Low-level resistance of *Cyperus iria* L. to ALS-inhibiting herbicides occurring in the State of Rio Grande do Sul

Baixo nível de resistência de *Cyperus iria* L. a herbicidas inibidores da ALS ocorrentes no Estado do Rio Grande do Sul

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Abstract

This work aimed at determining possible resistance of three biotypes of *Cyperus iria* to inhibiting herbicides of acetyl-CoA carboxylase enzyme (ALS). An experiment at greenhouse was conducted out where suspected resistance biotypes were multiplied through seeds in pots filled with soil. The treatments were arranged in a trifactorial (AxBxC) design, where factor A consisted of three biotypes of *Cyperus iria*, from three locations of the state of Rio Grande do Sul (named Santa Maria 1, São Borja 3 and Cachoeira do Sul 7). Factor B corresponds to three herbicides (pyrazosulfuron-ethyl, ethoxysulfuron and bentazon). Factor C refers to herbicide doses (zero, 50% of registered dose; registered dose, two, four and eight times registered dose of herbicides). For resistance measurement, it was used resistance factor calculation (RF) and for dose-response curves through regression analysis, RF on dry mass (DM) of plants. It was observed that São Borja 3 and Cachoeira do Sul 7 biotypes have a low resistance factor (1 < RF < 10) to pyrazosulfuron-ethyl and ethoxysulfuron herbicides. These showed lower susceptibility to ethoxysulfuron (RF = 5.49-6.76) than pyrazosulfuron-ethyl (RF = 2.21-2.47). The clear distinction in the susceptibility of biotypes, include responses of chemical control to field due to the long period of use of ethoxysulfuron herbicide. It is concluded that this factor characterizes low-level resistance, being a cross-resistance type, thus demonstrating the ineffectiveness of ALS - inhibiting herbicides to control this species.

Additional keywords: cereals; clearfield; control; ciperácea; rice flatsedge.

Resumo

Objetivou-se neste trabalho averiguar a possível resistência de três biótipos de *Cyperus iria* a herbicidas inibidores da enzima Aceto Lactato Sintase (ALS). Deste modo, realizou-se um experimento em casa de vegetação, em que os biótipos suspeitos de resistência foram multiplicados, via sementes, em vasos preenchidos com solo. O delineamento experimental conduzido foi um trífatorial (AxBxC), sendo o fator A constituído por três biótipos de *Cyperus iria*, oriundos de três localidades do Estado do Rio Grande do Sul (denominados Santa Maria 1, São Borja 3 e Cachoeira do Sul 7). O fator B corresponde a três herbicidas (pirazossulfurom-ético, etoxissulfuron e bentazona). O Fator C submete às doses dos herbicidas (zero; 50% da dose de registro; dose de registro; duas, quatro e oito vezes a dose de registro dos herbicidas). Para a aferição da resistência, foi utilizado o cálculo do fator de resistência (FR) e curvas dose-resposta através de análise de regressão, a partir da massa seca (MS) das plantas. Verificou-se que os biótipos de *Cyperus iria* São Borja 3 e Cachoeira do Sul 7 possuem baixo fator de resistência (1 < FR < 10), para os herbicidas pirazossulfurom-ético e etoxissulfuron. Estes, demonstraram menor suscetibilidade ao herbicida etoxissulfuron (FR = 5,49-6,76) do que ao pirazossulfurom-ético (FR = 2,21-2,47). A evidente distinção na suscetibilidade dos biótipos contempla as respostas do controle químico a campo, devido ao longo período de utilização do herbicida etoxissulfuron. Conclui-se que este fator caracteriza baixo nível de resistência, sendo ainda esta do tipo resistência cruzada, justificando a ineficácia de herbicidas inibidores da ALS no controle desta espécie.

Palavras-chave adicionais: cereais; ciperácea; clearfield; controle; junquinho.
Introduction

Currently rice is a product of great importance in human nutrition, being the food retail of more than 3 billion people. It has a total cultivation area about 158 million hectares worldwide and is the second most cultivated cereal. The Southern region of Brazil is responsible for most domestic production and the state of Rio Grande do Sul has approximately 67.5% of all rice produced in the country. On the Western border of the state concentrates the largest production of cereal (CONAB, 2015). Therefore, it has a strategic role in solving problems regarding food security (SOSBAI, 2014).

Crops suffer interference from several factors during the development period from germination to physiological maturity, with impact on productivity. In the same environment, cultivated plants compete with weeds for water, light, heat, carbon dioxide, oxygen and mineral nutrients. These essential factors for plant development may become limited, and in this adverse scenario, the plant species that use more efficient these resources will have a greater advantage in its development (Silva & Silva, 2007). The presence of these plants, however, can indirectly affect crop, serving to host insects, diseases, harvesting interference and other (Karam et al., 2010). It can also cause decrease in product value due to contamination with undesirable seeds and waste of weeds. In cereals, these factors can increase the moisture of grain mass. Thus, the adoption of strategies for integrated weed management is essential for success in control.

*Cyperus iria* is an annual plant, with trine stem, narrow leaves and reproduces by seed. It prefers moisture and rich soils, popularly known as rice flatsedge or sedge. Germination occurs in spring, with fast development completing two cycles in rice growing period (Kissmann, 1997). It is estimated that losses in productivity of irrigated rice caused by competition with plants of the genus *Cyperus* sp. are up to 48% (Oerke et al., 1994). This interference can negatively impact the number of fillers, reducing from 13% to 27% in rice plants when in competition with *Cyperus esculentus* L. (Erasmo et al., 2000).

The chemical management for control of weeds in irrigated rice is primarily performed with ALS inhibiting herbicides (ALS). These herbicides cause inhibition of the synthesis of branched amino acids, stopping protein synthesis and consequently causing interference in DNA synthesis, immediately in cell growth. The plant sensitive to the inhibitor mechanism of action becomes weak, acquiring a chlorotic appearance and finally dying just 21 days after application. Thus, the selection pressure imposed by this action mechanism on the weeds is high, especially after the introduction of Clearfield® system in rice cultivation in Rio Grande do Sul.

For the control of *Cyperus iria*, in the current Clearfield® system are mainly recommended products whose action mechanism inhibits ALS enzyme. They are included in the chemical groups of imidazolinones, sulfonylureas, pyrimidinylthiobenzoates and triazolopyrimidine to control this weed. Consequently, the excessive use of ALS-inhibiting herbicides causes greater selection pressure imposed on weeds, thus favoring the development of resistance to these herbicides.

Resistance to a given herbicide is the ability of a biotype to survive and to reproduce after exposure. This ability to resist is inherent and heritable, i.e., there is a selection pressure in which only resistant biotypes will remain. Excessive use of a unique herbicide, leads to this scenario of resistant plants in the field (Bonny, 2011). The global picture is worrying, there are 457 confirmed cases of resistance, consisted of 246 species (143 dicotyledonous and 103 monocotyledonous), involving 22 of the 25 action sites known by herbicides (Heap, 2015). Studies have shown the existence of *Cyperus difformis* L. biotypes resistant to ALS inhibitors herbicide, indicating cross-resistance to bensulfuron-methyl and bispyribac-sodium (Osuna et al., 2002).

In this context, the evolution of the first resistant biotypes of this Cyperaceae in irrigated Rice areas in Rio Grande do Sul, plus the rotation of herbicides and other control methods should be intensified in order to establish more effective management programs focused on these resistant biotypes.

Material and methods

For this study, three suspected *Cyperus iria* biotypes of resistance to ALS inhibitors herbicide were collected. The collection sites corresponded to three rice producer municipalities in the state of Rio Grande do Sul: Santa Maria, Cachoeira do Sul and São Borja.

The experiment was conducted in 2013/14 crop year, in a greenhouse at Universidade Federal de Santa Maria - Department of Biology, to examine the resistance of *Cyperus iria* biotypes to three herbicides, two inhibitors of Acetolactate Synthase (ALS) and one Photosystem II inhibiting herbicide. It was completely randomized design with four replications and treatments arranged in AxBxC trifactorial scheme.

The A factor corresponded to three *Cyperus iria* biotypes denominated: Santa Maria 1 (SM1) susceptible, São Borja 3 (SB3) and Cachoeira do Sul 7 (CH7) suspected of resistance. The B factor corresponded to two ALS inhibiting herbicides: pyrazosulfuron-ethyl (Sirius® 250 SC) (containing 250 grams of active ingredient per liter of commercial product and its registered dose for rice flatsedge control is 20 grams of active per hectare), ethoxysulfuron (Gladium®) (containing 600 grams of active ingredient per liter of commercial product and its registered dose for rice flatsedge control is 60 grams of active per hectare) and one Photosystem II inhibiting herbicide: bentazon (Basagran® 600) (containing 600 grams of active ingredient per liter of commercial product and its...
registered dose for control rice flatsedge is 960 grams of active per hectare). The C Factor corresponded to increasing doses of herbicides (zero, 50% of registered dose, registered dose, two, four and eight times the herbicide registered dose) (Agrofit, 2015).

The seeds of accessions were multiplied in polyethylene pots with 7.5 L capacity, coated in plastic bags, thus preventing water loss and herbicide when irrigated. These were filled with 2.5 kg of organic substrate and 4.0 kg of medium texture sand, sterilized, composing a sand-substrate system. The seeds were placed at 1 cm depth, tagged with millimetric ruler. The plants remained in greenhouse, watered every two days for 30 days. The average climatic conditions during the application were 27.3 °C and 61.2% of relative air humidity.

The treatment application was performed when *Cyperus iria* were at development stage of three to four fully developed leaves. It was used CO₂ backpack pressurized sprayer provided with a bar of 1.5 meters containing four nozzles of Teejet XR 110.02, pressure of 25 lbs pol⁻² and application rate of 150 L ha⁻¹.

The substrate used was Plantmax® with the following chemical characteristics: water pH (1:1) = 5.47; P = mg dm⁻3; K = 600 mg dm⁻3; V = 68.7%; Zn = 22.62 mg dm⁻3; Fe = 210.3 mg dm⁻³; Mn = 21.4 mg dm⁻³; Cu = 0.79 mg dm⁻³; Ca = 9.64 cmol c dm⁻³; Mg = 3.95 cmol c dm⁻³ and Al = 0.24 cmol c dm⁻³.

The removal of samples to determine dry mass (DM) was performed 20 days after application (DAA), due to clear visualization of phytotoxicity symptoms in rice flatsedge plants caused by herbicide treatments by visual analysis (data not shown). The assessment of the effects of treatments on plants was carried out at 20 days after treatments with number counting of plants per pot, and samples were referred for drying at 60 °C until constant mass. Dose-response curves were obtained by percentage analysis of dry mass compared to zero dose. The resistance factor (RF) was calculated from MSⱼ of the suspected biotypes of resistance compared to tested susceptible biotype, where RF values above 1 (RF > 1) characterizes the level of resistivity. For the confirmation of a high-level resistance, value of RF should be greater than or equal to 10 (FR ≥ 10), and for cases with low resistance, RF should be less than 10 (RF < 10) (Heap, 2005). All data were submitted to the ′F′ test in the analysis of variance to verify the interactions among factors and posteriorly, data were adjusted to non-linear regression model of log-logistics type using the model proposed by Seeefeldt et al. (1995).

\[
Y = \frac{A}{1 + \left(\frac{X}{B}\right)^{C}}
\]

In which: Y is the dry mass at 20 DAA, X is herbicide dose and A, B and C are curve parameters, so A is the difference between the maximum and minimum point of the curve, B is the providing dose of 50% variable response and C is the curve slope. The parameters required in the equation were obtained by plotting dry mass data (DM) of plants compared to the control. The plots were obtained from a basic matrix data analyzed in SigmaPlot® program 11 version.

### Results and discussions

From the obtained results, it can be stated that São Borja 3 (SB3), Cachoeira do Sul 7 (CH7) and Santa Maria 1 (SM1) biotypes were susceptible to bentazon herbicide in the registered dose (960 g of ai ha⁻¹), with reduction below of 50% in dry mass assessment (DM) at 20 days after application (Figure 1).

![Figure 1 - Dose-response curve of bentazon herbicide treatment applied in *Cyperus iria*.](image)

It can be concluded through the dose-response curves that biotypes of *Cyperus iria* São Borja 3 and Cachoeira 7 showed low-level resistance to pyrazosulfuron-ethyl herbicide, whose action mechanism inhibits ALS enzyme (ALS) (Figure 2). Works showed high-level resistance (RF≥10) to pyrazosulfuron-ethyl herbicide for biotypes of *Cyperus difformis* L. existing in irrigated rice crops (Galon et al., 2008, Agostinetto et al., 2011). However, Santa Maria
1 biotype showed susceptibility to pyrazosulfuron-ethyl, when evaluated at registered dose (20 g ai ha\(^{-1}\)) (Figure 2).

**Figure 2** - Dose-response curve of pyrazosulfuron-ethyl herbicide treatment applied in *Cyperus iria*.

São Borja 3 and Cachoeira do Sul 7 biotypes also showed low-level resistance to ethoxysulfuron herbicide, at registered doses (60 g ai ha\(^{-1}\)) and doses higher than the registered (Figure 3). Similar studies with ethoxysulfuron herbicide were carried out with suspected biotypes of *Cyperus difformis* L. of resistance to ALS-inhibiting herbicides. The results of dose-response curves showed that plants had a low-level resistance to this herbicide (Agostinetto et al., 2011). However, other studies on *Cyperus difformis* L. accessions collected in rice crops showed high-level resistance to ethoxysulfuron (Ruiz-Santaella et al., 2004). In this experiment, Santa Maria 1 biotype was susceptible to the same evaluated doses of ethoxysulfuron herbicide (Figure 3).

**Figure 3** - Dose-response curve of ethoxysulfuron herbicide treatment applied in *Cyperus iria*.

Low level resistance is conceptualized as one that it does not take into account the maximum recommended dose (ai) of the herbicide, as biotypes of this species may show different responses, not necessarily indicating that the herbicide does not efficiently control using the recommended dose (Christoffoleti, 2001, Christoffoleti & Ovejero, 2008). Practically, low-level resistance to herbicides occurs when the dose required to reduce 50% of the resistant biotype dry mass is less than ten times the dose required to reduce 50% of sensitive biotype dry mass, resulting in resistance factor less than ten \((RF < 10)\) (Heap, 2005). In this context, cases of low-level resistance to glyphosate have been detected in the state of Rio Grande do Sul for *Eleusine indica*, showing \(RF = 1.37\) at 20 days after treatment application (Vargas et al., 2013).

From the analysis of dose-response curves conducted on plants treated with ethoxysulfuron and pyrazosulfuron-ethyl, it can be concluded that the resistant accessions of *Cyperus iria* São Borja 3 and Cachoeira 7 have cross-resistance to ALS-inhibiting herbicides, and this low level resistance due to the resistance factor is less than ten \((RF < 10)\). In this study, it did not include an evaluation of the resistance mechanism, and further studies are necessary to identify if resistance occurs by modified site action or as a hypothesis to be tested: resistance by metabolism.

According to data, it was obtained significant values for resistance factor \((RF>1)\) of suspected biotypes (São Borja 3 and Cachoeira 7, by reducing the dry mass by 50% when compared to the susceptible biotype (Santa Maria 1). The results show low-level resistance \((RF<10)\) for São Borja and Cachoeira 7 biotypes when subjected to different doses of tested products (Table 1). In the test with bentazon, the registered dose \((960 \text{ ai ha}^{-1})\) controlled all tested accessions when subjected to analysis of variance \((p>0.05)\) (data not shown).

It is observed by the results obtained from the analysis of resistance factor (Table 1), that resistant biotypes showed less susceptibility to ethoxysulfuron herbicide \((RF = 5.49 \text{ to } 6.76)\) compared to susceptible biotype than pyrazosulfuron-ethyl \((RF=2.21-2.47)\). These results corroborate with the field observations. Areas, in which were collected resistant biotypes, have a history of long use of ethoxysulfuron herbicide to control *Cyperus iria*. This explains the lower susceptibility to this herbicide due to long period of use when compared to pyrazosulfuron, even if the results show cross-resistance to the two herbicides. Currently in these areas, the registered dose of ethoxysulfuron and pyrazosulfuron has controlled less than 50% of the *C. iria* population even being very effective herbicides in control it (data not shown).
Similar results of cross-resistance were found in studies on biotypes of *Cyperus difformis* L. resistant to ALS-inhibiting herbicides, indicating cross-resistance tobensulfuron-methyl and bispiribac-sodium (Osuna et al., 2002).

In order to have a satisfactory control, bentazon herbicide showed to be an effective option for post-emergent control, according to the results of response curves regarding alternatives in the management programs for these areas with *Cyperus iria* occurrence with low-level resistance to ALS enzyme, which may be within Clearfield® system, i.e., performing the rotation of herbicides. These results were similar to data obtained in irrigated rice area, which show the efficiency of bentazon use in control of *Cyperus difformis* L., both susceptible and resistant biotypes to ALS-inhibiting herbicides (Galon et al., 2008).

**Table 1** - Resistance factor (RF) according to the product dose which reduced 50% of dry mass of *Cyperus iria* biotypes, when evaluated at 20 days after application.

<table>
<thead>
<tr>
<th>Biotype</th>
<th>(1)MS50</th>
<th>(2)RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB3</td>
<td>303.74</td>
<td></td>
</tr>
<tr>
<td>CH7</td>
<td>554.95</td>
<td>1.83</td>
</tr>
<tr>
<td>SM1</td>
<td>636.61</td>
<td>2.10</td>
</tr>
<tr>
<td>SB3</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>CH7</td>
<td>34.68</td>
<td>2.21</td>
</tr>
<tr>
<td>SM1</td>
<td>38.84</td>
<td>2.47</td>
</tr>
<tr>
<td>SB3</td>
<td>29.18</td>
<td></td>
</tr>
<tr>
<td>CH7</td>
<td>160.15</td>
<td>5.49</td>
</tr>
<tr>
<td></td>
<td>197.36</td>
<td>6.76</td>
</tr>
</tbody>
</table>

(1) = product dose which reduced 50% of the dry mass, (2) = Resistance factor, (3) = Susceptible biotype, (4) = Despite the presented resistance factor, there was control superior to 80% in all biotypes (data not shown here).

The occurrence of *Cyperus iria* with some level of resistance to ALS - inhibiting herbicides had not been reported to Rio Grande do Sul, but at field level there are reports of the inefficiency of herbicides cited above in Cyperaceae biotypes, occurring in some regions of the state (data not shown).

**Conclusions**

Biotypes of *Cyperus iria* São Borja 3 and Cachoeira do Sul 7 have a low level of cross-resistance to pyrazosulfuron-ethyl and ethoxysulfuron, which the resistance factor for biotype São Borja 3 was (RF = 2.21 and 5.49) and for Cachoeira do Sul 7 biotype (RF = 2.47 and 6.76), respectively.

**References**


