Herbicide resistance status of sourgrass

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Abstract: Sourgrass [*Digitaria insularis* (L.) Mez ex Ekman] is one of the most challenging herbicide-resistant weeds in the grain-producing areas of Southeast, Central-west, and Northeast of Brazil. This species is a perennial grass that is highly competitive with the C4 photosynthetic pathway. It easily adapts to different environments and reproduces through both seed and rhizomes. The objective of this review was to compile what is known about sourgrass biology, the state of herbicide resistance and its associated mechanisms, and main weed management strategies. The high seed viability, ease of dispersal and the strong propensity to develop resistance to herbicides like glyphosate and acetyl coenzyme A carboxylase (ACCase) inhibitors in the prevalent no-tillage system in Brazil make the sourgrass one of the most difficult weeds to control. Due to the great genetic variability, the resistance mechanisms conferring glyphosate-resistance among sourgrass populations range from reduced absorption, altered translocation, enhanced metabolism, target-site mutations, and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) overexpression. In addition, Trp2027Cys mutation was found to confer cross-resistance to ACCase inhibitors. Sourgrass management strategies include herbicide rotation, herbicide tank mix or sequential applications, as well as the use of mulch to reduce infestations. These measures must be implemented before crop sowing because the range of management options is greater than in after crop sowing. Additionally, the best control of sourgrass is achieved when management is carried out during the early growth stages, before the plants develop rhizomes and form clumps.

Keywords: 5-enolpyruvylshikimate-3-phosphate synthase; ACCase inhibiting herbicides; *Capim-amargoso*; Multiple resistance; Resistance mechanisms

1. Introduction

The genus *Digitaria* (Poaceae) is comprised of approximately 300 plant species distributed in both in tropical and subtropical regions worldwide (Canto-Dorow, 2001; Barroso et al., 2021). In Brazil, there are 26 native and 12 exotic species of *Digitaria* spp., inhabiting most of the regions favorable to agriculture (Lopez-Ovejeto et al., 2017). Among these species, *Digitaria insularis* (L.) Mez ex Ekman (sourgrass) stands out due to its wide distribution in the country (Mondo et al., 2010). This species, commonly known as *capim-amargoso*, also referred to as *capim-flecha* or *capim-açu* in Brazil (Barroso et al., 2017), is native to the American continent with distribution from the southern United States to Argentina and the Antilles (Barroso et al., 2021). Furthermore, this weed has been introduced to tropical Asia and several Pacific islands (Chadhokar, 1976). Sourgrass derives its common name from its bitter (*amargo*) taste (Lorenzi, 2000), which makes it unpalatable to livestock. This weed has been reported to have negative impact on pasture production in the Markham and Ramu valleys of Papua New Guinea (Chadhokar, 1976).

While sourgrass has a wide distribution in tropical regions worldwide (Figure 1), no other country experiences losses in agricultural production as large as Brazil, where it occurs with significant intensity in the Southeast, Center-west and Northeast regions of the country (Lopez-Ovejeto et al., 2017). This weed is a perennial grass with a C4 photosynthetic pathway that thrives in pastures, annual and perennial crops, as well as marginal areas (Machado et al., 2008; Gazola et al., 2019). Sourgrass is a diploid (2n = 36) species that exhibits rapid growth, remarkable adaptability, and competitive capacity throughout the year (Gemelli et al., 2012). Its shallow fibrous root system allows it to survive in environments with varying types and intensities of resource limitations for growth and development (Locorini et al., 2015). This species reproduces and disperses through both seeds and rhizomes (Machado et al., 2008). Sourgrass produces a large quantity of hairy and lightweight seeds, enabling them to be carried over long distances by the wind, and they exhibit a high germination capacity (Mendonça et al., 2014; Anunciato et al., 2022). Furthermore, when plants develop rhizomes, clumps (*touceiras*) of plants are formed that are difficult to control (Lorenzi, 2000; Zabiole et al., 2016; Silva, Mendes, 2020).

Sourgrass used to be a relatively easy weed species to control with herbicides; however, the expansion of no-tillage areas and the rapid adoption of herbicide-
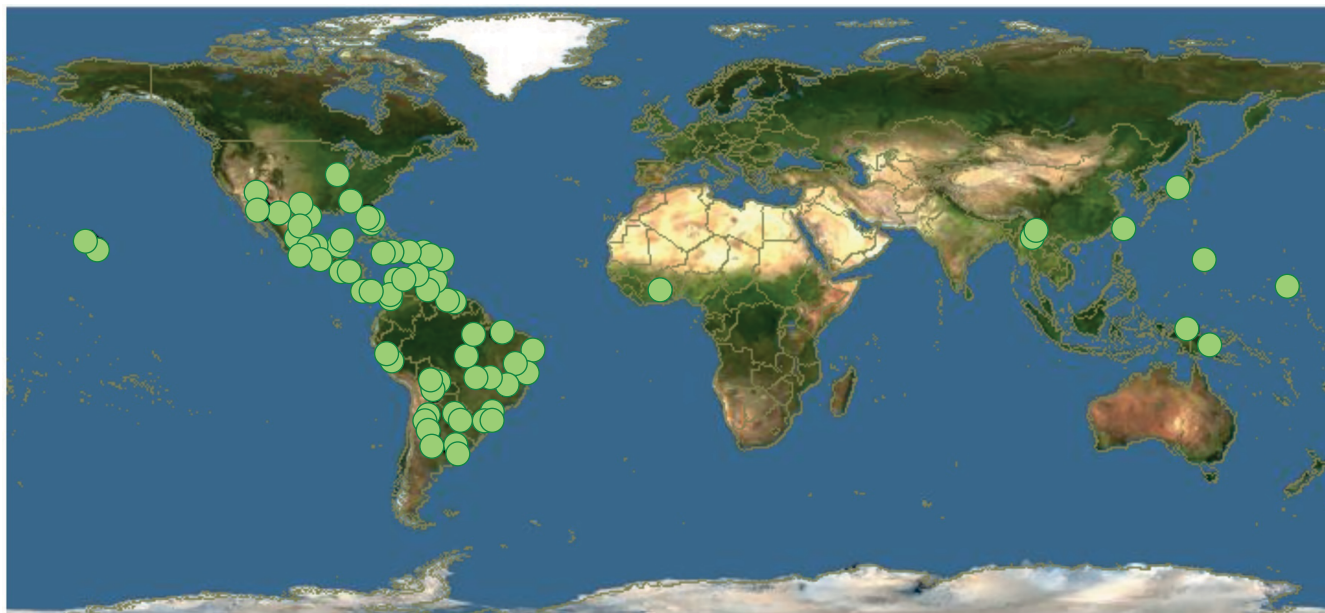
Source: Discover Life (2023)

Figure 1 - Global distribution of *Digitaria insularis* (L.) Mez ex Ekman

resistant genetically modified (GM) crops have made it one of the primary weed challenges in Brazil (Adegas et al., 2017; Lopez-Ovejero et al., 2017). Soybean, corn and cotton are the main GM crops produced in the country, where glyphosate is the most widely used herbicide (Alcántara-de la Cruz et al., 2020). This has led to the selection and spread of glyphosate-resistant (GR) biotypes of sourgrass (Gazola et al., 2020; Gonçalves Netto et al., 2021; Heap, 2023), because this species prevails in Brazil's main grain production systems, characterized by a double-cropping year of soybeans followed by corn, and then another cycle of soybeans followed by corn (Gonçalves-Netto et al., 2021).

Due to the significant yield losses that sourgrass causes in Brazilian agriculture, substantial efforts have been invested in characterizing the factors involved in its herbicide resistance, dispersal, and management (Lopez-Overejo et al., 2017; Carvalho et al., 2012; Barroso et al., 2015; Silveira et al., 2018). When herbicide resistance is diagnosed, studies on control alternatives become crucial to ensure the success management of herbicide-resistant weeds and to prevent yield losses (Silva, Mendes, 2020; Correia et al., 2015; Correia, Durigan, 2009). Due to the increase and dispersion of GR sourgrass populations throughout the main Brazilian agricultural regions, the management of this species presents great challenges; therefore, the exploration of new approaches to minimize the problem is required (Barroso et al., 2021; Silva, Mendes, 2020). Therefore, the objective of this review was to compile the extensive body of knowledge available about the biology, resistance status, and resistance mechanisms of sourgrass, while also exploring the main management alternatives for this species.

2. Biology and physiology of sourgrass

Sourgrass is an upright herbaceous grass with striated stems, long internodes, leaves featuring long and hairy sheaths, and a membranous ligule (Barroso, Hijano and Alves, 2017). This plant exhibits a high capacity for competition. The initial growth of the sourgrass is slow until 45 days after emergence (DAE) (Lorenzi, 2000). After this period, the plants develop rhizomes, which results in rapid and aggressive growth, forming clumps through tillering (Zabiole et al., 2016), reaching heights of up to 1.5 m (Barroso et al., 2021; Silva, Mendes, 2020). In addition, this species can reproduce both sexually and asexually (Mondo et al., 2010). Sourgrass panicles are showy and produce a large number of seeds, with more than 100,000 seeds being produced during the hottest months of the year. This seeds, due to their lightweight and hairy nature, can be easily dispersed over long distances by wind (Figure 2) (Silva, Mendes, 2020). Sourgrass rhizomes are short and thick, covered with short, densely hairy cataphylls, leading to the formation of clumps of plants that aid in their propagation and dispersal (Machado et al., 2008). Another characteristic that contributes the successful establishment of this weed is the ability to adapt to acidic and poor soils. As a result, it is found in the most varied Brazilian regions throughout the year (Lorenzi, 2000; Machado et al., 2008).

Sourgrass seeds exhibit high viability, germinating throughout the year in a wide range of temperatures and light intensities (Mendonça et al., 2014). They are positively photoblastic, and optimal germination (up to 90% at 10 days) occurs between 30 to 35 °C. However, sourgrass can germinate within a temperature range of 5 to 40 °C. Under moderate temperature conditions (20–30 °C), germination is influenced by light, reaching germination rates of ~70%
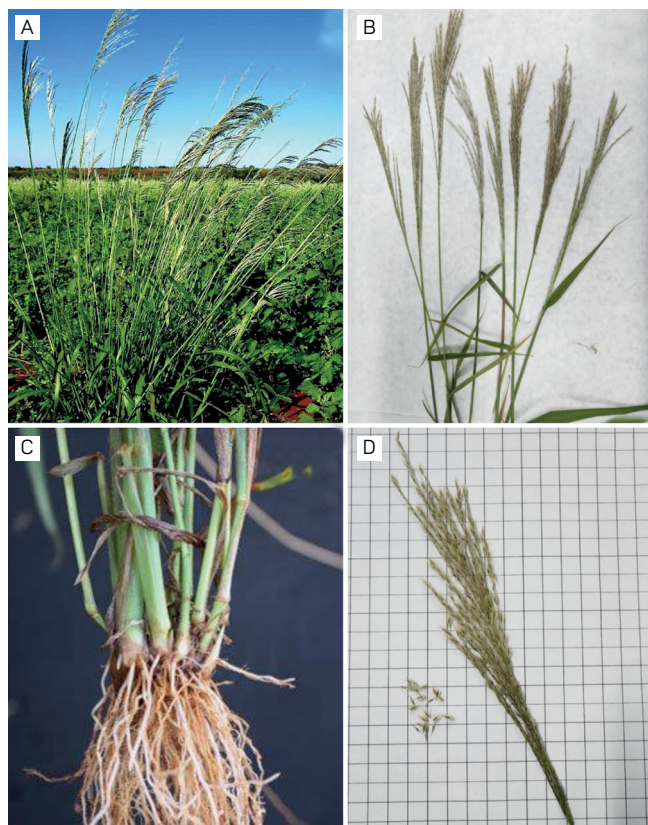
Source: Adapted from Silva and Mendes (2020)

Figure 2 - Morphological characteristics of *Digitaria insularis*: A) perennial plant, B) panicle, C) tillering, D) seeds

Source: Machado et al. (2006)

Figure 3 - Root system of *Digitaria insularis* plants at 45 days after emergence. A) general view of the roots; B) details of rhizome formed (with yellow circle)

within 5 days at a photoperiod of 8–12 h (Pyon et al., 1977). Temperature requirements during sourgrass germination are linked to changes in the seed coat, which affect permeability to water and gas exchange. Additionally, temperature fluctuations can impact the balance of substances that either inhibit or promote seed dormancy (Mondo et al., 2010). Furthermore, sourgrass seeds exhibit some tolerance to water stress during germination, allowing them to effectively germinate and emerge under conditions of low soil moisture (Mondo et al., 2010).

The highest percentage and speed of sourgrass emergence occur when the seeds are buried 1 to 3 cm deep in the soil (Pyon et al., 1977; Martins et al., 2017). Consequently, plowing and harrowing can be effective control methods for this species when the seeds are buried deeper than the aforementioned depth. Due to slow initial growth (Lorenzi, 2000), sourgrass seedlings are most vulnerable for control with herbicides during the first 45 DAE (Figure 3). However, once the seedlings develop rhizomes, their control becomes challenging due to the increased accumulation of reserve nutrients and tissue differentiation (Machado et al., 2008; Timossi, 2009). Plants originating from rhizomes, which are rich in starch, grow vigorously (Machado et al., 2006), exhibiting a greater number of stomata and laminar thickness compared to plants from seeds (Silva, Mendes, 2020; Zabiole et al.,

2016). Additionally, sourgrass plants originating from rhizomes show differences in stomata, vascular bundles, parenchyma, xylem/phloem ratio, and trichomes, which diminish their susceptibility compared to plants from seeds (Silveira et al., 2018). These differences are attributed to the presence of rhizome starches, which act as a barrier to herbicide translocation, facilitating the rapid regrowth of treated plants (Tuffi Santos et al., 2004).

The flowering of the sourgrass occurs between 63 to 70 DAE (Machado et al., 2006), but flowering may occur earlier under high light conditions (Pyon et al., 1977). During this period, it produces and disperses seeds with low levels of dormancy, making emergence dependent on factors like soil moisture conditions and depth (Machado et al 2008). Additionally, sourgrass displays insensitivity to photoperiod for flowering. However, the longer the photoperiod, the faster the panicles form, leading to a greater accumulation of dry matter in an individual plant (Pyon et al., 1977).

3. Negative Impacts of sourgrass on agriculture

Sourgrass is one of the main weeds infesting Brazilian agricultural areas, predominantly in summer annual crops of the Central-west, South and Southeast regions (Lopez-Ovejero, 2017). However, it also affects perennial crops such as citrus, coffee and eucalyptus forest plantations (Barroso et al., 2021), in addition to urban areas, especially in municipalities near agricultural production areas (Gazola et al., 2019).

Sourgrass is a highly competitive plant that can lead to yield losses due to weed interference with crops. In maize-producing areas infested with this weed, yield was 32% lower compared to a plot free of sourgrass (Gemelli et al., 2013). More precise estimates determined that densities of 7, 15, and 30 plants m^{-2} reduced maize yield by 23, 38, and 50%, respectively (Barroso et al., 2016). In soybean cultivation, the impact on yield varies depending on the biological origin of the sourgrass plants, in addition to the plant density. In experimental plots where sourgrass plants were obtained from seed, densities of 2.1 plants m^{-2} reduced

soybean yield by 0–375 kg ha^{-1}, while 6 plants m^{-2} resulted in yield reductions of 600 and 1,300 kg ha^{-1} compared to sourgrass-free plots, where the average yield was 3,350 kg ha^{-1} (Gazziero et al., 2019). In other words, losses can reach up to 39% when sourgrass plants come from seeds. In field situations with plants from rhizomes, where the average yield of sourgrass-free plots was 2,250 kg ha^{-1}, soybean yield losses were observed starting from 1.2 clumps m^{-2} (up to 350 kg ha^{-1}). With five and ten clumps m^{-2}, yield decreased by 600–1,100 and 750–2,000 kg ha^{-1}, respectively (Gazziero et al., 2019). This demonstrates that sourgrass plants originating from rhizomes are more competitive and can cause yield reductions of nearly 90%. In coffee cultivation, a density of 16 plants m^{-2} reduced the growth of the coffee trees by 41% (Carvalho et al., 2013).

Competition mainly occurs for potassium and nitrogen, the two main macronutrients required by sourgrass, elements that can constitute up to 50% of the nutrients in crops (Carvalho et al., 2013). The problem is exacerbated when sourgrass exhibits glyphosate resistance, as GR plants do not incur fitness penalties compared to glyphosate-susceptible (GS) plants (Martins et al., 2017; Pereira et al., 2017). These characteristics collectively make the control of sourgrass very challenging (Bauer et al., 2021).

At the beginning of the 2010s, the majority of GR sourgrass populations were concentrated in the southern states of Brazil, particularly in the well-established soybean production areas. However, within a short period, numerous other GR populations were identified in the central and northern regions of the country, where soybean cultivation is relatively more recent (Lopez-Ovejero, 2017; Gonçalves-Netto et al., 2021). In 2017, an estimated 8.2 million ha of soybean were infested by GR sourgrass (Figure 4) (Adegas et al., 2017), accounting for 24.1% of the ~33.98 million ha planted area with this crop (Alcántara-de la Cruz et al., 2020). This percentage is similar to the 25.95% found in a later study, where it was observed that the distribution of GR sourgrass throughout the main Brazilian soybean-producing areas is irregular, ranging from 5% in Minas Gerais to more than 80% in Rio Grande do Sul (Gonçalves-Netto et al., 2022).

Of the 8.2 million ha infested with GR sourgrass, 5.5 million ha were infested with sourgrass alone, and 2.7 million ha with both GR sourgrass and GR *Conyza* spp. (Adegas et al., 2017). The cost of weed management in areas infested only with GR sourgrass increased 165% to 290%, with an average increase of R$ 318.3 ha^{-1}, compared to areas without GR sourgrass populations, where the average cost of weed management was R$ 120.0 ha^{-1} for that year. In areas infested with GR populations of this species and *Conyza* spp., the cost increased 222% to 400%, with an average increase of R$ 386.7 ha^{-1}, and in some cases, reaching R$ 479.5 ha^{-1} (Adegas et al., 2017).

According to the *Companhia Nacional de Abastecimento*, the total planted area for the 2022/2023 agricultural cycle was 77 million ha in Brazil (CONAB, 2023), which was
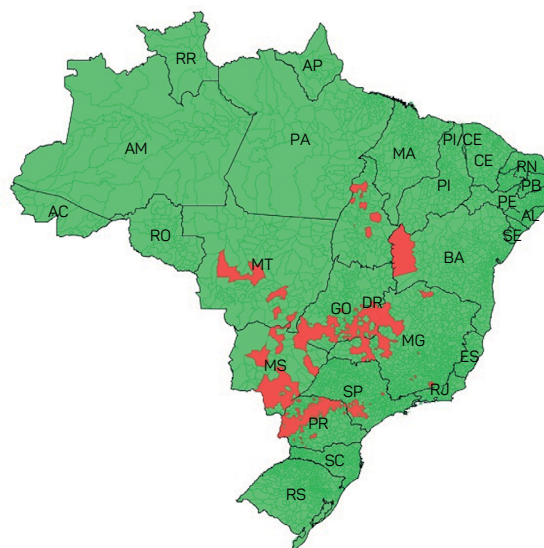
Figure 4 - Regions (red) infested with glyphosate-resistant *Digitaria insularis* in Brazil Source: Embrapa Trigo (2015)

similar to the planted area in 2017 (Alcántara-de la Cruz et al., 2020). The average increase of the weed management cost for the entire soybean area planted infested with GR sourgrass was R$ 2,792,180,000 (Adegas et al., 2017). While it cannot be assumed that all crops, either annual and perennial, had similar infestation levels with GR sourgrass as soybean, but extrapolating the values of this crop suggests that ~18.6 million ha of the total planted area in Brazil was infested by GR sourgrass populations in 2017, resulting in a weed management cost of R$ 6,333,841,707. As areas infested with GR sourgrass have continued to increase since 2017 (Gonçalves Netto et al., 2021), it can be conservatively be stated that at least 25% of the country's planted area has some degree of GR sourgrass infestation. However, unofficial sources have already indicated that as of 2022, approximately 80% of Brazil's agricultural area had some degree of GR sourgrass infestation (Bruna, 2018). Thus, when factoring in inflation and the rising prices of inputs (herbicides and fuels), the average increase in the cost of managing glyphosate resistance of this weed currently may exceeds R$ 10 billion (equivalent to USD $1.97 billion based on the direct exchange rate as of September 27, 2023) in the country.

4. Herbicide resistance status of sourgrass

Sourgrass was previously a weed species of little agronomic importance and was relatively easy to control using various herbicides (Silva, Mendes, 2020). However, the expansion of no-tillage areas, which favor the formation of clumps of sourgrass, and the rapid and extensive adoption of GM crops, have led to increased herbicide use. In these production systems, glyphosate is the most important herbicide (Green, 2018). This herbicide efficiently controls sourgrass plants that have not yet

formed rhizomes (Gazola et al., 2016). However, the effectiveness of glyphosate decreases when rhizomes have developed and clump plants have formed (Zabiole et al., 2016; Raimondi et al., 2019). In addition, the intensive use of this herbicide has led to the selection of GR populations (Lopez-Ovejero et al., 2017).

GR sourgrass has become one of the most competitive and significant weed in Brazil (Andrade Jr et al., 2018). In an effort to improve control of this species, growers increased both the doses and the frequency of glyphosate applications (Martinelli et al., 2022). However, this practice, combined with limited options for rotating herbicides with different mechanisms of action (MoA), has resulted in the emergence and spread of numerous GR populations of sourgrass in Argentina, Brazil, and Paraguay (Table 1). The first report of GR sourgrass occurred in 2005 in Paraguayan cotton, sunflower, corn, and soybean plantations (Heap, 2023). In Brazil, the first GR population was identified in soybean fields in Guaíra, western Paraná, in 2008. Since then, numerous studies have documented the occurrence of GR sourgrass populations in nearly all agricultural areas of the country (Lopez-Ovejero et al., 2017; Gonçalves Netto et al., 2021). In 2014, glyphosate resistance in this species was reported in Argentina (Table 1) (Heap, 2023).

The rapid spread of GR sourgrass populations raises concern in the Brazilian agricultural sector, mainly due to the scarcity of information related to the frequency and dispersal of the species (Silva, Mendes, 2020). The mechanisms of how the dispersal of GR populations has occurred in Brazil are unknown, but there is speculation that it might be linked to anthropogenic activities, mainly through the movement of machinery that was not sanitized or was not properly sanitized (Lopez-Ovejero et al., 2017; Gonçalves Netto et al., 2021). Molecular studies have indicated that the first GR sourgrass populations found in Brazil (Guaíra-Paraná) have a close genetic relationship with populations from Paraguay, which subsequently spread to other Brazilian states. However, there is evidence that some populations evolved resistance to glyphosate through independent selection processes (Takano et al., 2018), by high local selection pressure, playing an important role in the evolution of GR sourgrass populations throughout the country (Gonçalves Netto et al., 2021).

One of the strongest pieces of evidence that local management can contribute to the selection of GR sourgrass biotypes pertains to the hormetic effects (growth stimuli) induced by glyphosate (Brito et al., 2018), as herbicide hormesis can potentially influence the evolution of herbicide resistance (Belz et al., 2022). Because the application of a pesticide can results in a wide range of doses, spanning from 0% to 760% of the field dose (Velini et al., 2017). Thus, many weed plants are exposed to doses higher and lower than the recommended field dose of herbicide by direct application or by drift. The exposure of plants to herbicide subdoses can result in hormetic effects that improve the vegetative, phenological and reproductive development of weeds (Belz et al., 2022). In the case of sourgrass, it has been demonstrated that individuals, whether GR or GS, exposed to hormetic doses of glyphosate (ranging from 1.4 to 45 g ae ha^{-1}), flower up to 9 days earlier than untreated plants. Additionally, in treated plants, the seed weight increases by up to 29%, the germination rate is practically doubled from 37% in control to 70% at doses ranging from 5.6 to 22.5 g ae ha^{-1}, and the speed of germination occurs up to 10% earlier than in untreated plants (Anunciato et al., 2022).

The necessity to control GR sourgrass populations has led to the rotation of herbicides, primarily acetyl coenzyme A carboxylase (ACCase) inhibitor graminicides (Palharani et al., 2023). However, these herbicides pose a risk of selecting for resistance. In 2016, populations of sourgrass were reported with resistance to fenoxaprop-P-methyl and haloxyfop-P-methyl in the states of Mato Grosso and Mato Grosso do Sul. Shortly thereafter, in 2020, sourgrass populations exhibiting multiple resistance to glyphosate and clethodim, and cross-resistance to fenoxaprop-P-ethyl and haloxyfop-P-methyl were identified in both Center-West region of Brazil and the Department of Alto Paraná in Paraguay (Table 1) (Heap, 2023; Krzyzaniak et al., 2023).

Table 1. Summarize of the herbicide resistance status of *Digitaria insularis* globally (Heap, 2023)					
Year	Country	Crop	Herbicide	Mechanisms of Action*	Authors
2005	Paraguay (Alto Paraná)	Corn, cotton, soybean and sunflower	Glyphosate	EPSPS inhibitors	Adegas F, Bagateli J, Gazziero D, Viveiros EM, Nascimento E, Osipe R, Rodrigues C and Salas P A.
2008	Brazil (Paraná)	Corn and soybean	Glyphosate	EPSPS inhibitors	Adegas F and Gazziero D.
2014	Argentina (Santa Fe)	Soybean	Glyphosate	EPSPS inhibitors	Marzetti M.
2016	Brazil (Midwest Region)	Soybean	Fenoxaprop and haloxyfop	ACCase inhibitors	Melo MSC
2020	Brazil (Midwest Region)	Soybean	Glyphosate, fenoxaprop and haloxyfop	EPSPS + ACCase inhibitors	Christoffoleti P, Oliveira T and Melo MSC
2020	Paraguay (District of Hernandarias)	Soybean	Clethodim, glyphosate, and haloxyfop	EPSPS + ACCase inhibitors	Albrecht AP, Albrecht LP and Krzyzaniak P

* EPSPS – 5-enolpyruvylshikimate-3-phosphate synthase and ACCase – acetyl coenzyme A carboxylase

A survey conducted in the Brazilian Cerrado (Goias, Minas Gerais and Distrito Federal) documented the occurrence of multiple resistance to haloxyfop-P-methyl and glyphosate, identifying a homogeneous and widespread distribution of resistance to the EPSPS inhibitor (> 90% of the 56 biotypes tested) (Correia et al., 2020). In contrast, resistance to the ACCase inhibitor remained low (2.5%). Haloxyfop-P-methyl resistant sourgrass populations were found in the municipalities of Abadia dos Dourados in Minas Gerais State; and Montevideo, Padre Bernardo and Rio Verde in Goiás State. This indicates that although the incidence of resistance to ACCase inhibitors remains low, these resistant sourgrass biotypes are spreading or evolving throughout the main agricultural regions of the country. Infestation levels with sourgrass biotypes resistant to ACCase inhibitors could reach similar levels to those observed with glyphosate if appropriate management measures are not implemented to prevent the emergence and spread of this resistance.

5.   Resistance mechanisms of sourgrass

Understanding resistance mechanisms is essential for implementing appropriate management strategies (Alcántara-de la Cruz et al., 2020). For example, when resistance is conferred by target-site (TS) mechanisms, the rotation of MoA may be sufficient for control. If there is no cross-resistance, herbicides from a different chemical family of the same MoA can be used. If resistance is conferred by non-target site (NTS) mechanisms, management is more complex, often resulting in multiple-resistance to different MoAs, further reducing chemical management options. In cases of reduced absorption or translocation, adjuvants can improve the performance of the herbicide that selected the resistance (Palma-Bautista et al., 2021). Vacuolar sequestration of glyphosate, mediated by ATP-binding cassette transporters, can be competitively inhibited by alternative substrates (Ge et al., 2014). Furthermore, this is a saturable mechanism dependent on environmental conditions (Ge et al., 2011). Therefore, increasing the herbicide dose or applying it in colder periods can contribute to managing GR weeds with this mechanism.

Metabolic resistance, mediated by cytochrome P450 enzyme complexes, glutathione-S-transferases, or glycosyl transferases, is one of the most challenging (Rigon et al., 2020). However, this type of resistance can be reversible through the use of enzyme inhibitors, such as malathion, phorate, pyperonyl butoxide (PBO), and 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) (Busi et al., 2017; Oliveira et al., 2018; Palma-Bautista et al., 2023). These inhibitors can be used during the intercropping period to avoid crop damage, even when using the same herbicide that selected the resistance. In cases of resistance presenting both TSR and NTSR mechanisms, selecting management strategies, both chemical and non-chemical, must be approached with caution.

As can be seen, management of herbicide resistance varies according to the specific mechanisms at play. Implementing management without knowing the herbicide mechanism (s) involved can contribute to increasing herbicide resistance levels or selecting for multiple- or cross-resistance. Although studies are scarce, Brazilian scientists have made significant efforts to characterize resistance mechanisms in sourgrass, particularly those that confer resistance to glyphosate.

In the first study characterizing resistance mechanisms in GR sourgrass populations collected in the São Paulo State in 2009, reduced absorption, impaired translocation, metabolism of the herbicide, along with the Pro-106-Ser mutation in the *EPSPS* gene, were found to be the mechanisms responsible for glyphosate resistance (Carvalho et al., 2012). In other populations, mutations and differences in glyphosate absorption were observed, although not in its translocation (Melo, 2015), while populations collected from different regions of the São Paulo State exhibited mutations and *EPSPS* gene amplification (Galeano et al., 2016). In the most recent study, which included GR sourgrass populations from various states, it was challenging to characterize the specific mechanisms responsible for resistance (Melo et al., 2019). This suggests that other mechanisms not yet studied in sourgrass, such as vacuolar sequestration (Ge et al., 2010), cell exclusion (Pan et al., 2021), or even undiscovered mechanisms, could also be involved in the glyphosate resistance of sourgrass.

Information regarding mechanisms that confer glyphosate resistance in sourgrass has been subject to controversy among some Brazilian weed scientists. They have pointed out that there is no consensus for the mechanism responsible for conferring resistance to glyphosate, and that it remains unknown (Carvalho, Nicolai, 2016). However, these divergent results reveal that resistance to glyphosate in sourgrass can be governed by various mechanisms, either acting individually or in combination within a single plant or population. Therefore, it is essential to characterize resistance mechanisms individually in each population (Alcántara-de la Cruz et al., 2020). This information is crucial for developing appropriate integrated management programs, as control strategies can vary in complexity depending on whether the mechanism conferring resistance is of the TS, NTS, or both types of mechanisms. The difficulty in elucidating the resistance mechanisms is likely linked to the high genetic variability of sourgrass, which has an overall polymorphism rate of 56.6%. There is a high dissimilarity between populations because sourgrass is a species with cross fecundity, resulting in a varied genetic pool in reproduction (Martins et al., 2016).

Information on resistance to graminicides is still limited, and in the only study that characterized the mechanisms in sourgrass, it was found that the Trp2027Cys mutation in the *ACCase* gene confers low cross-resistance to pinoxaden and high-resistance to haloxyfop-P-methyl (Takano et al.,

2020). Fortunately, no sourgrass populations resistant to these herbicides displaying NTS resistance mechanisms have yet been found or reported. Otherwise, herbicide options for controlling this weed could become even more limited. This is because a mechanism like enhanced metabolism mediated by cytochrome P450 could potentially confer multiple resistance to herbicides with MoAs that have never been used for managing sourgrass.

6. Management methods of sourgrass

Few herbicides are available for the control of sourgrass, but starting in the 2010s, with the increasing reports of GR sourgrass, various research groups conducted extensive investigations to evaluate different herbicides, either individually or in mixtures, for the management of this weed (Barroso et al., 2017). A single application of paraquat, herbicide that was removed from the Brazilian herbicide market in 2021 (Agência Nacional de Vigilância Sanitária, 2020), did not completely eradicate sourgrass plants and resulted in regrowth (Zabiole et al., 2016). Diquat was not an efficient option for controlling sourgrass (Silva, Mendes, 2020). On the other hand, the use of ACCase inhibitors, especially "FOPs" (aryloxyphenoxypropionates) herbicides, has led to the rapid selection of resistant biotypes (Takano et al., 2020). When resistant sourgrass populations are presents, control becomes even more challenging, especially in cases of multiple resistance, which necessitates more complex and costly management strategies.

Considering the phenological stage of sourgrass plants is essential for achieving effective management (Silva, Mendes, 2020). Glyphosate efficiently controls GS sourgrass seedlings and GR seedlings are severely affected. However, GS seedlings over 45 days old, which have begun to tiller and clump together, also become difficult to control (Andrade Jr et al., 2018). Clethodim, fluazifop-p-buthy, tepraloxydim, clethodim, fenoxaprop-P-methyl, paraquat, haloxifop-P-methyl and imazapyr, when tested on sourgrass plants with up to two tillers, have demonstrated control levels exceeding 90% (Bauer et al., 2021; Correia et al., 2012; Correia et al., 2009; Zobiole et al., 2016; Barroso et al., 2014; Petter et al., 2015). However, once the plant has formed clumps and developed numerous propagation structures, control efficacy decreases to approximately 50% due to regrowth (Procópio et al., 2006). This is further exacerbated by the accumulation of high dry biomass and lignin content of plant tissues, which hinder herbicide translocation and action at the roots (Gilo et al., 2016). Therefore, it is recommended to implement control measures during the early phenological stages, when plant tissues are less developed, allowing for better absorption and translocation of herbicides (Zobiole et al., 2016).

The use of herbicides with different MoA in tank mixtures or sequential application is a common strategy, but it should be employed cautiously. In areas infested with grasses and broadleaf weeds, it is common to apply glyphosate or ACCase inhibitors mixed with synthetic auxins. However, it is important to note that 2,4-D and dicamba can antagonize ACCase inhibitors (Pyon et al., 1977), reducing translocation and increasing metabolism of 'FOPs' herbicides (Martins et al., 2017; Carvalho et al., 2021). Furthermore, when dealing with resistance, the first changes in the management should involve substituting active ingredients or combining herbicides with different MoA. The best results for control of GR sourgrass populations in Brazil after crop emergence have been achieved using ACCase inhibitors, either applied alone or in combination with glyphosate (Zobiole et al., 2016; Barroso et al., 2014; Carvalho et al., 2021; Melo et al., 2017). However, it is highly advisable to control sourgrass before crop sowing during the inter-season period (Silva, Mendes, 2020; Barroso et al., 2017). This can be achieved through the application of one or more herbicides, preferably systemic ones (Rudell et al., 2023; Kniss et al., 2022). This approach helps to reduce competition in the initial stages of crop development. Alongside early-stage herbicide applications to sourgrass, it is essential to employ cultural practices that prevent seed production.

6.1 Chemical control

Before planting crops, it is advisable to desiccate sourgrass, generally with more than one herbicide (sequential) application. Even though sourgrass is resistant to glyphosate, this herbicide still contributes to the control of other weed species and is included in applications alongside with ACCase inhibitors. Such as: clethodim, sethoxydim or haloxyfop-P-methyl (Gilo et al., 2016; Melo et al., 2017) are used before crop sowing. For plants with up to 3 or 4 tillers, a single application of glyphosate with graminicides effectively control sourgrass. However, in the case of clumped plants, sequential applications of broad-spectrum herbicides like glufosinate are necessary to manage regrowth. To control mature plants, it is advisable to mow them at a height of less than 20 cm, followed by the application of herbicides (typically a mixture of glyphosate and an ACCase inhibitor) when the regrowth reaches 15 cm (Raimondi et al., 2019). This practice depletes the nutritional reserves of perennial plant rhizomes, hindering subsequent regrowth. Mowing also reduces the need for herbicide applications after crop emergence, but implementation over large areas may be impractical.

During sowing or in the second desiccation before planting, herbicides with a residual activity that act in pre-emergence of weeds can be used for sourgrass control (Timossi, 2009; Andrade Jr et al., 2018; Patel et al., 2023; Tropaldi et al., 2017). Several preemergent herbicides are commonly used for this purpose such as: atrazine; clomazone; diclosulam; flumioxazin, alone or mixed with imazethapyr/diclosulam; S-metolachlor, alone or mixed with diclosulam; and trifluralin (Barroso et al., 2021; Silva, Mendes, 2020; Barroso et al., 2017; Gemelli et al., 2013;

Andrade, 2019). Acetochlor, a very long-chain fatty acid (VLCFA) inhibitor, is effective to control sourgrass in maize conventional tillage, but in no-tillage, exhibits high sorption in straw (Ferri et al., 2006). These herbicides are preferred because there are few or no cases of resistance registered in the country. However, it is essential to use these herbicides carefully depending on the next crop that will be planted in the area. For example, atrazine is not selective for soybean, while some ALS inhibitors are not selective for maize. Additionally, the dissipation half-life time (DT_{50}) of the herbicide must be considered to ensure it degrades before planting the next crop. Therefore, applications should be made 7 to 90 days before sowing, depending on the specific herbicide used.

After crop emergence, controlling sourgrass becomes challenging, especially when individual plants have regrown after desiccation, as there are limited herbicide options safe for the crops in question. Consequently, herbicide choices are restricted to those that are selective for the specific crop being grown. In soybean, ACCase inhibitors such as clethodim; sethoxydim; haloxyfop-P-methyl; and the acetolactate synthase (ALS) inhibitor imazapyr (in cultivars with Cultivance technology) have been employed in combination with glyphosate (Barroso et al., 2021). For maize, atrazine, mesotrione and nicosulfuron are recommended for sourgrass control (Zobiole et al., 2016; Barroso et al., 2014; Melo et al., 2017). The use of ALS inhibitors in mixture with glyphosate, mesotrione + atrazine + glyphosate, and nicosulfuron + atrazine has proven to be a very effective method for controlling sourgrass during pre-sowing soybean desiccation (Palharani et al., 2023). However, the effectiveness of atrazine against sourgrass is limited to its early developmental stage (Melo et al., 2017). As sourgrass plants mature, controlling them becomes more challenging due to the lack of herbicides with MoA that target both annual and perennial grasses (Silva, Mendes, 2020). Managing sourgrass becomes especially problematic in crop rotation that includes maize, since the crop has characteristics similar to those of the weed.

In the short term, there are no prospects for developing new management tools or discovering novel herbicides to aid in the control of herbicide resistant-sourgrass during the crop development cycle. Preserving the efficacy of ACCase inhibitors is crucial, as these herbicides are the primary means of controlling GR sourgrass (Takano et al., 2021). Some protective measures that may extend the useful life of the active ingredients of this MoA may include using them only in specific situations, ensuring appropriate environmental conditions, maintaining application equipment in good condition and calibrated, as well as avoiding both overdosing and underdosing to prevent the emergence and spread of new sourgrass biotypes resistant to ACCase inhibitors.

6.2 Non-chemical control

Non-chemical methods can effectively reduce sourgrass competition with the desired crop. These strategies encompass mechanical and cultural approaches, mainly for sourgrass plants from rizhomes. The management practices include manual removal of clumps, crop rotation, anticipation or delay of sowing dates, modified crop spacing, and the use of straw as mulch. The mulch act as a physical barrier to weed emergence and the growth of seedlings (Petter et al., 2015; Raimondi et al., 2019). Sourgrass seeds have limited longevity in the soil, and the presence of mulch significantly reduces their germination rates (Patel et al., 2023; Mechi et al., 2018). This makes mulch a valuable component of integrated weed management practices.

Utilizing different cover crops such as black velvet bean, pigeon pea, and Uroclhoa, and applying straw mulch at rates exceeding 4 t ha^{-1}, has proven highly effective in controlling sourgrass. These methods achieve control and germination inhibition rates exceeding 90% (Petter et al., 2015; Barroso et al., 2021). The supply of straw can serve as a long-term solution to combat sourgrass infestations of plants from seeds, as well as the regrowth of plants from rhizomes. For instance, straw amounts of 3 t ha^{-1} of sugar cane and corn reduced the germination of both GR and GS sourgrass biotypes by 90 and 86%, respectively. Nine tons ha^{-1} of sugarcane straw prevented the germination of sourgrass seeds. Additionally, the implementation of preventive measures such as sanitization of agricultural implements, cleaning of ditches and fences is essential to reduce the initial sources of infestation and the dispersion of propagules, thus contributing to successful sourgrass management.

Biological weed control remains relatively unexplored, mainly due to the challenges of implementing it on a large scale (Roberts et al., 2022). Recent studies have documented sourgrass infection by fungi, shedding light on the potential of biological control as a non-chemical alternative for managing this weed. In early 2022, sourgrass plants found in the municipality of Ubá, Minas Gerais, exhibited severe foliage blight symptoms, which increased in size as the plants matured. These symptoms were caused by *Bipolaris/Curvularia*-complex specifically *Bipolaris yamadae* (Alves et al., 2023). Additionally, *Colletotrichum truncatum* was recently identified as the causative agent of severe anthracnose in sourgrass (Tikami et al., 2023). However, *C. truncatum* is the main species of fungus associated with anthracnose in soybean (Boufleur et al., 2021), suggesting that sourgrass could serve as a host for this disease in soybean (Tikami et al., 2023), potentially limiting its use as a biological control agent.

7. Conclusions

Sourgrass remains and will continue to be one of the most significant challenges in managing herbicide

resistance in the southern hemisphere of the Americas. With at least 25% of Brazilian agricultural areas affected by herbicide-resistant sourgrass populations, leading to a surge in weed management costs of up to 400%, it is advisable to implement year-round management measures. Taking into account the biological and ecological characteristics of sourgrass, a combination of chemical control and non-chemical methods must be implemented.

When dealing with herbicide-resistance sourgrass populations, it is essential to avoid making generalizations because each case is unique and influenced by several factors. To development effective management strategies, it is crucial to accurately identify resistant biotypes through field surveys and characterize their specific resistance mechanisms. Unfortunately, due to the lack of this information, alternative herbicides are often misused, leading to increased resistance to glyphosate and the emergence of sourgrass biotypes resistant to ACCase inhibitors. It is also important to ensure that research results integrating aspects of biology, resistance mechanisms, and management measures are effectively communicated to farmers.

It is best to initiate management before planting the crop, utilizing a variety of herbicide action mechanisms that do not harm (carryover) the crop of the next growing season. This is important because weed management options after crop emergence are limited. Additionally, preventive measures such as controlling initial outbreaks and maintaining the cleanliness of machinery, ditches and fences are essential for successfully managing herbicide-resistant sourgrass biotypes. Preserving the effectiveness of ACCase inhibitors is also critical because there is a shortage of new herbicides and weed management tools for controlling sourgrass.

Author's contributions

All authors have read and agreed to the published version of the manuscript. KFM and RAC: conceptualization. GSA, HMS, and AJMS: methodology. KFM, MFGFS, and RAC: validation. GSA, HMS, KFM, AJMS, MFGFS, CAC, and RAC: investigation. MFGFS, and RAC: resources. GSA, HMS, KFM, and RAC: data curation. GSA, HMS, KFM, AJMS, CAC, MFGFS, and RAC: writing—original draft preparation. RAC, KFM, and CAC: writing—review and editing. KFM, CAC, and RAC: visualization. MFGFS, and RAC: supervision, project administration, and funding acquisition.

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