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# Herbicide Resistance in Brazil: Status, Impacts, and Future Challenges

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## Abstract

Brazil is a large producer and exporter of crops in global terms. Weeds may be responsible for ~14% of crop losses, depending on the crop system. Herbicides occupy 58% of the Brazilian pesticide market; however, the continuous use of these products and the high selection pressure have led to the emergence of weeds resistant to herbicides. Today, there are 51 weed species reported as being resistant to herbicides in Brazil, of which 17 involves cross and multiple-resistance. Acetolactate synthase (ALS), acetyl coenzyme A carboxylase (ACCase) and 5-enolpiruvylshikimate-3-phosphate synthase (EPSPs) inhibitors are the herbicidal groups with the most resistance cases. Soybean, corn, rice, wheat and cotton present 30, 12, 10, 9 and 8 cases, respectively, occurring mainly in herbicide-resistant crop fields from the Southern and Central West regions of the country. To better understand the dimensions of herbicide resistance, in this chapter, we will explore the size of agricultural activity in Brazil, the pesticide market and the use of herbicides in the main crops. In addition, the agronomic, scientific-technical and economic aspects that have contributed, directly or indirectly, to the selection of resistant weeds will be discussed in order to have an overview of the economic impact of herbicide resistance management.

**Keywords:** Brazilian pesticide market, glyphosate-resistant crops, herbicide resistance mechanisms, integrated weed management, management cost of weed

## 1. Introduction

Brazil is one of the leading manufacturers and exporters of food, fibers and energy, being one of the largest producers of coffee, maize, grapes, oil plants, oranges (fruit and juice), soybeans, sugarcane and meat [1]. These agricultural commodities have a crucial role in the development and agribusiness of the country, being the focus of Brazilian production and exports [2]. Agricultural pests limit global food security by reducing crop yields [3, 4]. The crop losses caused by pest can be over 80% if they are not controlled. Even when pests are controlled, crop yield losses range from 23 to 38% [4]. To reduce these losses, synthetic pesticides have become the main pest management tool globally [5].

Brazil has the fourth largest cultivation area worldwide, after India, China, and the United States; however, to ensure its agricultural productivity, Brazil has become in the largest pesticide market since 2011 [6]. The pesticides consumption increased 300% from 1991 to 2010 in this country [5]. Although weeds are responsible for ~14% of crop losses, depending on the agricultural system and crop situation [4], herbicides represent ~58% of the Brazilian pesticide market, where only the herbicide glyphosate occupied 35% of pesticide sales [7]. This increase has been largely due to the cultivation of herbicide resistant (HR) crops, mainly those resistant to glyphosate (GR) [8]. In addition, the loss of the glyphosate patent by Monsanto in 2000, and consequently a reduction of its price, was decisive for its widespread use as the main tool to control weeds in GR crops as well as another agricultural systems [9]. The almost exclusive reliance of glyphosate to control weeds, but not only, selected for glyphosate resistant weeds forcing to test/use alternative herbicides to control them.

Know the cause of the herbicide resistance, i.e., characterize the resistance mechanisms that govern it, is important for the proper choice of management methods [10]. However, of the 51 cases of herbicide resistance recorded for Brazil [11], only in few cases such resistance mechanisms have been studied [12–16]. Of the 17 cases of multiple or cross-resistance reported in Brazil, 14 occurred in the last 10 years. The most worrying case is *Conyza sumatrensis*, which was found as being resistant to the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) and photosystem I and II (PSI and PSII), protoporphyrinogen oxidase (PPO) inhibitors and synthetic auxins in a GR-soybean field from Assis Chateaubriand, Paraná [11]. Considering these data, the trend is that cases of herbicide resistance, mainly of the multiple resistance, continue increasing in the coming years in Brazil, if little effort is devoted to understanding the cause of herbicide resistance.

In this chapter, we will describe the current overview of the situation of resistance to herbicides in Brazil, discuss the agronomic, scientific, technical and economic factors that have contributed, directly or indirectly, to increase cases of herbicide resistance, as well as the future trends of these agronomic issues according to the weed management measures that are currently being implemented in the country.

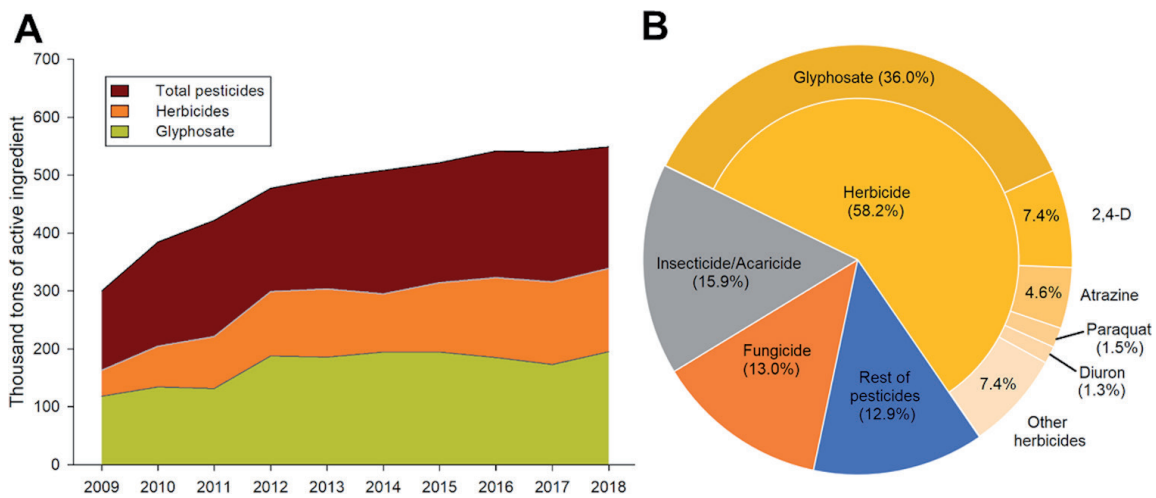
## 2. Pesticide use in Brazil

Brazil, with 77.8 million ha (8.9% of the national territory) in 2018 and with the goal of obtaining 85.7 million ha in 2029 [17], is one of the largest agricultural powers in the world. The area planted in Brazil represents only 3.4% of the global planted area, while countries like India, United States, China and Russia contribute with 9.68, 9.06, 8.96 and 8.38%, respectively [18]. However, Brazil is the main consumer of pesticides since 2011 (20% of the global market in 2017) [6, 7, 19].

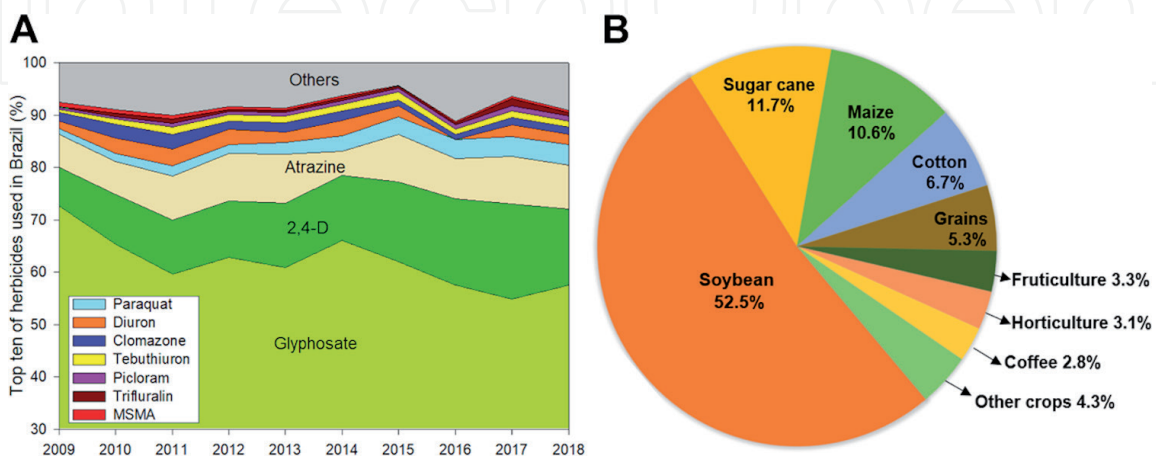
Pesticide consumption has almost doubled from 300.5 thousand tons of pesticide active ingredient in 2009 to 549.3 in 2018 in Brazil (**Figure 1A**). According to the pesticide trade reports of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) [7], the use profile of pesticides has maintained a growth and similar trend in use in the last 10 years (2009–2018), where the sale of insecticides/acaricides and fungicides accounted for 28.9% of the national market, and the other classes of pesticides (nematicides, bactericides, adjuvants, growth regulators, etc.) occupied only 12.9%. However, the most striking is that herbicides are the products that dominate the national pesticide market with 58.2%, i.e., of every 10 kg of pesticides sold, 5.8 kg were herbicides. Additionally, the

herbicide market has been dominated by five active ingredients (glyphosate, 2,4-D, atrazine, paraquat, and diuron), but glyphosate accounts for 36% of the national market (**Figure 1B**). Sales of glyphosate grew from 118.5 thousand tons of active ingredient in 2009 to 195.0 in 2018, i.e., increased 65% (**Figure 1A**).

As already noted, herbicides were the main pesticide class used in Brazil between 2009 and 2018, with oscillations from 52.4% (2011) to 62.5% (2012). The top 10 active ingredients used in this period were: 2,4-D, atrazine, paraquat, diuron, clomazone, tebuthiuron, picloram, trifluralin, MSMA, with some peaks in specific years of clethodim, hexazinone, and triclopyr, but the main herbicide has been glyphosate, consuming more than 50% of the herbicide market in the country (**Figure 2A**). In percentage terms, glyphosate consumption decreased 15% from 2009 (73%) to 2018 (58%) in favor of the use of other herbicide active ingredients that increased sales such as atrazine, 2,4-D and paraquat. The last active ingredient, with an average of 2.6% in the period 2009–2018, presented a regular increase in its sales going from occupying 1.2% of the herbicide market in 2009 to 3.9% in 2018. Already 2,4-D and atrazine have presented a variable preference on the



**Figure 1.** (A) Commercialization of pesticides (tons of active ingredient × 1000) from 2009 to 2018 in Brazil. (B) Pesticide market share (%) according to their biological activity. Charts were constructed from the pesticide trade reports of the IBAMA [7].

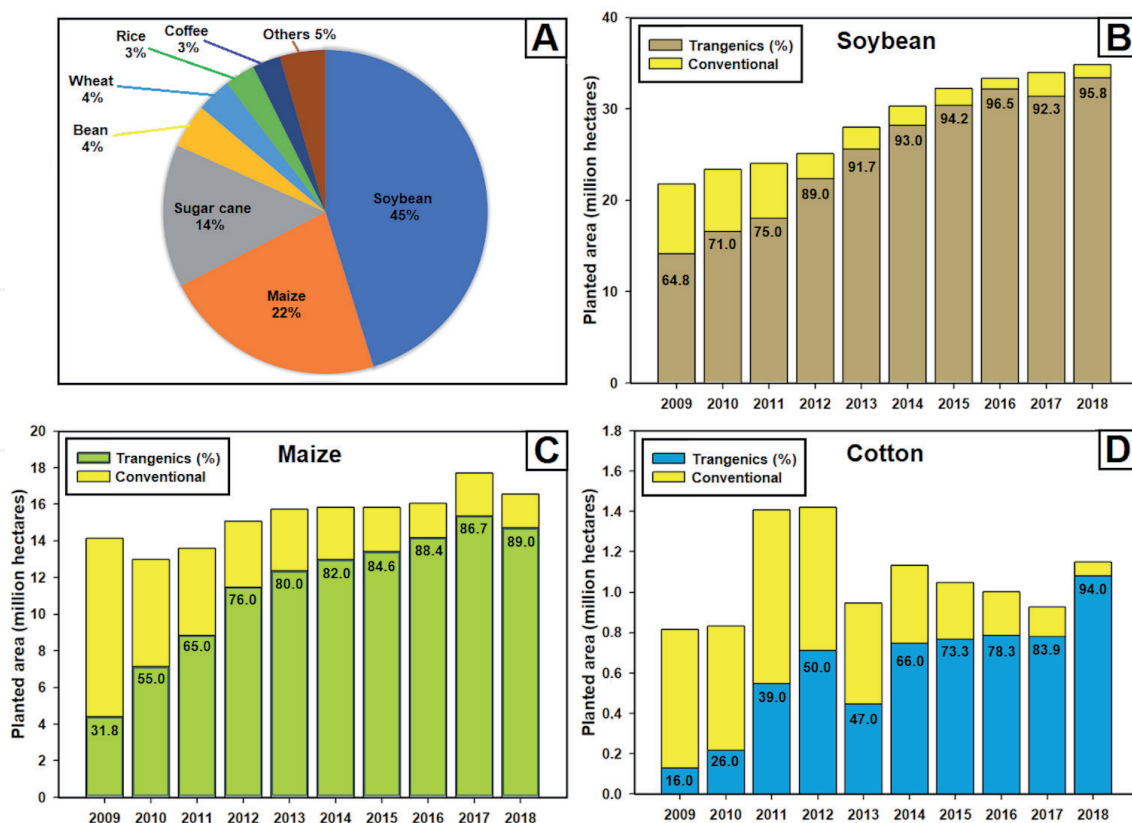


**Figure 2.** (A) Percent evolution of the herbicide market in Brazil from 2009 to 2018. (B) Percentage of pesticides occupied in the main production systems of the country. Charts were constructed from the pesticide trade reports of the IBAMA [7].



part of the farmers. For example, 2,4-D (average of the period 12.7%) occupied 7.4% of the herbicide market in 2009, however, in 2017 it reached 18.2%, while atrazine (7.8%) represented the 4.7% in 2014 reached its highest peak in 2013 with 9.4% (**Figure 2A**). A large part of pesticides used in Brazil (81%) is destined to the production of four crops. Soybean is the main consumer being responsible for 52.2% of sales, followed by sugarcane (11.7%), maize (10.6%), and cotton (6.7%) (**Figure 2B**) [20].

The increase in the use of pesticides is related to the evolution of agricultural production, mainly to the increase of agricultural areas destined to monoculture of transgenic crops, i.e., crop varieties that carry traits of resistance to herbicides (HR), insects and diseases, mainly the events that stack glyphosate resistance (GR) traits [21]. According to the Instituto Brasileiro de Geografia e Estatística (IBGE) [22], more than 45% of Brazil's cultivated area is occupied by soybean followed by maize (22%) and sugarcane (14%) (**Figure 3A**), which contributed 62% of the value of agricultural production in 2017 [1]. Between 2009 and 2018, soybean, maize, and cotton showed increases in cultivated area of 60, 17, and 41% [21]. However, the highest growth was observed in relation to the area destined for the cultivation of GR crops. For example, in 2008 there were 14.1 million hectares (64.8%) of GR soybean, but in 2018 the area destined for GR soybeans had more than doubled, occupying 33.4 million hectares (95.8%) (**Figure 3B**). The total area destined for the cultivation of maize showed a lower growth, but the area cultivated with GR varieties tripled in the same period from 4.4 million hectares (31.8%) in 2009 to 14.7 (89%) in 2018 (**Figure 3C**). The total cultivated area of cotton had highs and lows in this period, where the area devoted to the cultivation of GR varieties remained constant between 2012 and 2017 with ~0.75 million hectares.



**Figure 3.**

(A) Percentage of planted area by type of crop in 2015, and total area (million ha) and percentage occupied by transgenic varieties resistant to herbicides of soybean (B), maize (C), and cotton (D). Charts were constructed from the municipality productivity reports of the IBGE [22] and the Conselho de Informações sobre Biotecnologia [21].

However, the area of conventional varieties was reduced from 50 to 16% in the same period. Today, 94% of the area devoted to cotton production is occupied by GR varieties (**Figure 3D**).

The increase in area cultivated with GR varieties has impacted the pesticide market, since more than 70% of pesticides are used in the cultivated area with these crops. However, pesticide statistics do not provide information on how defenses are used in individual crops; therefore, it is not possible to conclude how pesticide use has changed as a result of large-scale adoption of GR varieties [23]. However, this scenario, specifically the herbicide market, reflects the great concern of farmers about the interference of weeds in the agricultural production, but also, how the use and high dependence of these products have had a direct impact on the selection and emergence of weeds resistant to herbicides.

### 3. History and status of herbicide resistance

The rapid acceptance of GR crops, but not only, the addition of new productive areas and the increasing difficulty in obtaining labor in the fields, has established herbicides as the main control tool, even in integrated systems of weed management. This almost exclusive dependence on herbicides for weed management has contributed to the selection of herbicide resistant weeds with higher frequency. Herbicide resistance is the inherited ability of a plant to survive following application of the commercially used dose of the herbicide recommended for its control [24]. Currently 262 weeds (152 dicots and 110 monocots) have presented 512 unique cases (species x site of action) of herbicide resistance worldwide in 93 crops in 70 countries [11]. In Brazil, there are 51 weed species resistant to herbicides confirmed.

The Brazilian situation of weed resistant to herbicides, mainly to the acetoacetate synthase (ALS) and acetyl coenzyme A carboxylase (ACCase) inhibitors, in conventional soybean cultivation in the mid-2000s was already considered unsustainable due to control difficulties, high cost and low efficiency of the available herbicides to control weed resistant species. The solution to this problem was the introduction of GR soybean varieties [23, 25]. Therefore, to understand the current status of herbicide resistance, it is important to note that GR crops were officially approved in 2005 in Brazil, although GR soybean was irregularly introduced and cultivated in Rio Grande do Sul since 2000. Therefore, the chronological appearance of herbicide resistant weeds is divided into two periods: the pre-glyphosate era preceding 2005 when the use of herbicides was more diversified, and the post-glyphosate era, beginning after approval of GR crops involving an almost exclusive use of glyphosate. In the pre-glyphosate era, from 1993 to 2004, 16 cases were reported, of which only one case presented multiple resistance to two sites of action. In the post-glyphosate era, 35 cases have been reported, of which 16 are cases of multiple resistance. The weed genera with the most resistance cases are *Amaranthus* (7), *Conyza* (8), and *Lolium* (5) (**Figure 4**).

The main groups of herbicides with resistance are the ALS, ACCase, EPSPs, and PSII inhibitors with 30, 9, 16, and 7 cases, respectively (**Figure 5A**). The crop systems with more frequency of herbicide resistance were soybean (30), maize (12), rice (10), wheat (9), and cotton (8) (**Figure 5B**). The Southern, comprising the states of Paraná, Santa Catarina and Rio Grande do Sul, and the Central-West (only in Mato Grosso and Mato Grosso do Sul) regions present 82% of the cases, being Paraná the state where more cases of herbicide resistance were reported (**Figure 5C**). Most of these cases were found in GR crop fields and occurred after 2005, i.e., in the post-glyphosate era, evidencing the drastic changes that GR crop technology caused in weed management.

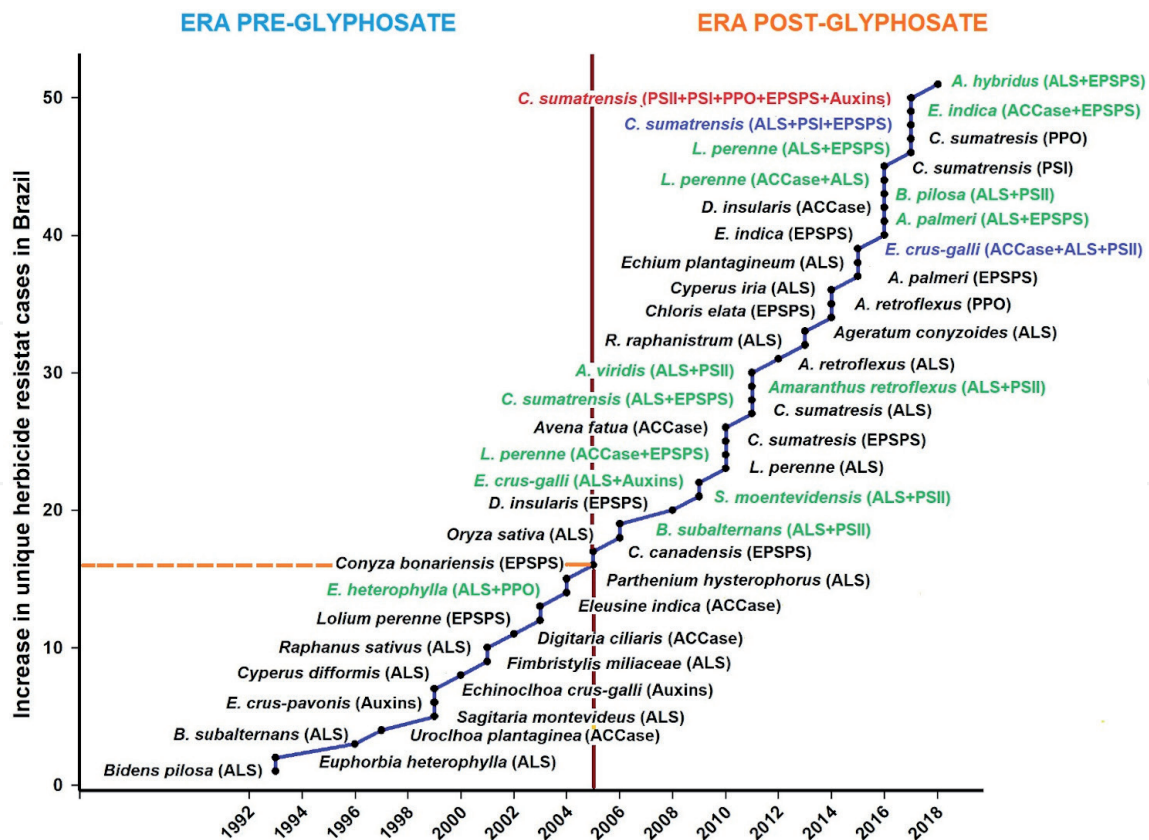


Figure 4.

History of reports of herbicide-resistant weeds in Brazil. Vertical bar indicates the official introduction of transgenic crops resistant to glyphosate. Chart was constructed from the information available in the International Survey of Herbicide Resistant Weeds database [11].

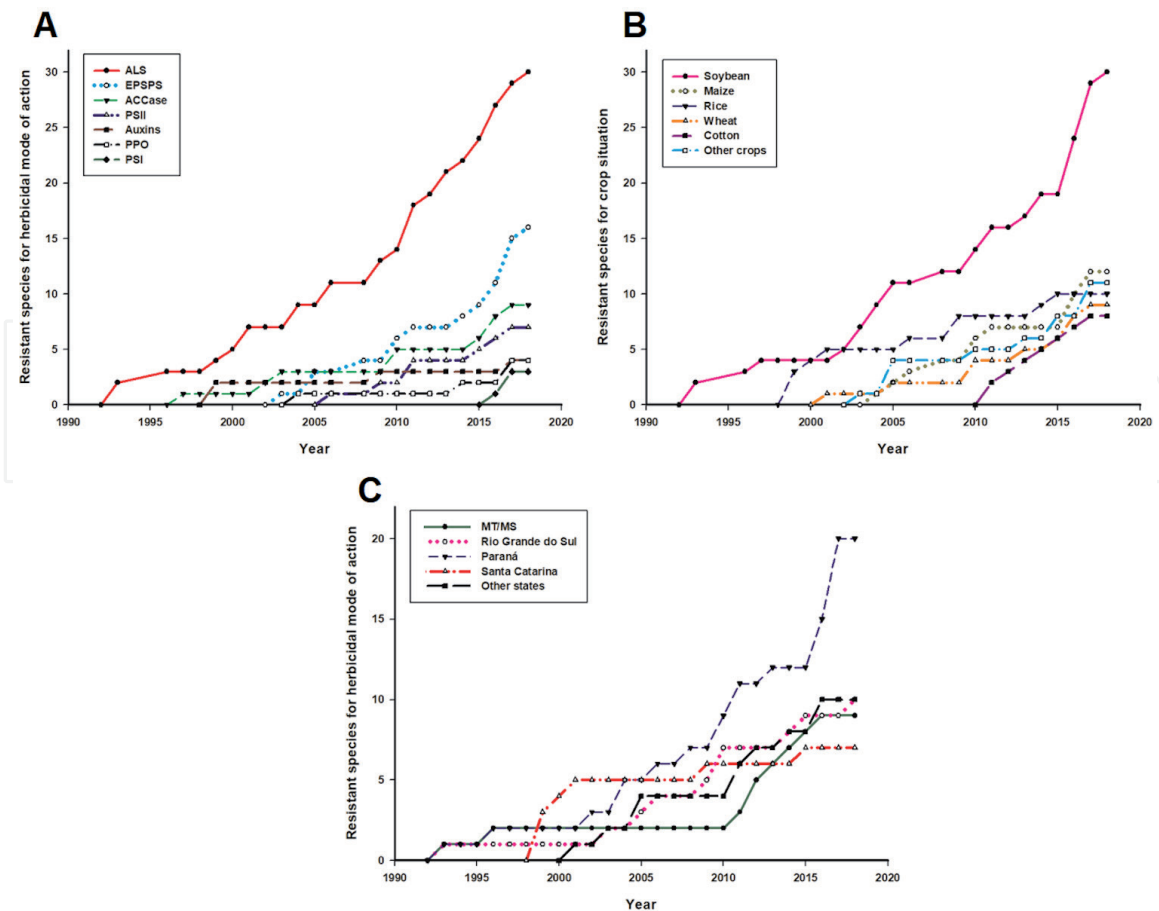
### 3.1 Resistance to ALS inhibitors

The first cases of resistance to ALS inhibitors were *Euphorbia heterophylla* and *Bidens pilosa* reported in 1993 in soybean areas in the states of Mato Grosso do Sul and Rio Grande do Sul, which showed cross-resistance to sulfonylureas and imidazolinones [26]. After, resistant biotypes of *B. subalternans* (1996) [27], *Parthenium hysterophorus* (2004) [28], *Conyza sumatrensis* (2011) [29] and *Ageratum conyzoides* (2013) were found in Paraná. The latter species was also reported in cotton in Mato Grosso [11]. However, the greatest resistance challenges to ALS inhibitors are found in irrigated rice cultivation. The species reported with ALS resistance in this culture are: *Sagittaria montevidensis* (1999) [30], *Echinochloa* sp. (1999) [31], *Cyperus difformis* (2000) [32], *Fimbristylis miliaceae* (2001), *Oryza sativa* (2006), and *Cyperus iria* (2014) [33] in Rio Grande do Sul and Santa Catarina.

Cases of resistance in rice cultivation are associated with the rapid adoption of Clearfield® technology (crops tolerant to imidazolinones, a chemical group of ALS inhibitors), which were introduced in 2002 in areas of southern Brazil [34]. Although the emergence of new resistant species after the adoption of Clearfield® cultivars did not increase significantly, the dispersion of weed populations resistant to ALS inhibitors, mainly of red rice, was favored by genetic flow of cultivated rice to red rice, representing a great agricultural, economic, and social restriction in the use of Clearfield® technology [35].

Other specific, but not least, cases of resistance to ALS inhibitors are *Raphanus sativus* (2001), *Lolium multiflorum* (2010), and *R. raphanistrum* (2013), found in wheat and barley in Rio Grande do Sul and Paraná; and *Amaranthus retroflexus* (2012) in cotton in the states of Mato Grosso, Mato Grosso do Sul and Goiás [11, 36, 37].





**Figure 5.** History of reports of herbicide-resistant weeds in Brazil per mode of action of herbicide (A), crop situation (B), and state of first record (C). MT/MS are the abbreviation of the states Mato Grosso and Mato Grosso do Sul. Charts were constructed from the information available in the International Survey of Herbicide Resistant Weeds database [11].

### 3.2 Resistance to EPSPs inhibitors

Currently, nine weed species have been reported with glyphosate resistance in Brazil, some of these species have multiple resistance to other modes of action [11]. *Lolium multiflorum* (2003) was the first species identified with glyphosate resistance in orchards and vineyards from Rio Grande do Sul [38]. After, *Conyza bonariensis* (2005), *C. canadensis* (2005) [39], *C. sumatrensis* (2010) [40], *Digitaria insularis* (2008) [12], *Chloris elata* (2014) [13], *Amaranthus palmeri* (2015) [14], *Eleusine indica* [15], and *A. hybridus* (2018) [11] were identified with this resistance mainly in maize and soybean, and wheat fields, but also in citrus and coffee orchards in the states of Mato Grosso, Paraná, Rio Grande do Sul and São Paulo.

With the exception of *L. multiflorum*, the selection of glyphosate resistance in these species is related to the use of GR cultivars, which has also influenced their dispersion throughout the country. Resistant populations of *L. multiflorum* have gone from infesting apple orchards and vineyards to invading GR-soybean fields in the southern states of Brazil [41]. The species of the genus *Conyza*, which have a high invasive potential due to the large seed production, the rapid and high germination capacity, cause great damage to agriculture, and due to their poor interspecific differentiation, it can be an exchange of resistant alleles between species [42]. However, *D. insularis* has been, among glyphosate resistant species, one of the main problems to be faced; therefore, greater efforts have been made to characterize the factors involved in its resistance, dispersal and management [12, 43–48]. Molecular studies showed that the first glyphosate resistant *D. insularis* populations found in the country (Guairá—Paraná) came from Paraguay



and were dispersed to other states of Brazil, partly due to their biology and perennial capacity, but mainly due to anthropogenic activities, such as the lack of cleanliness of agricultural implements, but also events of independent selection [47, 48].

Other weeds that pose a major challenge to Brazilian agriculture are species of the genus *Amaranthus*, as they are often reported with glyphosate resistance in GR fields in the United States and Argentina [49]. In addition, *Amaranthus* sp. can hybridize interspecifically facilitating dispersion of resistance alleles [50]. In Brazil, *A. palmeri* was reported to have glyphosate resistance in 2015 [11], when its multiple resistance to the ALS inhibitors was also corroborated [14]. However, the Instituto Mato-Grossense do Algodão had records of the occurrence of glyphosate resistant populations of this species since 2012 in the municipalities of Ipiranga do Norte and Tepurah, Mato Grosso, that was imported from Argentina in cotton harvesting machines in 2011 [51]. Recently, multiple resistance of *A. hybridus* to glyphosate and ALS inhibitors was also confirmed in Rio Grande do Sul in soybeans [11, 52]. With respect to the latter case, there is great concern because it is feared that it has also been introduced from Argentina, where populations of *A. hybridus* with this resistance profile carry mutations in the genes encoding the target enzymes [53]. In the case of glyphosate resistance, it is a triple mutation that confers high levels of resistance and that had not previously been observed in any other species [10, 54]. In addition, in Argentina there are also populations of the species with multiple resistance to 2,4-D and dicamba [55]. Therefore, if it is confirmed that the resistant populations of *A. hybridus* found in Brazil were introduced from Argentina, the scenario faced by Brazilian farmers in the coming years in relation to weed management will be very difficult.

### 3.3 Resistance to ACCase inhibitors

*Urochloa plantaginea* (1997) [56], *Digitaria ciliaris* (2002) [57], *Eleusine indica* (2003) [58], *Avena fatua* (2010) [11], and *D. insularis* [16] were reported with resistance to ACCase inhibitors, mainly in non-transgenic soybean fields. These findings demonstrate the importance of these herbicides for the control of grasses in soybean fields, due to the low availability of selective herbicides that effectively control these weeds in pre-emergence conditions, allied to the difficulties of using graminicides, since these products have high retention in the organic matter [29].

### 3.4 Resistance to other mechanisms of action

The majority of herbicide resistance cases reported in Brazil are included in the three groups of herbicides described above, following the global trend. However, cases of resistance to other modes of action have also been found. In 1999, *Echinochloa crus-gavonis* and *E. crus-galli* were reported with resistance to synthetic auxins, specifically quinclorac, in rice fields of Itajai, Santa Catarina [59]. *Amaranthus retroflexus* (2014) and *C. sumatrensis* (2017) were reported with resistance to PPO inhibitors [11]. The first showed fomesafen resistance and it was found in GR-soybean and -cotton fields of Mato Grosso; and *C. sumatrensis* presented resistance to saflufenacil in soybean fields in the western region of Paraná in the municipalities of Palotina and Assis Chateaubriand [11]. This last species had already been confirmed to be resistance to chlorimuron-ethyl (ALS inhibitor) in 2011 [60] and paraquat (PSI inhibitor) in 2016 [61] within the same region.

### 3.5 Cross- and multiple-resistance

Cross resistance is expressed when a weed resistant biotype shown resistance against two or more herbicides with the same mode of action, and multiple

resistance occurs when a weed resistant to a given herbicide manifests resistance to two or more different modes of action. Most cases of resistance to ALS inhibitors have cross resistance, that is, weeds resistant to imidazolinones often have a degree of resistance to sulfunylureas and vice versa [29]. *Eleusine indica* resistant to sethoxydim (cyclohexanediones) showed resistance to the ariloxifenoxipropionatos (FOPs) [58], and quinclorac resistant *E. crus-galli* showed cross resistant to others synthetic auxins [11]. Weeds with cross resistance represent a great challenge for Brazilian agricultural sustainability; however, weeds with multiple resistance are more challenging by reducing chemical alternatives for their control.

The occurrence of multiple resistance has increased significantly in recent years, and most of the reported cases occurred in the post-glyphosate era. The first case of multiple resistance was *E. heterophylla*, which was found in fields of maize and soybeans in 2004 and showed resistant to triclopyr and fomesafen (ALS + PPO) [62]. In 2009, *E. crus-galli* was found with resistance to synthetic auxins and ALS inhibitors in rice fields in Rio Grande do Sul [34]. Biotypes of *B. subalternans* (2006) and *B. pilosa* (2016) were found to be resistant to atrazine (PSII inhibitors) and ALS inhibitors in soybean and maize fields from Paraná [63]. Among the cases that involves glyphosate resistance are *C. sumatrensis* (2014), *A. palmeri* (2015) and *A. hybridus* (2018) as dicots, that also shown resistance to the ALS inhibitors and were found in soybean fields [11, 14, 60], and *L. multiflorum* (2010), *D. insularis* (2016), and *E. indica* (2016) as monocots with resistance to the ACCase inhibitors. However, the most worrying case is *Conyza sumatrensis* reported in 2017, which was found as being resistant to EPSPs, PSI, PSII, PPO and synthetic auxins in a GR-soybean field from Assis Chateaubriand-PR [11].

This brief account shows the global scenario of the current situation of herbicide resistance in Brazil; however, it is far from reality, because only the first occurrence of a unique case (species x site of action) is reported, while in countries like the United States and Australia, there are multiple reports for the same unique case of herbicide resistant occurring in different regions. For example, the case of *A. palmeri* resistant to glyphosate have more than 30 reports along of the United States [11]. To have an idea of the real problem in Brazil, we have as an example the study conducted by Lopez-Ovejero et al. [45], who determined the frequency and dispersion patterns of glyphosate resistant *D. insularis* revealing the existence of 1299 (of 2596) populations with different resistance levels to this herbicide distributed only in the areas of soybean production. In the scientific-academic environment it is commonly said that it is more difficult to find a population susceptible to the glyphosate of *C. sumatrensis* or *D. insularis* than a resistant one. In addition, from the botanical point of view, more species of the Amaranthaceae, Asteraceae, Cyperaceae, and Poaceae families have high potential to select for resistant to the inhibitors of ALS, ACCase, EPSPs, PPO, and synthetic auxin herbicides in the coming years [64].

#### 4. Herbicide resistance: the problem and the cause

Genetic factors such as genetic variability (mutations localized in a single locus), heredity patterns (dominance of genes enable rapid dispersion), type of pollination (cross-pollination allows for greater genetic recombination and recessive alleles are more easily established in autogenous species), flow gene (transfer resistance characteristics to a susceptible population) and number of resistance genes involved; and bioecological factors such as short life cycle, high seed yield, low dormancy, multiple generations per year, mechanism of propagule dispersion, extreme susceptibility to herbicides, population size, and low biodiversity are key factors in the selection of herbicide resistant weed populations [65]. However, in this section

only the agronomic, economic, and even scientific-technical factors that may have contributed to the increase in herbicide resistance in Brazil will be discussed.

#### 4.1 Agronomic factors

Among the agronomic factors that favored the rapid selection of resistance are the characteristics of the herbicide used and the cultural practices. Some herbicide chemical groups have a higher risk of selecting for resistance, especially those with a single mechanism of action or detoxification way (high specificity). High dose applications provide greater selection pressure for resistant weed individuals. The greater persistence of a herbicide also favors the selection for resistance, since the period of exposure is longer, therefore, the ideal is that the herbicide only has effect in the critical period of competence. Reduced crop rotation (monoculture), lack of alternative herbicides, nonuse of herbicide mixtures or sequential applications, nonremoval of weeds from field that escaped herbicide control, and poor inclusion of nonchemical methods are major cultural practices that can lead to emergence of herbicide resistance [66].

In Brazil, a large part of crop production systems is intensive, and today effective weed management without herbicides is inconceivable in the short term in these systems [67]. In addition, a large part of the agricultural areas is occupied with HR crops, resistant to glyphosate or imidazolines, as described in Section 2. The adoption and the use of these technologies caused great changes in weed management, which in most cases, implied the substitution of different herbicidal molecules, that were traditionally used before the insertion of HR crops, by the almost exclusive herbicide associated with said technology in question, at least in the first years after its adoption [23, 68]. For example, in the United States, glyphosate applications replaced a large part of previously used herbicides in GR crops [69].

In Brazil, during the first years after the adoption of GR crops, glyphosate was used in various steps of the production process (chemical fallow (pre-planting), weed management (single or sequential), and desiccation) in doses ranging from 2 to 8 L ha<sup>-1</sup>, and in some cases, those doses exceeded 10 L ha<sup>-1</sup> per application [69]. In other cases, many GR soybean farmers delayed the management of weeds that germinated before planting in order to control them with post-emergent applications of glyphosate made on the crop when the competition between the soybean and weeds had already begun [23, 69].

The almost exclusive use of glyphosate quickly showed deficiencies in weed control [23]. Species such as *A. palmeri*, *Conyza* sp., *C. elata*, *D. insularis*, and *E. indica* selected for resistance to this herbicide, forcing farmers to use other herbicides in areas cultivated with GR crops [70]. Herbicides such as 2,4-D, ACCase inhibitors, and ALS were retaken for weed control during pre-sowing (chemical fallow) and crop development, and glufosinate, diuron, and paraquat for desiccation. Currently, glyphosate is applied in isolation only 14% of the time [71]. At the same time, the relative amount of glyphosate used per hectare decreased. For example, 118.5 tons of glyphosate were sold in 2009 and there were 18.6 million ha of GR crops (14.1 soybean + 4.4 maize + 0.13 cotton), and by 2018, there were 49.2 million ha of GR crops (33.4 soybean + 14.7 maize + 1.15 cotton) and 195.1 tons of glyphosate were sold. Considering that only these three crops consume 70% of pesticides market of Brazil, in 2009, 4.46 kg of glyphosate ha<sup>-1</sup> year<sup>-1</sup> were used, while in 2018, that amount was 2.78 kg of glyphosate ha<sup>-1</sup> year<sup>-1</sup>, i.e., there was a reduction of at least 26% (Figure 1A and 2A). On the other hand, sales of herbicides such as 2,4-D, paraquat, atrazine, increased between 2009 and 2018 (Figure 2A). However, the increase in the use of herbicides with different mode of action, applied in mixture or in sequence with glyphosate, has contributed to the emergence of weeds with multiple resistance.



Paraquat and diuron are considered as bodyguard of glyphosate and are essential tools for Brazilian farmers to hamper the spread of glyphosate resistant weeds [72]. However, the use of paraquat is only authorized until 2020 by the Agência Nacional de Vigilância Sanitária (ANVISA) after several studies demonstrated that this herbicide can cause Parkinson's and irreversible damage to the genome [73]. Therefore, this legal determination will represent a new challenge in relation to the management of glyphosate resistant weeds, not only in GR fields.

Currently, soybean farmers are anxious and have high expectations with the introduction of new varieties of transgenic soybeans of the technologies Enlist E3™ (2,4-D + glyphosate + glufosinate) and Intacta 2 Xtend® (dicamba + glyphosate) that, have stacked traits of resistance to lepidoptera and up to three herbicides and, will be available in the Brazilian market for commercial use as of the 2020/2021 and 2021/2022 cycles, respectively [74, 75]. On the other hand, the use of synthetic auxins has also been questioned. According to the Instituto Brasileiro do Vinho (Ibravin), the 2,4-D drift used to control weeds in pre-planting of GR soybeans caused damage estimated in R\$ 100 million only in 2018 in vineyards of Rio Grande do Sul [76]. Moreover, the use of auxinic herbicides needs to be done with caution, especially in periods with lower temperatures, since any problems related to the application technology, which allows the contact of *Digitaria sp.* plants with low doses of these herbicides, will promote the re-growth of these weeds, which will hinder its control and favor the dispersion of this species [77].

Crop rotation is a consolidated weed management strategy in most of the grain-producing agricultural regions of Brazil. However, it often involves the use of the same technology, i.e., GR soybean is replaced by GR maize and vice versa. This situation is due to the high competitiveness of global commodity markets, which have led farmers to specialize in the production of one or few closely related crops, avoiding the implementation of more complex crop rotations (grain by vegetables). The efficiency obtained by the specialization, which allows the use of the same seeder, combine and marketing infrastructure, has led to the widespread adoption of monocultures [67]. This limited crop rotation (grains by grains), has impacted on the use of herbicides because the number of applications is doubled per agricultural year (3–5 applications per agricultural summer or winter cycle), since second crop requires similar agricultural tasks to the first crop. This practice has increased the herbicide selection pressure on weed populations, but also have provoked the occurrence of voluntary plants from the previous crop, which are difficult to control because they have a similar herbicide resistance profile as the current crop, reducing the crop yield [78, 79].

Direct sowing systems in conjunction with other cultural weed control practices such as cover crops and crop rotations reduce weed population densities [67]. Brazil is one of the few countries that have widely adopted the direct sowing system. This production system reduce the annual weed density compared to conventional agricultural systems [67]; however, the appearance of biannual (*Conyza sp.*) or perennial (*C. elata*, *D. insularis*, and *E. indica*) weeds have been favored [29, 40, 66], which under continuous selection pressure of glyphosate selected for resistance; therefore, weed management strategies more complex are required.

## 4.2 Scientific and technical aspects

The Brazilian technical and scientific community specialized on weed science is very large, and their efforts to prevent, monitor, identify, and establish management programs of herbicide resistant weeds, as well as to alert farmers about the occurrence of new cases in order to reduce their dispersion are also very large. Symposiums, congresses, and multiple regional, national, and even international



extension activities are frequently held to improve the sustainability of the main production systems, bringing together farmers, researchers, agricultural companies (machinery, pesticides, seeds, etc.), politicians, and agronomy students. In this way we can affirm that the Brazilian agricultural community knows in depth the negative impacts of herbicide resistance. The main efforts of the scientific community are addressed in combating the “problem,” as evidenced by the greater amount of research papers proposing alternative management strategies of herbicide resistant weeds. For example, in 2019, of the 150 articles published in *Planta Daninha* (scientific journal published by the Brazilian Society of Weed Science), 22 papers addressed issues related to alternative methods of weed management (chemical, nonchemical, or combined measures), four articles reported the occurrence of new herbicide resistant cases, and only two articles fully or partially characterized the mechanisms of resistance involved (Material S1), i.e., studied the “cause” of herbicide resistance. *Planta Daninha* is not the only scientific journal where Brazilian researchers publish their results, but if it is the main one; therefore, these data reflect the trend in which they invest their main efforts to combat herbicide resistance.

Knowing the “problem” is one thing, but knowing the “cause” is another. Herbicide resistance would be equivalent to referring to a headache. In both cases, the “problem” is known, but the “cause” is unknown. We often underestimate headaches (which can be caused by muscle tension, stress, anxiety, head trauma, etc.) by resorting to self-medication or requesting medication from the pharmacist on duty, who asks a series of questions and recommends some type of analgesic. The pharmacist has not identified the “cause” of the problem, but his recommendation could totally or temporarily relieve the headache and, at the same time, we avoid the consultation with a specialist doctor. Similarly, agricultural field technicians have a deep understanding of the negative impact of herbicide resistant weeds and often recommend different management alternatives; however, they do not know the “cause” of herbicide resistance. Implementing herbicide resistance management measures without knowing the cause of it, by characterizing the resistance mechanisms that govern it would be equivalent to self-medicating. In many cases, herbicide resistant weeds are satisfactorily controlled initially, but often the problem worsens over time, resulting in cases of cross and/or multiple resistance. This analogy allows us to infer, that in many cases, Brazilian technicians, and even weed scientist, have acted more as pharmacists than as doctors. This scenario can be added that many field professionals (agronomists and sales agents of pesticides) act without professional ethics prescribing pesticides in a superhuman rhythm [80]. In Brazil, farmers need of prescriptions to purchase these products; however, a professional is often an employer of cooperatives or reseller pesticide offices, so he needs to sell supplies to guarantee his employment [80]. A study carried out by the Agência de Defesa Agropecuária do Paraná (ADAPAR) showed that at least 30% (600 of 2000) of the field professionals signed daily between 7 and 17 prescriptions between 2015 and 2017, i.e., they signed 1–2 prescription for every hour of work. According to ADAPAR, this fact is technically inviable due to the long distance between properties, because to sign a prescription, the field professional must visit the crop fields [80].

In theory, Brazilian weed scientists know the cause of herbicide resistance, i.e., they are familiar with the possible physiological, biochemical, and molecular mechanisms that can confer it. However, studies aimed at characterizing these mechanisms are scarce, often conducted only with the first population(s) that confirmed the occurrence of a given case of herbicide resistance. In addition, the resistance mechanisms characterized in a herbicide resistant population have been adopted in a generalized manner by technicians and other weed researchers,

assuming that new occurrences of a case of herbicide resistance already reported (species x herbicide) will have the same mechanisms observed in the first resistant populations. This conclusion may be partially correct when a new occurrence is found in agricultural areas near where the first occurrence was found (dispersion) [48]. However, resistance within the same area and between geographically distant areas may be due to independent herbicide resistance selection events [81], so the resistance mechanisms involved may be different. When multiple studies on the characterization of resistance mechanisms have been carried out on the same weed, the results have been interpreted in a controversial way by the scientific community. For example, in the case of glyphosate resistant *D. insularis* there is no consensus of the mechanism that governs such resistance [82]. In the first populations of glyphosate-resistant *D. insularis*, collected in the state of São Paulo in 2009, the reduced absorption, translocation and metabolism of the herbicide, and a gene mutation (at the Pro-106 EPSPs gene position referred to as Pro-182) were the mechanisms conferring that resistance [12]. After, differences in absorption, but not in the translocation of glyphosate and the occurrence of mutations were observed in other populations [83, 84], while collected populations in different regions of the State of São Paulo presented mutations and enhanced activity of the EPSPs [85]. In the most recent study, including glyphosate resistant *D. insularis* populations collected in different states, it was not possible to characterize the mechanism (s) involved in the resistance [86]. These results show that resistance to a given herbicide can be governed by different mechanisms, acting in isolation or together, in the same species. In addition, these divergent results observed in the different studies show that each new occurrence must be evaluated individually, therefore, this information should not be used to generate a consensus on the mechanisms involved in the resistance of a given case (herbicide x species).

Knowing the mechanisms that govern herbicide resistance is fundamental to plan a proper management strategy, since in some cases, a specific mutation (target site mechanism) does not represent that a herbicide with the same mode of action to which resistance was observed can be used. For example, the Ala-122-Thr mutation in the ALS gene confers high resistance to imidazolinones, but does not confer resistance to sulfonylureas [87]. In the case of herbicide metabolism (non-target-site mechanism) regulated by the cytochrome P-450 enzyme complex, which can confer multiple resistance up to six or possibly more groups of herbicides [88], the use of the same herbicide to which resistance was reported it may be possible using a cytochrome P-450 inhibitor such as malathion or phorate before applying the herbicide in question [89, 90]. Paraquat is an alternative for the management of glyphosate resistant weeds only if resistance is not governed by vacuolar sequestration, since although they have different mechanisms of action, this non-target-site mechanism confers resistance to both herbicides [91]. It is important to note that the management of herbicide resistance is not as simple as described here, since it often involves the participation of different resistance mechanisms, but the timely and appropriate characterization of them could reduce the use of complex mixtures of herbicides in high doses, reducing the impact environmental [92].

Some Brazilian weed research groups have partnerships with weed scientists from the United States and Spain and other countries, who collaborate actively in studies that characterize the mechanisms of the most important cases of herbicide resistance of the country [12–16, 93]. However, these efforts are still insufficient and the previous information allows us to infer that in most cases, the management of herbicide resistance in Brazil has been faced in an inverse way, i.e., first, efforts are made to test and propose different alternatives to solve the “problem” and, in some cases, then try to characterize the “cause.” This would be equivalent to placing the shoes first and then the socks. Therefore, to face the problem of herbicide

resistance, more efforts must be made to characterize the resistance mechanisms involved in each case, and only later, evaluate different alternative management strategies that are technical and economically viable.

### **4.3 Economic factors**

In this aspect there are two great scenarios. On the one hand, there are the pesticide manufacturers that are valued according to the volume of sales of their products; therefore, they invest their efforts in “conquering” more farmers every day so that they use their products and thus have greater presence in the market and consequently greater prestige. On the other hand, there are farmers who in turn want to obtain the highest profit margin with the least investment, often, in the short term.

As highlighted in the previous section, Brazilian scientists focus their efforts on developing herbicide-resistant weed management strategies, mainly through the applications of herbicides with different modes of action applied in tank mix or in sequence [71]. These investigations are often funded by pesticide manufacturers. Although the conclusions are not biased, objectively reflecting which treatments are the best alternative to control certain weed resistant herbicide(s) in a particular production system, and the researchers also do not recommend the use of commercial formulations of a specific manufacturer, obviously the intention of the financing pesticide manufacturer is to increase the sales of its products and technologies.

Weed researchers evaluating alternative management programs often find at least one efficient control option, both for the level of control achieved ( $> 80\%$ ) and for the period that a treatment maintains the level of control, i.e., there are solutions to the “problem,” and Brazilian weed scientists never have stopped looking for new herbicide management alternatives. However, if research is abundant in this regard in the country, why do cases of herbicide resistance continue to increase? The answer to this question is possibly related to the fact that in most of these studies the costs (herbicides + cost of operations + worker’s payment) of the resistance management programs evaluated are not considered. In addition, the yield ( $\text{kg ha}^{-1}$ ) that a given management program can guarantee to the farmer is rarely determined. A specific case that addresses these two aspects (cost vs. yield) is the study developed by Piasecki et al. [94], who evaluated 16 treatments, of which 11 did not show differences in soybean yield ( $3600\text{--}3750 \text{ kg ha}^{-1}$ ), but there were differences in the costs of each treatment, since they were composed by 3 or 4 herbicides. In that study, the highest yield of soybean ( $3888 \text{ kg ha}^{-1}$ ) was achieved with the treatment consisting of glyphosate + chlorimuron-ethyl + 2,4-D + saflufenacil (T13:  $1080 + 25 + 670 + 50 \text{ g ia ha}^{-1}$ ), which had a cost of  $\text{R\$ } 180.00 \text{ ha}^{-1}$ . However, the best relative economic return was obtained with the treatment of glyphosate + chlorimuron-ethyl + 2,4-D (T12:  $1080 + 22.5 + 670 \text{ g ia ha}^{-1}$ ), which presented a yield of  $3749 \text{ kg ha}^{-1}$  and cost only  $\text{R\$ } 85.00 \text{ ha}^{-1}$ . This study did not include the costs related to the application operations, but contrasting the cost of a management program with the crop yield can be an additional tool for the farmer, so that he can estimate his profit margin and decide whether or not to adopt given weed management program.

This situation is also reflected in the type of HR crop technology used by farmers. For example, Liberty Link® technology (glufosinate resistant crops) is available in Brazil since 2016/2017 cycle [95]; however, its use is low compared to GR crops, since glufosinate is, in average, three times more expensive than glyphosate. Total glufosinate sales exceeded  $1000 \text{ tons year}^{-1}$  in 2017 (1137 tons) and 2018 (1450 tons), but they are still very far from glyphosate sales (173,150 and 195,056 tons in 2017 and 2018, respectively) [7]. This shown that farmers often prefer to continue living with glyphosate resistance than to adopt a new but more expensive technologies, i.e., the adoption of an HR technology is motivated by the cost–benefit ratio



by saving costs devoted to pest control guaranteeing high yields [2]. Therefore, the success of Enlist E3™ and Intacta 2 Xtend® technologies, which will be available in the Brazilian market from 2020/2021 and 2021/2022 crop cycles, respectively, will depend on their final cost; meanwhile, farmers will continue to be reluctant to adopt integrated management measures for herbicide-resistant weed control or new HR technologies [96, 97]. The Brazilian scientific community has the task of demonstrating to the farmers that, although the implementation of an integrated weed management program is complex and expensive initially, in the long term it is profitable and environmentally sustainable [98].

## 5. Economic impacts of herbicide resistance

The economic impact of herbicide resistance management is related to the need to use alternative herbicides with different modes of action, yield losses caused by competition, but mainly to the weed species resistant to being controlled [25]. The cost of alternative herbicides varies according to the choice of farmer, as there is often more than one herbicide option available. Yield losses caused by competition vary according to weed and crop competitive ability, number of plants per area, vegetative stage of crops and weeds, soil fertility, and water availability, among other factors. Therefore, estimating the real economic impact of herbicide resistance on Brazilian agricultural activity is difficult.

Embrapa's Herbology Research Group (GherbE) has been continuously monitoring herbicide-resistant weeds in grain production systems in Brazil since 2010, through questionnaires and consultations with technical assistance, farmers, and other researchers; seed collection from areas suspected of resistance with subsequent tests for resistance in a greenhouse; field experiments; and visits to areas with suspected resistance. Resistance monitoring by GherbE researchers was made possible through the joint implementation of the projects "Identification and characterization of glyphosate resistant weeds in Brazil" and "Integrated management of herbicide resistant weeds in soybean production systems" [99]. Relevant information is now available showing the potential economic impact of glyphosate resistant weeds on soybean production and the most representative results are summarized here [25, 99, 100].

The average cost of nonresistant weed control in 2017, restricted to two post-emergence glyphosate applications and one for desiccation, was estimated in R\$ 120.00 ha<sup>-1</sup>. In a scenario of glyphosate resistant *L. multiflorum* infestation, in addition to glyphosate, it is necessary to add a graminicide (ACCase inhibitor), increasing the average cost to R\$ 177.65 ha<sup>-1</sup>. If the infestation is of *Conyza* sp., the use of a latifolicide such as 2,4-D increases the average cost to R\$ 170.50 ha<sup>-1</sup>. In areas infested with *D. insularis*, a weed more difficult to control than *L. multiflorum*, requires the use of graminicides in both postemergence and desiccation, and may be interspersed with contact herbicides such as paraquat and glufosinate, increasing the average management cost of this species up to R\$ 318.35 ha<sup>-1</sup>. However, in mixed infestation scenarios, herbicide resistance management is complicated because herbicide options are reduced. For example, infestations of *Conyza* sp. and *L. multiflorum* require selective herbicides for cultivation during soybean vegetative phase, with flumioxazin and trifluralin being the main options, while for desiccation 2,4-D and paraquat are required for control of *Conyza* sp. and *L. multiflorum*, respectively. The average control cost in this scenario may reach R\$ 197.55 ha<sup>-1</sup>. If the infestation is of *Conyza* sp. and *D. insularis*, the control cost can be up to R\$ 386.65 ha<sup>-1</sup>, i.e., R\$ 266.65 ha<sup>-1</sup> more expensive compared to one scenario without resistance. These estimates do not consider the possible occurrence of multiple resistance of *L. multiflorum* and *D. insularis* to graminicides or *Conyza* sp.



to latifolicides, scenarios in which the cost of management is more expensive and restricted in relation to the alternative herbicide options available [25].

According to GherbE monitoring, 59% of soybean area (20.1 out of 34.0 million ha) had infestations of glyphosate-resistant populations of *Conyza* sp., *D. insularis* and/or *L. multiflorum* in 2017. *Lolium multiflorum* affected 4.2 million ha in the southern states, and this weed occurred simultaneously with *Conyza* sp. in 3.4 million ha. The areas infested by *Conyza* sp. and *D. insularis* were estimated at 7.7 and 8.2 million ha, respectively, of which 2.7 million ha correspond to mixed infestations of these two species [100]. On the GherbE website it can visualize distribution maps of these weeds in the different agricultural regions of Brazil [99]. Analyzing the infested area and the control cost according to the infesting weed species, the average cost of resistance management was R\$ 4,918,820,000.00 in 2017 [25]. If a conservative 5% yield loss by weed competition is added, the total cost of herbicide resistance in Brazil exceeds R\$ 9 billion annually in soybean cultivation alone [25].

## 6. Future trends, challenges, and conclusions

Brazil is a consolidated agricultural power; however, the large size of its agricultural activity, especially the intensive production, makes it highly dependent on pesticides for the management of phytosanitary issues, which has led to the emergence of pests resistant to these products.

Much of the Brazilian agricultural activity (68.4%) is focused on the production of grains (52.5% soybean, 10.6% maize, and 5.3% other grains) by cultivating herbicide-resistant crop varieties. The introduction, rapid adoption, and high dependence on these technologies and their associated herbicides (58% of the national pesticide market) caused major changes in weed management practices, contributing to the selection of herbicide resistance weeds.

Today, herbicide resistance is a fait accompli in Brazil; however, the problem is not rooted in the cultivation of herbicide resistant crops but in the inappropriate use of these technologies as a whole, mainly related to off-season applications and herbicide overdose. Clearly, weed management practices must be constantly changed to prevent or delay the emergence of resistant plants in an area. However, the high specialization of farmers to grow, manage and market one or few crops with similar agricultural tasks limits the implementation of alternatives weed management measures as well as reduce more complex crop rotations (i.e., grains by vegetables instead of grains by grains), since transferring their production system to other crops requires investments in professional training, infrastructure, new agricultural implements as well as in the creation of new marketing networks; otherwise, farmers have no guaranteed economic return. In addition, farmers prefer to continue living with the herbicide resistance, and they are reluctant to adopt integrated weed management measures or new herbicide resistant crop technologies for herbicide resistance control if their profit margins are not severely compromised.

The management of the herbicide resistance may represent an increase ranging from 100 to 350% ha<sup>-1</sup> of the costs devoted for weed control in relation to fields with no resistance. However, the dimensions of this phytosanitary issue is incalculable, as five glyphosate resistant weeds (*C. bonariensis*, *C. canadensis*, *C. sumatrensis*, *D. insularis*, and *L. multiflorum*), occurring only in soybean, infested  $\geq 25\%$  of the total planted area (20.1 out of 77.8 million ha) of Brazil, and caused R\$ 9 billion of losses in 2017. To know the true economic impact of herbicide resistance, the areas of other crops infested by these glyphosate resistant weeds, as well as areas affected by the other 46 cases of herbicide resistance (species x herbicide x crop situation) reported in Brazil should also be considered.

Brazilian technical and scientific community specialized on weed science continually made great efforts to prevent, monitor, identify as well as discuss and establish new weed-resistant weed management strategies. However, in most cases, herbicide resistance has been fought in an inverted way, i.e., it has been tested/implemented for solutions to the problem without determining the cause; therefore, if little effort continues to be devoted to characterize the resistance mechanism involved in each case of herbicide resistance before implementing weed management strategies, new occurrences of herbicide resistance weeds, mainly with cross- and multiple-resistance, will continue to appear in the coming years in Brazil.

Besides inherent biological factors of weeds to select herbicide resistance, agronomic, economic and scientific-technical factors have, directly or indirectly, contributed to increasing cases of herbicide resistance. These factors are generally linked to each other but they often are analyzed separately. Therefore, in order to achieve sustainable weed management, future studies aimed at addressing herbicide resistance problems by evaluating different weed management programs should consider these factors, as well as practical and economic aspects for their large-scale implementation.

The Brazilian weed science community have the great challenge of demonstrate to farmers that the implementation of integrated weed management programs may be expensive initially, but in the long term it is profitable and environmentally sustainable.

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