Chapter 1

Weed Resistance to Herbicides in Rice Fields in Southern Brazil

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Additional information is available at the end of the chapter

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1. Introduction

Rice (*Oryza sativa* L.) is the main staple food for a great part of the world population, and together with corn and wheat represents most of the cereals produced and grown worldwide [1]. With the growth of the world population, especially in East Asian countries, there are concerns about if rice production will be sufficient to meet the demand in the future [1]. There is the need to increase crop productivity levels, but there are both limitations for the opening of new agricultural areas, and issues regarding environmental pollution and use of natural resources.

The annual rice production in Brazil is 11.6 million tons [2], occupying an average area of 2.43 million ha per year with yields averaging 4.73 t ha^{-1} (Table 1). The southern states of Rio Grande do Sul (RS; 1.05 million ha) and Santa Catarina (SC; 0.15 million ha) contribute with more than 77% of the rice production with about 51% of the cultivated area in Brazil. Average grain yields obtained in the last five years in the RS and SC were around 7.26 t ha^{-1}, almost 55% higher than the national average [2]

The intensification of rice cropping systems in the same area promotes an increase in infestations by weeds. The fields of irrigated rice in southern Brazil provide a special habitat for weeds. During some months of the hot season, in addition to temperature and luminosity suitable for plant growth, there is also abundant soil moisture, which favors the development of weeds. This makes weeds responsible for losses in yield and grain quality, due to the direct interference they cause to the crop [3]. The weeds also cause other indirect negative effects in the production system, such as losses in nutritional value of pastures, interference in cover crops and even depreciating the land value [4-6].

In the fields of southern Brazil, the increase in weed occurrence is well characterized, mainly due to the fact that the irrigated rice was – until recently – almost the only cultivated crop in lowlands. To reduce the impact of weeds in rice, farmers have adopted some technologies. At first, there were modifications in the soil management system, shifting from a conventional plough-and-harrow to other forms of soil cultivation, such as minimum-till, no-till and the water-seeded rice system. Secondly, there was the adoption of ALS-tolerant rice cultivars (Clearfield technology - CL®) and last, the increase in the area of Roundup Ready soybeans in drained lowlands has also contributed to the weed management in rice fields. Herbicides, however, are still heavily used as the main form of weed control in almost all irrigated rice fields in RS state. In complement, organic rice is growing in adoption, but actually is restricted to small fields. The certified organic smallholders account for 400 producers, in an area of about 3,400 ha dispersed in the RS state.

It is known that the average regional yields (7.26 t ha^{-1}) are below those obtained in field trials and in high technology farms. Even though new cultural techniques are often used to control weeds, poor weed control is one to be highlighted among the probable causes of grain yield variability. According to results of [7] and [2] it is estimated that about 1 million tons of rice are lost annually in Brazil, which is roughly equivalent to 8% of the national production of this cereal, even after using all methods available for weed management. This corresponds to an annual loss estimated of about US$ 200 million.

Cropping season	**Area (1000 ha)**			**Yield (kg ha^{-1})**			**Production (1000 t)**		
	Brazil	RS	SC	Brazil	RS	SC	Brazil	RS	SC
2002/03	**3186**	960	145	**3254**	4890	7195	**10367**	4696	1043
2003/04	**3654**	1039	151	**3511**	6064	6630	**12960**	6433	1000
2004/05	**3916**	1050	154	**3377**	5912	6800	**13355**	6333	1050
2005/06	**3018**	1040	156	**3884**	6610	7050	**11722**	6872	1099
2006/07	**2967**	954	156	**3813**	6726	7050	**11316**	6419	1099
2007/08	**2875**	1067	153	**4200**	6902	6650	**12074**	7362	1018
2008/09	**2909**	1106	150	**4332**	7150	6950	**12603**	7905	1040
2009/10	**2765**	1080	150	**4218**	6781	7060	**11661**	7321	1057
2010/11	**2820**	1172	150	**4827**	7600	6625	**13613**	8904	996
2011/12	**2455**	1053	150	**4728**	7350	7180	**11600**	7740	1078

* Source: [2]

Table 1. Historical cultivated area, grain yield and production of rice in Brazil and in the states of Rio Grande do Sul (RS) and Santa Catarina (SC), from 2002 to 2012.

Due to the particular regional characteristics, there are many ways of soil, water and plant management in irrigated rice in southern Brazil. The main system is minimum-till (around 60% of the area) in which the soil is plowed, harrowed, leveled and the levees are done in the autumn, right after the harvest of the summer crop, with chemical desiccation in spring before rice planting, done with a no-till drill in dry soil. Another system is the conventional seeding, where all the tillage is done just before planting rice, in dry soil. Finally, about 20% of the fields are cultivated with the water-seeded system, performed mainly in small farms (up to 30ha) in which rice is sown pre-germinated over a field already flooded (schemes on Figure 1). The system of manual or mechanic transplanting rice seedlings from the nursery to the puddled and flooded field – very common in the Asian paddies – is almost not used in Brazil.

In the last few years, there was a continuous increase in the soybean area in the lowlands of RS, and currently this crop occupies around 250,000ha in rotation with rice (all RS state have approximately 4.19 million hectares of soybean). Probably in the following years, soybean will spread up to 0.5 million hectares in the lowlands of RS, limited by poor soil drainage conditions. Glyphosate-tolerant soybean has changed the scenario of resistant-weeds in rice fields and will be discussed later in this article.

1.1. The main weeds of irrigated rice in southern Brazil

The main weeds in flooded rice fields in Brazil are commonly classified into narrow- and broad-leaved weeds. The major representatives of narrow leaves are weedy rice (*Oryza sativa*), barnyardgrass (*Echinochloa* sp.), the aquatic grasses (*Leersia hexandra* and *Luziola peruviana*), and the sedges (*Cyperus difformis*, *C. esculentus*, *C. ferax*, and *C. laetus*).

Recently, there was an increase in the occurrence of Alexander grass (*Brachiaria plantaginea*), crabgrass (*Digitaria horizontalis*) and goosegrass (*Eleusine indica*) in the rice fields. These monocotyledonous weeds, common in dry fields in crops such as corn, sorghum and soybeans, are expanding due to the increase in crop diversification in lowland areas, to the continued use of ALS inhibitors and the abandonment of propanil herbicide in the rice fields. Some places also reported the presence of perennial weeds such as Olive hymenachne (*Hymenachne amplexicaulis*), ribbed murainagrass (*Ischaemum rugosum*), Mexican sprangletop (*Leptochloa uninervia*), Fall panicum (*Panicum dichotomiflorum*), Knotgrass (*Paspalum distichum*) and *Paspalum modestum*. These perennial plants grow in areas with an excess of moisture.

As broadleaved weed representatives, there are the jointvetches (*Aeschynomene* spp.) and in some areas some species of morning glory (*Ipomoea* spp.), water pepper (*Polygonum hydropiperoides*) and alligator weed (*Alternanthera philoxeroides*). The aquatic weeds, associated mainly with fields grown in the water-seeded system (with pre-germinated seeds) are globe fringerush (*Fimbristylis miliacea*), arrowheads (*Sagittaria montevidensis* and *S. guyanensis*), water hyacinth (*Eichornia crassipes*), kidneyleaf mudplantain (*Heteranthera reniformis*) and the Ludwigia complex (*Ludwigia elegans*, *L. longifolia* and *L. octovalvis*).

Many of these species are difficult to control and severely compete with the crop for resources available in the environment if no control method is adopted. In addition, barnyardgrass,

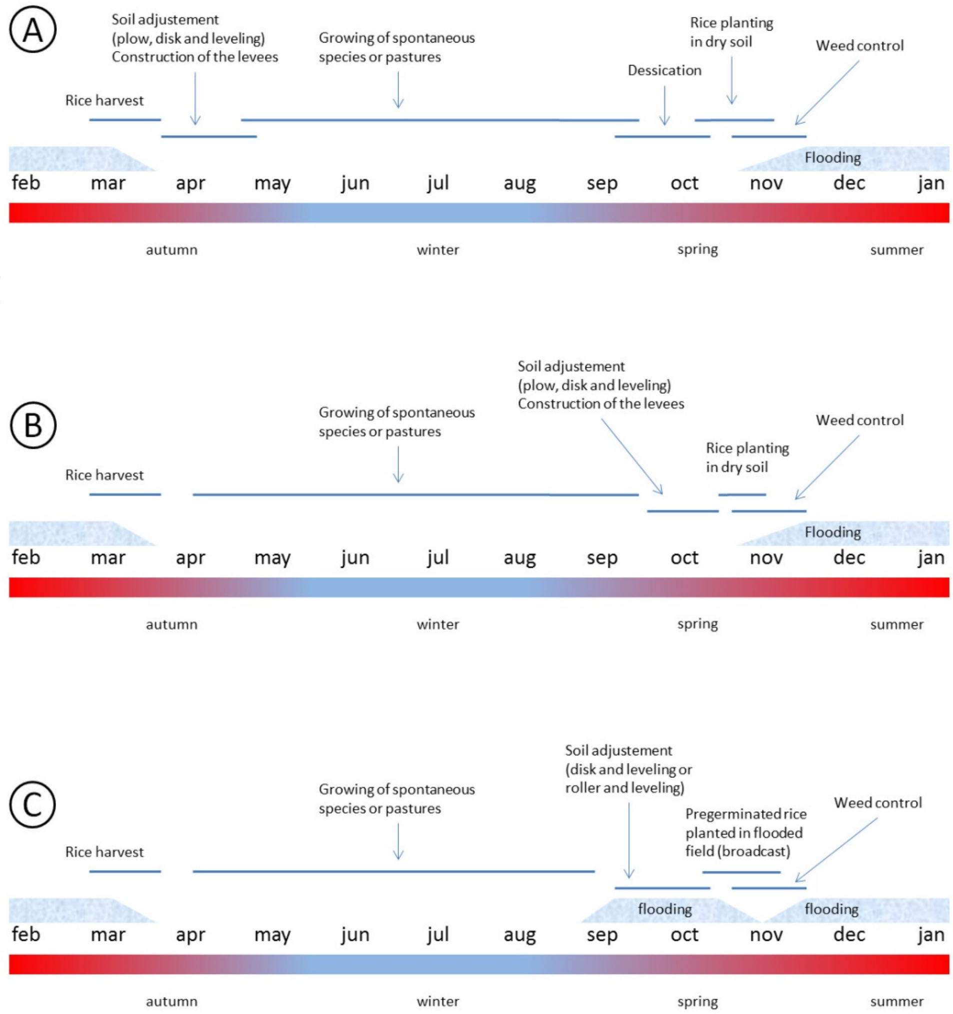

Figure 1. A simplified scheme of the three main production systems of irrigated rice in southern Brazil. (A) Represents the minimum-till system; (B) represents the conventional system; (C) represents the water-seeded system. The schemes illustrate part of a very common two-year sequence of rice cropping.

weedy rice, globe fringerush, arrowhead and some sedges have acquired resistance to herbicides (Table 3).

1.2. How does resistance to herbicides appear in rice fields?

The adoption of herbicide-tolerant rice has increased considerably in the last few years. The results of this unprecedented change in agriculture have been many, but perhaps most dramatic is the simplification of weed-control tactics; growers can now apply a single herbicide group (ALS-inhibitors) at higher rates of active ingredient without concern for injury to the crop. Regardless, the number of chemical groups of herbicides applied has declined, thus increasing the ecological implications such as reducing the biodiversity of arable land, facilitating population shifts in weed communities and the evolution of herbicide-resistant biotypes. Historically, a number of significant changes in agricultural systems have occurred with significant impact on weed communities.

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The use of herbicides for weed control has become a common practice in agriculture worldwide. Once, this technology was used mainly by big farmers; it is currently becoming a common practice even among smallholders. Nowadays, weed control in irrigated rice relies almost exclusively on herbicides, mainly because chemical control has been efficient, relatively cheap, readily available and professionally developed. Thus, other methods of control have been left as a second choice or under certain circumstances may present themselves unattractive or unfeasible. It needs to be noted that the strong presence of pesticide suppliers has almost banned the use of other forms of weed management but the herbicides in irrigated rice fields. The widespread and almost exclusive use of the chemical method of weed control in rice promotes changes in the weed flora, from quite easily controlled broadleaved weeds to more hostile grass weeds [8-9]. The recurrent use of herbicides with the same site of action can select individuals that are genetically capable of surviving a dose of a given herbicide which normally would kill or suppress the species [10]. Herbicide resistance is the inherent ability of a species to survive and reproduce following exposure to a dose of herbicide normally lethal to its wild type. Resistance is not directly caused by herbicides, rather, it appears from the selection of natural mutation or minor pre-existing population of herbicide-resistant plants (selection pressure imposed by herbicides) [11] or in rice cases, gene flow from herbicide-resistant to weedy rice [12-14].

As at other places worldwide, in the rice fields of south of Brazil, the continuous use of herbicides has led to the evolution and appearing of herbicide-resistant (HR) weeds, and this is an additional problem in the pest management context. Chemical weed control is used in almost all areas and the scenario in the short-past, at present – and probably to the future – is a continuous intensification of the rice cropping systems. This intensive system, combined with the continued use of herbicides with the same mechanism of action, has resulted in the development of resistant weeds. The resistance of weeds to herbicides in that region was confirmed by several institutions, namely EMBRAPA, EPAGRI, IRGA, UFRGS, UFPEL and UFSM.

2. Main herbicide resistant weeds occurring in rice in Southern Brazil

There are already reported cases of herbicide-resistant biotypes of the main weeds such as *Oryza sativa* (red rice or weedy rice), *Echinochloa* spp., *Cyperus difformis*, *C. esculentus*, *C. iria*, *Fimbristylis miliacea* and *Sagittaria montevidensis.* These weeds are common in almost all rice fields of Southern Brazil and at some places show resistance to ALS-inhibiting herbicides. Some barnyardgrass biotypes resistant to ALS-inhibitors also were resistant to quinclorac herbicide. One of the most important cases of resistant weeds is the occurrence of weedy rice resistant to the ALS-inhibiting herbicides used in the Clearfield® technology [15], because in this particular situation the weedy rice is from the same species as the crop (*Oryza sativa*).

2.1. First cases

Weed resistance to herbicides in rice fields of Southern Brazil was first registered in 1999 [16], with a biotype of arrowhead (*Sagittaria montevidensis*), which evolved resistance to four ALS-inhibiting herbicides. A short time after, other cases of resistance were reported with a new biotype of *Sagittaria* [17]; and also with barnyardgrass (*Echinochloa* spp.) resistant to the herbicide quinclorac [18]. Since then there was an increasing number of reports of weed resistance (Table 3).

2.2. The case of *Echinochloa crus-galli* resistance to the herbicide quinclorac

This species is a monocotyledon that survives in flooded environments, occurring normally in high levels of infestation. It is widely distributed in almost all rice fields of SC and RS. In addition, barnyardgrass presents morpho-physiological similarities with the crop in the early stage of development. The negative effects of its presence in rice include: the high capacity to compete with rice by resources as light and nutrients; the intrinsic difficulties related to control; the increases in the production costs; it causes rice lodging, difficulties in the harvest and depreciation of the product; it is a host of some pests in rice and this species can even decrease the commercial value of arable areas [3,19-20].

Barnyardgrass is also one of the most widely distributed weeds in the grain crops grown in rotation with rice in lowland areas, mainly represented by soybeans, some sorghum [21] and a little portion of areas with maize. In reference [4] reported that many of the ALS-resistant biotypes of *Echinochloa* showed faster initial development compared to susceptible ones. The authors also report that biotypes from different areas are distinct in terms of initial growing speed.

Due to the continuous use of herbicides with the same mode of action, often in the absence of crop rotation and lack of integrated management, barnyardgrass evolved resistance to several herbicides [22] and some biotypes have multiple resistance [23]. In reference [24] reported a biotype of barnyardgrass presenting cross-resistant to quinclorac (auxin-mimic herbicide) and to ALS inhibitors. Herbicides represent the main tool for weed control within the program of integrated management in rice fields of Southern Brazil. Among those used in rice, quinclorac (auxin-mimic) combines flexibility in the application (pre- and post-emergence) and normally offers good efficiency to *Echinochloa crus-galli* and *Aeschynomene rudis* control, low toxicity to humans and animals and high selectivity to rice. This active ingredient started to be used in rice production areas of RS and SC in the early 1990's, being used intensively until mid-1999, when complaints began to emerge about failures in barnyardgrass control. Studies confirmed the occurrence of resistance already in 2000 [18, 25].

2.3. The cases of weed resistance to ALS inhibiting herbicides

Similar to what happened with quinclorac in the past, in more than a half of all cultivated rice areas in RS state, the ALS-inhibitors were (and still are) vastly applied in the fields. This scenario was aggravated by the use of varieties tolerant to the herbicides belonging to this group (CL technology), aiming to achieve efficient control of weedy rice and barnyardgrass. The repetitive use of some ALS-inhibiting herbicides for 4 to 5 years after the launch of the CL technology resulted in resistance of barnyardgrass to the herbicides bispyribac-sodium, penoxsulam, imazethapyr+imazapic and imazapic+imazapyr [26].

2.4. Arrowhead — *Sagittaria montevidensis*

This is an aquatic weed often found in water-seeded or transplanted rice systems. Arrowhead is characterized as a weed that occurs in high levels in most areas of flooded rice in Santa Catarina [27]. This weed presents a low capacity to compete with rice as compared to other species which infest the crop [28]. However, the frequency of high infestations by arrowhead has resulted in increased use of herbicides for its control. In the RS, rice is mainly drill planted in dry soil, and flood irrigation starts about 20-25 days after emergence; in the State of SC almost 100% of its rice area is grown in the water seeded system, which favors arrowhead.

Several biotypes of arrowhead were found to be resistant to ALS inhibitors [29]. In Brazil, populations with cross-resistance to the sulfonylurea and pyrimidinyl thiobenzoates were identified in 1999 in areas treated with these products for about five consecutive years [16]. In reference [30] the authors found that the resistant biotype of arrowhead showed faster emergence, higher seed vigor and absorption of herbicides preferably by shoots instead of roots, when compared to the susceptible population.

Rice areas with arrowhead resistant to ALS inhibitors are common in Brazil due to the extensive and repetitive application of herbicides with this mechanism of action. A recent study revealed the occurrence in SC State of populations of this weed with cross-resistance to several ALS-inhibiting herbicides and multiple resistances to PSII inhibitors [31]. Currently, arrowhead resistant to ALS inhibitors is present in almost all municipalities which grow rice in Santa Catarina State.

In rice fields where the ALS-resistant biotype of arrowhead occurs, the herbicides carfentrazone-ethyl or bentazon can be used as alternatives for chemical control. Both herbicides applied alone or in tank mix allowed control levels of arrowhead superior to 92% at the pre-harvest of water-seeded rice in SC State [32]. It should be emphasized that planting rice at low- or lower-densities that the recommended [33] allows a more favorable environment for aquatic rice weeds, especially arrowhead. In reference [34] it was observed that a strong negative correlation between the planting density of the rice variety BRS 6-Chui and the infestation by arrowhead; in other words, the infestation was more serious as rice density was decreased. According to the authors, this suppression caused by higher rice densities is due to the increased ability of the crop to compete for light, which prevented the weeds from having access to adequate levels of radiation.

2.5. Nutsedges — *Cyperus difformis, C. iria* and globe fringerush — *Fimbristylis miliacea*

Some weed species of the family *Cyperaceae* infest rice fields in the RS and SC states, being responsible for reducing the potential yields of this cereal. *Cyperus difformis* appears as one of the most damaging weeds to rice. This species is distinguished by production of large quantities of seed (50,000 seeds plant^{-1}), promoting rapid infestation with high growth rates. This has, as a consequence, the formation of a large amount of green mass with high competitive potential with rice, especially in the initial stages of development of the crop [19].

The weed control in rice fields can be accomplished with the use of herbicides due to its ease of use and high efficiency. There are difficulties, however, in chemically controlling species of the genus *Cyperus*. Some species of *Cyperus* reproduce both by seeds and vegetatively (tubers and stolons) as in the case of *C. esculentus* and *C. rotundus*. Furthermore, the chemical control of *Cyperus* spp. in pre-emergence is especially problematic due to the scarcity of products to be applied in this modality. For controlling these species, post-emergence herbicides inhibiting the enzyme ALS, as bispyribac-sodium, penoxsulam, pyrazosulfuron-ethyl, ethoxysulfuron, cyclosulfamuron and azimsulfuron, can be applied. It is necessary also to respect the limit of growth stage at the time of application and to use adjuvants specific to each herbicide [33].

The control of *C. difformis* with ALS inhibitors, however, has presented problems due to the development of resistance [35,36]. The authors report that this is mainly due to the intensive cultivation of rice, associated with the use of herbicides with the same mechanism of action for several years, favoring the selection of resistant populations.

From the 1980's, the ALS-inhibiting herbicides have become very important tools for agriculture, and the widespread use of these products was mainly due to its high efficiency at low doses, low toxicity to animals, high selectivity for some crops and reduced environmental impact when compared to other pesticides [37]. These traits contributed to the increased use of these herbicides in various crops. Two years after these products were made available in the market, however, appeared the first case of a weed with resistance to this mechanism of action. Currently, there are 95 resistant species, distributed in 34 countries [38].

Results in reference [36] are shown in Figure 1. One biotype of *C. difformis* presented a high-level of resistance to the herbicide pyrazosulfuron-ethyl (sulfonylurea), and was also cross-resistant to the bispyribac-sodium (pirimidinyl thiobenzoate), both ALS inhibitors. Bentazon is an efficient alternative for the chemical control of the ALS-resistant biotype of *C. difformis* (Figure 2). The same authors point out that, for the management of populations of *C. difformis* resistant to ALS inhibitors in flooded rice areas, it is recommended the adoption of practices such as rotating herbicides with different mechanisms of action and management practices that may restrict the expansion of the resistant populations.

The mechanism involved in the resistance of *C. difformis* to pyrazosulfuron-ethyl is the insensitivity of the enzyme ALS to herbicides, which inhibit this enzyme, conferring high levels of resistance [39]. In [40] tested the herbicides pyrazosulfuron-ethyl, bispyribac-sodium, imazapyr, imazapic and penoxsulam on the species *C. iria* (Table 2), and also proved the resistance of this species to ALS inhibitors due to the low levels of control achieved with all herbicides. In the same study, bentazon (PSII inhibitor) controlled 100% of the biotype. Another study [41] also observed no efficient control of *C. iria* under application of 1x and 2x the recommended dose of pyrazosulfuron-ethyl, imazethapyr, imazapic or ethoxysulfuron.

For rice fields infested with biotypes of weeds resistant to ALS inhibitors, the most effective strategies are pointed out in the following. The application of glyphosate alone or mixed with pendimethalin or clomazone at the so-called "needle point" will ensure that the rice emerges free from the infestation of *Cyperus,* allowing also efficient control of several other weeds. The "needle point" is the rice germination stage immediately prior to the initiation of the emergence, depicted in Figure 3. Usually, when a very few rice seedlings start to emerge in the field indicates the needle point, and the non-selective herbicide should be applied on that day. This

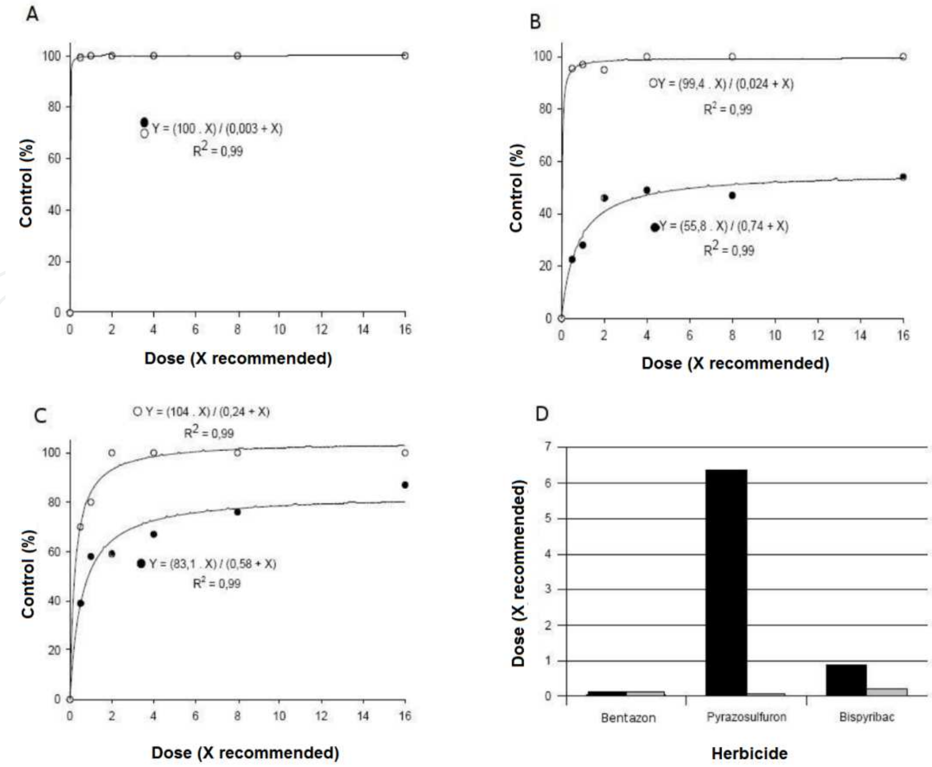

Figure 2. Control (%) of a biotype of *Cyperus difformis* resistant (●) or susceptible (○) to ALS-inhibiting herbicides by using PSII and ALS-inhibiting herbicides as a function of dose. [bentazon (A), pyrazosulfuron-ethyl (B), bispyribac-sodium (C)] In (D) the doses that control 50% of the population (LD_{50}) of the resistant (black bars) and susceptible (grey bars) biotypes are presented. Source: [36]

Herbicide	Control (%)		Dry Mass
	14 DAH[1]	28 DAH	(g plant^{-1})
Pyrazosulfuron-ethyl	15 b[2]	6 b	1,59 a
Bispyribac-sodium	6 bc	13 b	2,31 a
Imazapyr + imazapic	2 c	2 b	2,31 a
Penoxsulam	3 c	10 b	1,48 a
No application	0 c	0 b	2,79 a
CV(%)	18,09	38,16	27,41

[1] Days after application of herbicides. Means followed by the same letter, in the column, are not different (Tukey P>0.05). Source: Adapted from [40]

Table 2. Control efficiency and shoot dry mass of *Cyperus iria* as a function of the application of ALS-inhibiting herbicides.

should not affect the stand of rice plants in the field, as the majority of the seedlings will not be emerged on that day. This happens from three to five days after rice planting, depending on environmental conditions (soil moisture and temperature).

Although effective, a delay in the application of glyphosate + pendimethalin or clomazone for a single day from the needle point may cause severe damage to rice. This is particularly a problem if there are frequent rains forecasted for the five days following planting. So, technicians are highly encouraged to evaluate carefully the risk of this practice before recommending it for farmers. In addition, the application of glyphosate should not be done only at the needle point. There is the need for a previous desiccation of the area between 20 and 10 days before planting, which will allow control of the older weed plants.

Another option defined in [40] is the use of PSII inhibitors like bentazon or carfentrazone-ethyl in post-emergence. Carfentrazone, however, may cause severe damage to rice. In addition, both chemicals are contact-only herbicides, which means that a good coverage of the plants by using higher water volumes than the usual followed by flooding on the following day, should allow good results.

Figure 3. Rice seeds at distinct germination stages, from S0 to S3 (needle point). Source: FREITAS, T. F. S; GROHS, D. (SOSBAI, 2012).

The species *Fimbristylis miliacea,* popularly known as globe fringerush, belongs to the family *Cyperaceae* and is disseminated in various regions of the world. In Brazil it appears to be more common in the Southern coastal region infesting flooded rice [42]. The plant cycle is annual or perennial, depending on the environmental conditions; it presents seed dormancy, and germinates in any season if water is available. In RS and SC, the species is distributed all over the rice producing areas. It is observed that the higher infestations occur generally in areas with no uniform irrigation. The population and crop management determine the potential damage in yields due to globe fringerush, but the average losses can be about 73% under high infestations [27].

There were only three reports of resistance of *F. miliacea* to herbicides in the world, and the first record was in Malaysia in 1989 with biotypes resistant to 2,4-D; the second in 2001, in

Brazil, with biotypes resistant to pyrazosulfuron-ethyl and cyclosulfamuron, and more recently in 2010 in Venezuela, also with resistance to ALS-inhibiting herbicides [38]. It is known that biotypes of this species are resistant to ALS inhibitors in Brazil, especially in SC, but the mechanism of resistance is still unknown.

2.6. The case of weedy rice (red rice) resistant to ALS-inhibiting herbicides

Among the major weeds infesting rice, weedy rice can surely be highlighted as the one which most limits the potential yield of rice [43]. The direct losses resulting from competition exerted by weedy rice in rice paddy fields is estimated at about 20% [43].

There are also several indirect losses, such as raised cost of production, depreciation of the market value of cultivated areas and of the harvested product, equipment damage and reduction in generation of jobs, further reducing the profitability of farming [44]. The degree of interference of weedy rice varies with the level of infestation, soil and climatic conditions, cultivar traits, coexistence period and biotype found in the area [45].

The control of red rice with herbicides has become possible after the development of rice genotypes tolerant to the herbicides from the imidazolinone group (ALS-inhibitors) [46]. The same authors also reported that effective chemical control of red rice is almost impossible using conventional genotypes, because of the morpho-physiological similarity between the cultivated and the weedy rice. Despite the Clearfield® system providing a great advantage in terms of weed control, the adoption of herbicides from the ALS group associated to this technology resulted in selection of resistant genotypes of this weed [15]. Thus it is evident that the continued use of the Clearfield® system in rice areas of Rio Grande do Sul favored the development of populations of red rice resistant to imidazolinone due to its repeated use in the absence of crop rotation or others tools.

The introduction of rice cultivars tolerant to imidazolinone herbicides probably resulted in gene flow of the resistance to wild rice genotypes [47-48]. The occurrence of weedy rice populations resistant to herbicides may be caused by gene flow between cultivated varieties and weedy rice [12,13]. A research at RS has indicated that pollen dispersal occurs between cultivated rice and transgenic rice at levels below [49] and others studies [50-51] showed gene flux between rice CL varieties and weedy rice rates of 0.042 and 0.065%, respectively. It should be noted that even with low rates of gene flow, there might be a considerable increase in the frequency of resistant individuals in the population, given the high degree of infestation of cultivated areas [15]. Another study shows that the gene flow as low as the rate of 0.008% originated 170 individuals of red rice per hectare with resistance [13].

In reference [15] with populations of red rice from the six most rice-producing regions of the RS, the occurrence of resistant biotypes to herbicides from the imidazolinone group (imazethapyr and imazapic) was confirmed to occur in all regions under the Clearfield® system. The predominant mechanism of herbicide resistance in weedy rice in RS and SC is the target site insensitivity due to changes in nucleotide sequence of the ALS enzyme [14,52]. Gene flux was the main origin of imidazolinone herbicide resistance, but independent selection occurred in 1.1 % of the evaluated weedy rice plants [14]. The high frequency of weedy rice resistant plants carrying the $G_{654}E$ mutation, which is the same mutation responsible for the resistance in the rice cultivar largely used in Southern Brazil when the weedy rice plants were collected, suggests that gene flow is occurring from the rice cultivar to weedy rice [52].

2.7. A retrospect of the ALS-inhibiting herbicides and ClearField® technology use in irrigated rice fields of Southern Brazil

The use of Clearfield® (CL) technology in rice areas of southern Brazil began in 2002 with imidazolinone-resistant cultivars. Ten years later, more than 60% of irrigated rice in Rio Grande do Sul State carry the CL technology and are treated with these herbicides. The combined use of imidazolinone-resistant rice cultivars with the correspondent herbicides is often very effective, providing more than 95% of control of weedy rice in most cases [53]. This technology had permitted immediate benefits in terms of efficiency and easiness of weed control, mainly for weedy rice and the *Echinochloa* complex. However, at the beginning of the use of CL rice cultivars there were some difficulties that possibly favored the increasing of the number of the ALS-resistant weeds. First, due to high initial costs of the commercial seeds and of the herbicide, part of the fields was planted with saved-seeds and there were the use of not-registered, illegal herbicides, applied at elevated doses in some fields. Second, the CL rice cultivar was planted repeatedly in areas heavily infested with weedy rice, disregarding the official recommendations for the management, which suggested herbicide rotation, field management rotation and crop rotation in fields of irrigated rice [33].

Even though some weeds presented resistance to ALS-inhibitors before the adoption of the CL technology (Table 3), the selection pressure caused by the increasing use of the ALS-inhibitors should be associated with the emerging of weedy rice (*Oryza sativa*) resistant to ALS-inhibiting herbicides, only four years after the starting of the use of Clearfield® technology in southern Brazil [15] which occurred in USA [53]. The fields infested with these resistant biotypes represent a part of the whole area of rice cultivation, but all regions have dispersed resistant weedy rice and there is an increase in the number of cases of resistance. The farmers and assistants are at the present taking additional management strategies for this weed, as the crop and herbicides rotation to reduce the losses and constrains associated with the weed resistance. In Arkansas [53] after 5 years of imidazolinone-resistant rice technology, crop rotation and use of certified seeds are the main reason for rice fields being free of weedy rice.

2.8. Prevention of herbicide-resistant weeds in irrigated rice of Brazil

An herbicide-resistant weed biotype usually occurs in areas where the common practice for weed control is the repeated use of the same product, or the use of different herbicides but with the same mechanism of action. This is the main scenario at the beginning of the weed-resistance cases in rice fields - the high selection pressure - as reported by [37]. This situation is very common in the RS, where rice still is continuously grown as a mono-crop in most parts of the area. In the state of Santa Catarina, the areas are smaller and the management more varied, with farmers using both herbicides and cultural practices on weed management.

The adoption of best-practices in weed control is one of the main tools to prevent the occurrence of new cases of resistance. In reference [33, 68], some preventative measures to avoid or to

Species	Common name	Active ingredient confirmed	Sources*
Sagittaria montevidensis	arrowhead	Azimsulfuron, bentazon, bispyribac-sodium, cyclosulfamuron, ethoxysulfuron, imazapic+imazethapyr, metsulfuron, penoxsulam, pyrazosulfuron-ethyl	[16, 17, 31]
Echinochloa spp.	barnyardgrass	Quinclorac	[4, 18, 25, 54]
		Bispyribac-sodium, flucarbazone, imazapyr, imazethapyr, imazethapyr+imazapic, imazapyr+imazapic, nicosulfuron, penoxsulam, quinclorac	[55, 56, 60-65]
Cyperus difformis	nutsedges	Azimsulfuron, bispyribac-sodium, cyclosulfamuron, ethoxysulfuron, penoxsulam, pyrazosulfuron-ethyl	[35, 36, 39, 65]
Cyperus iria	nutsedges	Bispiribac-sodium, Ethoxysulfuron, imazapyr+imazapic, imazethapyr+imazapic, penoxsulam, pyrazosulfuron-ethyl	[40, 41, 57, 58]
Fimbristylis miliacea	globe fringerush	Azimsulfuron, bispyribac-sodium, cyclosulfamuron, ethoxysulfuron, penoxsulam, pyrazosulfuron-ethyl	[66-67]
Oryza sativa	weedy rice	Imazethayr +Imazapic Imazapyr	[15, 52]

* The sources lists only a part of the whole studies conducted with the species listed.

Table 3. Herbicide-resistant weeds reported in irrigated rice in Southern Brazil.

minimize the risks are the use of crop rotation, the use of herbicides in the correct time and when necessary; to perform the rotation of herbicides, using those with distinct mechanisms of action; and be aware of the results of herbicide applications, checking for escapes and shifts in weed population. When an escaped plant is observed it must be immediately eliminated, preventing the spread of this suspected resistant biotypes. These recommendations are not always adopted in all fields due to the various difficulties. A good exception is the case of the seed-producers: these farmers really care with the weeds in your fields and adopt the best-management practices in terms of weed control, because there are some weed species whose seeds are expressly prohibited in lots of commercial rice seeds, and its presence would condemn the entire field, preventing it to be sold as seed.

In areas where herbicide resistant weed populations occur, some simple – but important – management strategies are issued. It is recommended not to plant very early in spring, because due to low temperatures, weeds will emerge and grow faster than rice, offering an additional difficultly for control and increased competition. The soil could be prepared, or chemically desiccated, immediately before planting rice to eliminate the weed seedlings already emerged; the machinery should be cleaned when leaving an infested area; the herbicides with proven resistant biotypes should not be used, and resistant escaped plants should not be allowed to produce seeds, by means of the localized chemical desiccation or by manual rouging.

In lowlands of Southern Brazil, rice is the main crop and commonly shares areas with cattle production. The cattle can occupy the fields in winter (between two cycles of rice) and consume cold-adapted grasses and broadleaves belonging to the genus *Lolium*, *Trifolium*, *Vicia* and others; or, in summer when the main feed is composed by grasses such as red rice, barnyard-grass, some perennial grasses and others species. Integration crop-livestock in rice fields is an important form of weed management in the production system once they consistently reduce the seed production of some grasses and the number of viable seeds in the soil seed bank will decrease [69].

In recent years, however, soybean has increased in area in the lowlands, also being used as a cash-crop in these fields due to the high prices in the international market. Between one-fourth and one-third of the rice in RS is already rotated with soybeans and this crop is the main – and probably the best – option to the rotational scheme with irrigated rice in terms of increasing the soil fertility and reduction of some pests in rice. Almost all soybeans cultivated in RS are tolerant to glyphosate (Roundup Ready technology) and this herbicide offers very good control of annual grasses such as red rice and barnyardgrass. The consolidation of RR soybean was a step forward in the effectiveness of integrated pest management in irrigated rice in the RS state. The soybean is already used as the main tool of management in the cases of herbicide-resistant weeds occurring in irrigated rice fields, mainly in those well-drained areas. However, there are some concerns about the selective pressure driven by glyphosate, and about the spread of the resistant weeds to glyphosate, such as the Italian ryegrass (*Lolium multiflorum*) and the hairy fleabane (*Conyza* sp.), already present in various places in the south of Brazil.

In terms of herbicide rotation, in the fields with barnyardgrass resistant to ALS-inhibitors and/or auxin-mimic herbicides, the herbicides pendimethalin, trifluralin, thiobencarb, clomazone (in pre-emergence), quinclorac (in pre or post-emergence – avoid it in areas where auxin-mimic resistant biotypes occur), propanil alone or mixed with pendimethalin or clomazone (in early post-emergence) and ACCase inhibitors (in post-emergence of the crop) are good options [33]. There are, however, reports about biotypes of *Echinochloa* with multiple resistances to ALS inhibitors and other chemical groups in several countries of Latin America [70]. As a consequence, no abuses in the chemical control should be allowed, making this weed difficult to be controlled, demanding crop and chemical rotation along the years. It should be highlighted that the use of ACCase inhibiting herbicides in rice fields have promoted efficient control of *Echinochloa* biotypes, but there is the need for rotation of chemical groups to avoid the appearance of biotypes resistant also to this mode of action.

In reference [62] studying methods of application of clomazone and imazapic + imazapyr, reported that the application of clomazone alone or mixed with imazapic + imazapyr in the rice on "needle point" allow efficient control of ALS-resistant *Echinochloa* and the susceptible biotype was efficiently controlled by clomazone alone in the needle point, and by imazapic + imazapyr in all application times.

Several rice farmers use residual herbicides in mixture with glyphosate in the pre-planting desiccation, mainly in areas under minimum- or no-till system (sod seeding) and/or with delayed flooding. In these cases, the elimination of existing weeds is accomplished with glyphosate and the new cohorts of seedlings are controlled by the residual herbicides. One of the most widely used herbicides for this task is clomazone, which presents residual effects over several grasses, especially barnyardgrass [71]. Thus, the use of clomazone with glyphosate, either in the early pre-planting desiccation of sod seeding areas, or in the post-planting on “needle point”, is an effective tool for weed suppression. The application of glyphosate in the needle point was previously discussed, being illustrated in Figure 2.

Besides clomazone, pendimethalin may also be used at the “needle point” mixed with glyphosate aiming to suppress the emergence of *Echinochloa* spp. This pre-emergence herbicide plays an important role in the suppression of propanil-resistant junglerice in Central America [72, 73], whose genotypes still were not identified in Brazil. Pendimethalin thus can represent an important herbicide in the management strategy for the Brazilian ALS-inhibiting and Auxin-mimic resistant biotypes. In addition, propanil applied in early post-emergence, mainly mixed with clomazone or pendimethalin, are alternative choices depending on the level of the field infestation and effectiveness of the previously applied treatments [74]. In Brazil there are no reported cases of *Echinochloa* biotypes resistant to ACCase-inhibiting herbicides (Merotto and Noldin, personal information); thus, these herbicides are great options for post-emergence control of biotypes of *Echinochloa* resistant to ALS or Auxin-mimic herbicides. However, herbicides with this mode of action are considered of “high risk” for resistance evolution if not properly managed [70].

Managing herbicides properly within these options will allow farmers to have a 3-year rotation of herbicide, which will reduce both the occurrence of resistant biotypes, and the chance of appearance of a new resistant weed biotype. Farmers should request their technicians to plan the most proper herbicide rotation for every case. Used alone, none of the currently available cultural techniques provides an adequate level of weed control. However, when used in carefully planned combinations, extremely effective barnyardgrass control can be achieved [75].

3. Conclusions

Weeds resistant to herbicides have been of concern for scientists and farmers in the Rio Grande do Sul and Santa Catarina states of Brazil, since most herbicides used for chemical control are no longer effective in many fields. It is noteworthy to mention that the evolution of weeds resistant to herbicides is related to selection pressure, genetic variability of weeds, the number of genes involved, patterns of inheritance, gene flow and dispersal of the propagules. The elucidation of these factors becomes important for future predictions of proportions between resistant, tolerant and susceptible biotypes in the fields, and will require choosing more efficient management methods on these biotypes, aiming also to prevent the multiplication and dissemination of weed-related problems in the area.

In the case of rice, there are some intrinsic difficulties for adoption of full-integrated weed management with crop rotation because the condition of soil, with its susceptibility to be flooded and difficulties for fast drainage. The weed resistance to herbicides may cause losses to the rice production in many regions of Southern Brazil. Without the introduction of new herbicide mechanisms of action or better herbicide-resistance management, a technology that has allowed increases in agricultural productivity is at risk [76]. Despite the success attained in some cases, more research and investments must be directed to this field of study in irrigated rice in Brazil, especially in the Southern region, which is the main producer, so that the problem can be more understood and specific strategies to manage this problem can be established and applied by the farmers.

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