacea*. When excised plants induced to sprouting were subjected to low temperature, there was a twofold increase in fructan hydrolytic enzymes, suggesting the occurrence of two different control mechanisms for these enzymes, one triggered by defoliation and sprouting and the other by low temperature (Portes et al. 2008). Low temperature also enhanced gene expression and the activity of fructan exohydrolases and contributed to the maintenance of high levels of fructo-oligosaccharides. These data provide evidence of the involvement of low-chain fructans in cold tolerance in plants from the Cerrado (Asega et al. 2011) and emphasize the complex interaction of fructans and abiotic stresses.

Raffinose family oligosaccharides are also among the most notable class of soluble carbohydrates in plants, being widespread in the plant kingdom. They are accumulated as reserve compounds in seeds and in underground organs but also have a role in carbon transport and protection against abiotic stresses, including the prevention of oxidative damage and osmoprotection (Van den Ende 2013). Recent experiments with *Barbacenia purpurea* (Velloziaceae), a desiccation-tolerant species from Brazilian rock outcrops, indicated that soluble carbohydrates of the RFOs played a crucial role in the osmotic adjustment of potted plants under water suppression being accumulated at the final stage of a drying cycle of 20 to 24 days. These carbohydrates were considered important sources of carbon in the subsequent rehydration phase (Suguiyama et al. 2014), as were fructans accumulated in the rhizophores of *Chrysolaena obovata* (Asteraceae; Garcia et al. 2015).

Because many Cerrado herbs are rooted in the upper soil layers, which dry relatively fast during the dry season, it is feasible to suppose that soluble carbohydrates not only ensure the maintenance of favorable plant-water balance during the long dry season, but they also allow rapid leaf flushing and the fast completion of the life cycle following the onset of the wet season or after a fire event. But interestingly, the specific regulatory mechanisms behind the

mobilization and depletion of sugar reserves following a fire event or a drought period—and their role in maintaining plant metabolism under water stress—remain poorly understood.

Conclusions

Many aspects of the diversity and adaptive strategies of the Cerrado vegetation remain to be investigated, particularly the ground-layer vegetation. The herbaceous-subshrub component holds a substantial part of the floristic diversity of Cerrado and is prevalent in many of its physiognomies. A number of these species have a relatively high proportion of biomass belowground relative to their aerial organs, and the detailed morphology of the complex and diversified underground systems has been studied in several of these systems. They are responsible for resprouting during restoration after environmental disturbances because of the presence of buds and the high amounts of soluble carbohydrates readily mobilized, such as fructans of the inulin type, typically found in Asteraceae, and of the levan type, found in Amaranthaceae. Unlike temperate grasses, the Poaceae of the Cerrado do not accumulate fructans but do accumulate considerable amounts of free glucose, sucrose, and malto-oligosaccharides, possibly related to the evolution and geographic distribution of this highly diversified and widely spread family. Taken together, information presently available on the soluble carbohydrates in herbaceous species of the Cerrado—combined with understanding interactions among their morphological, ecological, and metabolic traits—may provide significant insights into the adaptive mechanisms that ensure coexistence and facilitate future persistence in highly diverse herbaceous floras in neotropical savannas.

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Supplemental material

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