Ciência Rural, Santa Maria, v.54:02, e20220384, 2024 http://doi.org/10.1590/0103-8478cr20220384
ISSNe 1678-4596
CROP PROTECTION

Resistance of barnyardgrass biotypes (*Echinochloa crus-galli*) to ACCase-inhibiting herbicides in the main rice-growing regions of the state of Santa Catarina, Brazil

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ABSTRACT: The resistance of *Echinochloa crus-galli* (barnyardgrass) to cyhalofop-p-butyl has already been confirmed in rice fields in Santa Catarina, Brazil. However, it is not known if this resistance affects other ACCase inhibitors. This study evaluated the occurrence of cross-resistance in *Echinochloa crus-galli* biotypes from the main rice-growing regions of Santa Catarina to ACCase inhibitors. The research was conducted in a greenhouse, using a completely randomized design with a factorial scheme that included three ACCase-inhibiting herbicides (cyhalofop-p-butyl, quizalofop-p-ethyl, and profoxydim) belonging to two chemical groups (aryl-oxifenoxi-propionates and cyclohexanediones), eight herbicide doses, and four biotypes evaluated in the F1 generation and two biotypes evaluated in the F2 generation. These biotypes were selected based on the results of a preliminary trial with 21 populations. The herbicides were applied when the plants presented two true leaves. After control evaluations, the lethal dose required to control 50% and 80% of the population (LD_{50} and LD_{80}) and the resistance factor (RF) were determined by nonlinear regression. The results showed that only one biotype from Tubarão, Santa Catarina met all statistical and agronomic criteria and had cross-resistance to ACCase inhibitors confirmed. In both generations, the RF was greater than 1.0, and the dose required to achieve 80% control exceeded the maximum recommended dose on the label. Resistance levels were higher for herbicides belonging to the aryl-oxifenoxi-propionate chemical group, with RF greater than 7.0. For the cyclohexanedione chemical group, the RF was less than 5.0.
Key words: aryloxyphenoxypropionates, cyclohexanediones, whole-plant experiment, *Echinochloa crus-galli.*

Resistência de biótipos de capim-arroz (*Echinochloa crus-galli*) aos herbicidas inibidores da ACCase nas principais regiões orizícolas do Estado de Santa Catarina

RESUMO: A resistência de capim-arroz (*Echinochloa crus-galli*) ao cyhalofop-p-butyl já foi confirmada em áreas de produção de arroz de Santa Catarina, entretanto não se sabe se essa resistência compromete outros inibidores da ACCase. O objetivo desse trabalho foi avaliar a ocorrência de resistência cruzada em biótipos de capim-arroz das principais regiões orizícolas do Estado de Santa Catarina a inibidores de ACCase. A pesquisa foi desenvolvida em casa de vegetação, em delineamento inteiramente casualizado com esquema fatorial, sendo: três herbicidas inibidores de ACCase (cyhalofop-p-butyl; quizalofop-p-ethyl e profoxydim) pertencentes a dois grupos químicos (ariloxifenoxipropionatos e ciclohexanodionas); oito doses dos herbicidas e quatro biótipos foram avaliados na geração F_1 e dois biótipos na geração F_2. Estes biótipos foram selecionados a partir dos resultados de um ensaio preliminar com 21 populações. A aplicação foi realizada quando as plantas apresentaram duas folhas verdadeiras. Após as avaliações de controle determinou-se, por regressão não-linear, a dose letal necessária para controlar 50% e 80% da população (DL_{50} e DL_{80}) e o fator de resistência (FR). Os resultados demonstraram que apenas um biótipo de Tubarão-SC atendeu todos os critérios estatísticos e agronômicos e teve a resistência cruzada a inibidores da ACCase confirmada. Nas duas gerações observou-se que o FR foi superior a 1,0 e que a dose necessária para atingir o controle de 80% excedeu a dose máxima recomendada em bula. O nível de resistência foi superior para os herbicidas do grupo químico ariloxifenoxipropionatos, com FR maior que 7,0. Para o grupo químico ciclohexanodionas o FR foi menor que 5,0.
Palavras-chave: ariloxifenoxipropionatos, ciclohexanodionas, dose resposta, *Echinochloa crus-galli*.

INTRODUCTION

The management of herbicide-resistant weeds is a challenge due to the limited number of herbicides that act on alternative sites of action (TAKANO et al., 2021). A total of 512 herbicide-resistant weeds (species x site of action) have been reported worldwide, compromising 23 of the 26 known herbicide mechanisms of action (HEAP, 2022). Of all resistance cases, 46 are related to barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], which is one of the main rice weeds worldwide (NORSWORTHY et al., 2013).

Received 07.06.22 Approved 04.21.23 Returned by the author 06.24.23
CR-2022-0384.R3
Editors: Leandro Souza da Silva André da Rosa Ulguim

Barnyardgrass (*Echinochloa complex*) belongs to the Poaceae family and is widely distributed worldwide, reported in Europe, Africa, Asia, Australia, the Pacific, North America, and South America, being considered the third most relevant weed species worldwide (HEAP, 2022). Barnyardgrass is an annual plant, reproducing by seeds, with rapid initial growth and highly competitive capacity (BASTIANI et al., 2015), with broad geographic distribution and morphologically similar to rice plants (ANDRES et al., 2007).

The *Echinochloa* genus includes approximately 250 annual and perennial species (BAJWA et al. 2015), originating from different countries and distributed in tropical and warm temperate regions of the world, is a polyploid plant (OSUNA et al., 2002). Losses generated by barnyardgrass infestation in irrigated rice fields can reach 90%, considering various factors such as weed plant density, rice sowing density, sowing time, cultivar used, and the timing of the beginning of irrigation (PINTO et al., 2002). In another study, GALON et al. (2007) reported that the presence of a single barnyardgrass plant per m2 reduced rice yield between 5 and 22%; while MENNAN et al. (2012) identified that the density of two *E. crus-galli* plants per m2 reduced grain yield by 10%.

Chemical control is one of the most widely used methods for controlling weeds, due to its high efficiency, little need for labor, time savings, and selectivity to certain crops. It is important to know the characteristics of the herbicide to be used, such as half-life, withholding period, and toxicity (MACIEL, 2014), as well as the application time. Chemical control can be performed in pre- or post-emergence applications (CARVALHO, 2013). Pre-emergence application occurs before weed emergence. Post-emergence application occurs after crop emergence and aims to act on weed plant development. During weed plant development, the probability of suffering losses due to competition is higher (AGOSTINETO et al., 2015).

Acetyl-coenzyme A carboxylase (ACCase) inhibitors have been widely used in irrigated rice to control Echinochloa species (FANG et al., 2020), three groups of ACCase inhibitor herbicides are available on the market: aryloxyphenoxypropionates (FOPs), cyclohexanediones (DIMs), and phenylpyrazole (DEN) (DAYAN et al., 2019). Over the years, herbicide-resistant weeds to ACCase inhibitors have become a growing problem worldwide, with the aggravating factor of multiple resistance, compromising and making management difficult in these areas.

According to HEAP (2014), a plant can exhibit cross-resistance or multiple resistance. Multiple resistance occurs when more than one resistance mechanism is present in the same individual, usually resulting from the sequential selection of resistance mechanisms by herbicides with different modes of action or through the accumulation of resistance genes through cross-pollination or gene flow. Cross-resistance is when a single resistance mechanism confers resistance to more than one herbicide, but this characteristic does not necessarily confer resistance to all chemical groups of herbicides with the same mode of action. Variable levels of cross-resistance to herbicides from different groups can exist (HEAP, 2014).

Cases of resistance to ACCase inhibitors in *Echinochloa* have been reported worldwide, as populations of this genus have been described as resistant. According to HEAP (2022), the occurrence of ACCase-resistant barnyardgrass is responsible for 23% of all cases of proven barnyardgrass resistance, and the first report of ACCase-resistant grasses in Brazil was in 1999 (HEAP, 2022). Recently, HWANG et al. (2021) reported a population of *Echinochloa crus-galli* resistant to cyhalofop-p-butyl, as well as reports of populations with multiple resistance to herbicides (MATZENBACHER et al., 2015; EBERHARDT et al., 2016; ROUSE et al., 2019; HAGHNAMA& MENNAN, 2020).

In Brazil, the record of *Echinochloa crus-galli* resistance to herbicides in paddy rice occurred in 2015, localized in Tubarão-SC, one of the main paddy rice-producing regions in Santa Catarina. In this case, the study focused on a population in a single generation that showed multiple resistance to the herbicides quinclorac, penoxsulam, and cyhalofop-butyl. Cross-resistance and resistance characterization for other chemical groups of ACCase inhibitors were not assessed (EBERHARDT et al., 2016). Therefore, this research evaluated the occurrence of cross-resistance to ACCase inhibitors in barnyardgrass biotypes in the main rice-growing regions of the State of Santa Catarina, following the criteria proposed by HRAC (2020) and SBCPD (2018).

MATERIALS AND METHODS

Seed collection

The viability of *Echinochloa crus-galli* panicles was identified by the Basic Local Alignment Search Tool (BLAST), which was developed to compare primary biological sequences against a database containing a large amount of information, returning the most similar and statistically significant sequences. In this study, to find regions of similarity between biological sequences, nucleotide sequences were compared with databases, obtaining a statistically significant result of 99.98% of the collected samples with *Echinochloa crus-galli*.

Samples were collected during the 2019/2020 harvest season in the North Coast, South Coast, and Itajaí Valley regions of the state of Santa Catarina (Table 1), where there were indications of control failure after the application of ACCase inhibitor herbicides. After collection, panicles were placed in labeled paper bags with the collector's name, municipality, collection location, infested area, infestation density, and chemical management used in the last crops (herbicides and doses). Later, the seeds were sent to CAV/UDESC for cleaning and standardization before storage in a refrigerator at 2-6 °C.

Table 1 - Representation of identification (nomenclature assigned to each biotype), collection municipality, density of weed plants, and total infested area at the time of seed collection.

Biotypes	Municipality	Location	plants m^{-2}	Area (ha)
CA 01	Paulo Lopes	27º 55' 15,68" S; 48º 39' 22,53" W	15	50
CA 02	Içara	28º 43' 21,64" S; 49º 13' 12,46" W	20	50
CA 03	Turvo	28º 57' 15,09" S; 49º 45' 13,58" W	5	22
CA 04	Paulo Lopes	27º 55' 13,30" S; 48º 39' 21,12" W	20	50
CA 05	Palhoça	27º 53' 55,46" S; 48º 41' 14,38"W	10	130
CA 06	Imaruí	28º 07' 59,50"S; 48º 45' 13,52"W	5	184
CA 07	Tubarão	28º 30' 06"S; 49º 01' 42"W	2	4
CA 08	Tubarão	28º 30' 55,91"S; 49º 00' 25,58"W	30	160
CA 09	Ermo	29º 00' 56,66"S; 49º 38' 19,77"W	5	55
CA 10	Praia Grande	29 º 14' 09,9"S; 49º 54' 05,6"W	2	15
CA 11	Tubarão	28º 33' 56,36"S; 48º 57' 34,19"W	5	325
CA 12	Tubarão	28º 27' 08,97"S; 48º 59' 41,84"W	10	120
CA 13	Rio do Campo	26º 55' 53,11"S; 50º 07' 47,95"	30	100
CA 14	Taió	24º 03' 41,20"S; 50º 14' 49,44"W	10	30
CA 15	Rio do Oeste	27º 11' 37,85"S; 49º 54' 17,62"W	10	26
CA 16	Pouso Redondo	27º 12' 14,63"S; 49º 53' 06,51"W	20	15
CA 17	Massaranduba	Verde ExpWsitWr	-	-
CA1B	Pouso Redondo	SítiW perens	15	8
CA2B	Rio do Campo	TaiWzinhW	15	24
CA3B	Rio do Oeste	AngicW	10	2
CA4B	Taió	Palera	8	2

Preliminary trial

A preliminary trial experiment was conducted in a greenhouse at the Center of Agroveterinary Sciences (CAV), State University of Santa Catarina (UDESC), in Lages-SC, Brazil. Barnyardgrass biotypes were sown in 12 dm^{-3} trays filled with commercial MecPlant substrate. A population of 50 plants was established for each repetition, adopting three repetitions per biotype. After plants reached the stage of 2-3 fully developed leaves, cyhalofop-p-butyl (Clincher™) was applied at a dose of 360 g ai ha^{-1} with mineral oil as an adjuvant (Assist™ EC) at a dose of 1.5 L ha^{-1}. This dose is commonly used in the field and is higher than the maximum recommended in the label for barnyardgrass. The application was held using a CO_2-pressurized backpack sprayer, equipped with four flat fan nozzles (AD 110 02), at a working pressure of 208 kPa, height of 0.5 m above the target, and application rate of 200 L ha^{-1}. The weather conditions during application were checked using a thermo-hygrometer-anemometer and indicated a temperature of 27 °C, relative humidity of 67%, and wind speed of 1.2 km h^{-1}. The trays were flooded 24h after herbicide application.

The plant survival rate was evaluated 21 days after application (DAA). The plants were considered alive if they had produced new green leaves after treatment and maintained active growth (NEVE & POWLES, 2005). After this evaluation, the biotypes that showed a survival rate ≥ 50% were selected for further steps.

Dose-response assays in two generations (F_1 and F_2)

Dose-response experiments were conducted in a greenhouse at the Center for Agricultural and Veterinary Sciences (CAV), State University of Santa Catarina (UDESC), in Lages-SC, Brazil. Experimental units consisted of 0.4-dm^3 plastic pots filled with commercial inert substrate and, after the establishment of barnyardgrass, thinning was performed, keeping four plants per experimental unit.

The experimental design was a completely randomized factorial design (4 x 3 x 8), with three replications. The first factor corresponds to four biotypes, namely CA01, CA02, CA05, and CA07 (selected from the preliminary trial), the second factor was three herbicides - cyhalofop-p-butyl (Clincher™) at the recommended dose of 360 g ai ha^{-1}; quizalofop-p-ethyl (Targa™) at the recommended dose of 120 g ai ha^{-1}, and profoxydim (Aura™) at the recommended dose of 170 g ai ha^{-1}, and the third factor corresponds to eight herbicide doses (0.00, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, and 16 times the recommended dose for each herbicide).

The application was performed when plants had 2-4 fully developed leaves, using a CO_2- pressurized backpack sprayer with AD 110 02 nozzles at a height of 0.50 m from the target, application rate of 200 L ha^{-1}, working pressure of 208 kPa, and speed of 3.6 m s^{-1}. The climatic conditions during application were checked using a thermo-hygrometer and indicated a temperature of 23 °C, relative humidity of 72%, and wind speed of 1.8 km h^{-1}. The pots were flooded 24 hours after herbicide application. Control was evaluated at 7, 14, and 28 days after application by a visual scale from 0 to 100%, wherein 0 means no control and 100% complete control (KUVA, et al., 2016).

Seeds collected in the field were considered the first generation (F_1). After the dose-response trial in the first generation (F_1), only one of the biotypes had surviving plants after exposure to herbicide doses equal to or greater than the recommended dose. The surviving plants were kept in a greenhouse to produce seeds; and subsequently, conduct the second generation (F_2). When barnyardgrass plants started producing seeds, panicles were isolated with porous fabric (tulle) and, after maturation, they were collected and sent to the laboratory where seeds were cleaned for storage.

In the second generation (F_2), the procedure was identical to that described for the F_1 generation, and it was conducted with two biotypes - CA01 and CA07, based on the results of the F_1 generation. The climatic conditions during application were checked using a thermo-hygrometer and indicated a temperature of 25 °C, relative humidity of 77%, and wind speed of 2.1 km h^{-1}. The experimental units were flooded 24 hours after herbicide application. Control was evaluated by a visual scale (KUVA, et al., 2016) from 0 to 100%, wherein 0 means no control and 100% complete control.

Alternative control

The experiment was conducted in a greenhouse at the Center of Agroveterinary Sciences (CAV), State University of Santa Catarina (UDESC), in Lages-SC, Brazil. It was performed using a completely randomized design (CRD) with four replicates. Experimental units were represented by 0.4-dm^3 plastic pots. The biotypes used were CA01 (S) and CA07 (R), and the treatments were applied in two modalities, pre- and post-emergence (Table 2). For the pre-emergence modality, the pots were filled with agricultural soil (humic aluminic Cambisol), with ten seeds being sown per experimental unit. For the post-emergence application, the pots were filled

Table 2 - Description of the herbicide treatments, application method, and dosage used in the alternative control study at CAV/UDESC, Lages-SC.

Treatment	Commercial product	Application mode	Dose (g ai ha^{-1})	Dose (p.c. ha^{-1})
T1: pendimethalin[1]	Prowl H_2O	Pre-emergence	1,600	4.0 L
T2:clomazone[1]	Gamit	Pre-emergence	432	1.2 L
T3: penoxsulam[2]	Ricer	Pos-emergence	60	0.25 L
T4: imazapyr + imazapic[3]	Kifix	Pos-emergence	73.5+ 24.5	0.14 kg
T5: bispyribac-sodium[4]	Nominee	Pos-emergence	50	0.125 L
T6: propanil[1]	Stam	Pos-emergence	3.600	4.5 kg
T7: quinclorac[5]	Facet	Pos-emergence	375	0.75 kg
T8: florpyrauxifen-benzyl[1]	Loyant	Pos-emergence	30	1.2 L
T9: control	-	Pos-emergence	-	-
T10: control	-	Pre-emergence	-	-

[1]no adjuvant will be added, [2] Veget'Oil™ adjuvant will be added at a dose of 1.0 L ha^{-1}, [3] Dash™ adjuvant will be added at a dose of 1.0 L ha^{-1}, [4] Iharagues-S™ adjuvant will be added at a dose of 1.0 L ha^{-1}, and [5] Assist™ adjuvant will be added at a dose of 1.0 L ha^{-1}.

with commercial inert substrate and, after thinning, four plants were left per experimental unit.

The application was carried out with a precision backpack sprayer, using AD 110 02 flat-fan nozzles, at a height of 50 cm above the target, a pressure of 220 kPa, and an application rate of 200 L ha^{-1}. After herbicide application, the pots were taken to the greenhouse, remaining without irrigation for 24 hours to avoid interfering with herbicide absorption. Control was evaluated by a visual scale (KUVA, et al. 2016) from 0 to 100%, wherein 0 represents no control and 100% completely controlled plants at 28 days after application (DAA). Shoot dry matter (SDM) was also evaluated by cutting all plants in the pot at ground level at 28 DAA, drying them in an oven at 65 °C, and after 72 hours, SDM was quantified using a precision balance (0.001 g).

Statistical analysis

The dose-response assay data were subjected to analysis of variance and f-test. When significant, the data were adjusted to non-linear log-logistic regression models, as indicated by SEEFELDT et al. (1998).

According to SEEFELDT et al. (1998), the log-logistic model is the most appropriate for dose-response studies. We used the three-parameter sigmoidal model, which is given by the equation below, wherein 'a' refers to the intercept, 'b' refers to the slope of lines to the axes, and 'DL_{50}' is the lethal dose required to control 50% of the population.

$$y = \frac{a}{1 + e -^{\left(\frac{x - DL50}{b}\right)}}$$

Using the DL_{50} values, a Resistance Factor (RF) was obtained, which corresponds to the ratio between the DL_{50} of the resistant biotype and the DL_{50} of the susceptible one. To assess the agricultural efficacy of the treatments, DL_{80} was also mathematically calculated, which is the herbicide dose required to control 80% of the population.

As criteria for confirming resistance, this study followed the information from HRAC (2020) and SBCPD (2018), wherein the biotype was considered resistant when it met the following three conditions: resistance factor was greater than one (RF > 1.0), recommended herbicide dose did not provide control superior to 80% (Recommended Dose < DL_{80}), and resistance was a heritable trait, maintained in the F_2 generation

RESULTS AND DISCUSSION

Dose-response experiments in two generations (F_1 and F_2)

The biotypes showed different sensitivity to the three herbicides at the tested doses in both generations (F_1 and F_2). The biotype from municipality CA01 (Paulo Lopes-SC) was completely controlled (100% control) with a dose equal to or lower than the recommended dose. It was sensitive to all three herbicides (cyhalofop-p-butyl, quizalofop-p-ethyl, and profoxydim). The same pattern can be observed (Table 3) for biotypes CA02 (Içara-SC) and CA05 (Palhoça-SC) tested in generation F_1.

Resistance levels were evaluated according to parameters proposed by GAINES &

Table 3 - Parameters of the logistic equation (a and b), the dose required for 50% control (DL_{50}), the dose required for 80% control (DL_{80}), resistance factor (FR), and determination coefficient (R^2) for the control variable at 28 days after application (DAA) of cyhalofop-p-butyl (CY), quizalofop-p-ethyl (QI), and profoxydim (PR) for the first (F_1) and second generation (F_2) of the barnyardgrass biotypes.

Biotype	Herbicide	----------F_1 generation----------					
		a	b	DL_{50} (g ai ha^{-1})	DL_{80} (g ai ha^{-1})	R^2	FR
CA01	CY	99.0589	0.0999	259.20	414.70	0.9997	-
CA02	CY	99.9453	0.0716	94.50	151.20	0.9998	0.36
CA05	CY	99.9244	0.1696	241.80	386.90	0.9983	0.93
CA07	CY	89.5227	1.4366	14760	2361.60	0.9782	5.7
CA01	QI	98.5000	0.0185	21.60	34.56	0.9921	-
CA02	QI	96.2500	0.0160	18.06	28.89	0.9321	0.83
CA05	QI	99.0000	0.0210	22.94	36.70	0.9983	1.06
CA07	QI	90.4809	1.2552	660.00	1056.00	0.9723	30.5
CA01	PR	98.1111	0.0141	32.30	51.70	0.9923	-
CA02	PR	99.8190	0.0930	51.40	82.20	0.9983	1.54
CA05	PR	98.5991	0.0526	36.00	57.60	0.9925	1.11
CA07	PR	101.0696	2.5536	518.50	829.60	0.9752	16.05
Biotype	Herbicide	----------F_2 generation----------					
		a	b	DL_{50} (g ai ha^{-1})	DL_{80} (g ai ha^{-1})	R^2	FR
CA01	CY	99.5976	0.0207	109.44	171.36	0.9876	-
CA07	CY	89.5885	1.2236	864	1656	0.9373	7.89
CA01	QI	98.5429	0.0299	22.80	36.50	0.9924	-
CA07	QI	93.5898	1.2965	176.00	281.60	0.8942	7.72
CA01	PR	96.4637	0.0696	35.70	54.40	0.9742	-
CA07	PR	90.7344	0.8387	151.30	242.00	0.8397	4.24

HEAP (2022), in which a biotype is considered moderately resistant when its resistance factor is below 10, and highly resistant when it is above 10. The biotype CA07 (Tubarão-SC), which had been previously reported as resistant (EBERHARDT et al., 2016), showed moderate resistance to the aryloxyphenoxypropionate herbicides (cyhalofop-p-butyl and quizalofop-p-ethyl), with a resistance factor greater than 7.0 and a lethal dose necessary to control 80% of the population (DL_{80}) ranging from 2.5 to 5.0 times the recommended dose on the herbicide label for cyhalofop-p-butyl (315 g ai ha^{-1}) and quizalofop-p-ethyl (120 g ai ha^{-1}).

AMARO-BLANCO et al. (2021) reported an *Echinochloa* spp. population with high resistance to ACCase inhibitors, with a dose 2.6 times higher than that recommended for cyhalofop-p-butyl being necessary to control 50% of the resistant population (DL_{50}), similar to what was reported in this research. Similarly, Eberhardt et al. (2016) observed that cyhalofop-p-butyl provided a minimum acceptable control (80%) with a dose 60% higher than the recommended dose on the label, thus expressing a higher resistance factor than that we found (61.9). According to LOPEZ-OVEJERO et al. (2005), high resistance factor values may be related to the high susceptibility of a sensitive biotype.

Unlike our findings, GUERRA et al. (2017) noted that a dose of 75 g ai ha^{-1} for quizalofop-p-ethyl provided a complete (100%) control of *Echinochloa* spp. and that increasing it resulted in greater control. In a study of a herbicide-resistant biotype of *Echinochloa crus-galli*, which also belongs to the aryloxyphenoxypropionate chemical group, HAMZA et al. (2012) found a resistance factor of 12.07. However, for populations of *Eleusine indica*, CHEN et al. (2021) reported a resistance factor of 14.2 for quizalofop-p-ethyl.

Regarding the cyclohexanedione group (profoxydim), the biotype CA07 expressed a resistance factor of 4.24, which is lower than those for the aryloxyphenoxypropionate group. To achieve 80% control (DL_{80}), the dose had to be 1.42 times higher than usual. Conversely, VIDOTTO et al.

(2007) and KALOUMENOS et al. (2013) reported over 95% control for *E. crus-galli* biotypes by applying profoxydim at a dose of 200 g ai ha^{-1}, ruling out resistance in that population.

ORTIZ et al. (2015) conducted studies with the same herbicide (profoxydim), but in biotypes of *I. rugosum*, and reported very high resistance factors (>90) that contrast with ours. FERRERO et al. (2012) studied 29 *Echinochloa* spp. biotypes in Italy and found only isolated cases of resistance, among which only 7% showed resistance to profoxydim, while the rest of the populations still showed susceptibility.

The results of DL_{50}, DL_{80}, and FR expressed from the dose-response tests in both generations (F_1 and F_2) confirm the cross-resistance of the CA07 biotype from Tubarão-SC, indicating a higher level of resistance to herbicides belonging to the aryloxyphenoxypropionate chemical group, with a significant compromise of the ACCase inhibitors group, making it difficult to control *Echinochloa* weeds in paddy rice crops, limiting the options of herbicides capable of controlling them.

Alternative control

The herbicides applied as an alternative control method did not efficiently control both biotypes (CA01 and CA07), and the analysis of variance was significant for the variable "control of *Echinochloa crus-galli* biotypes," with the interaction between the factors "biotypes" and "herbicides." At 7 days after application (DAA) for biotype CA01, the herbicides pendimethalin, and clomazone provided control of 98.75% and 99.5%, respectively, while plants treated with other herbicides showed low control, not exceeding 22.5% with the herbicide quinclorac. The same pattern was observed for biotype CA07, but the treatment with quinclorac was 0% (Table 4).

At 14 DAA, pendimethalin and clomazone provided effective control (100%) for both biotypes. Bispyribac-sodium and quinclorac generated controls of 37.5% and 69.5% for biotype CA01, respectively. For biotype CA07, florpyrauxifen-benzyl caused control of 73.25%, while the other treatments reached a maximum of only 15% (Table 4). SCHAEDLER et al. (2008) demonstrated that bispyribac-sodium provides a level of control greater than 90% for sensitive biotypes and those with suspected resistance; however, for a biotype resistant to quinclorac, they observed a control between 65% and 74%.

At 28 DAA, 100% control was maintained by pendimethalin and clomazone for both biotypes. For biotype CA01, treatments with Bispyribac-sodium and quinclorac generated a control rate of 41.5% and 78%, respectively, while the other treatments did not show good control rates, remaining below 30%. Florpyrauxifen-benzyl provided a control rate of 87% for biotype CA07, while the other treatments did not exceed a control rate of 30% (Table 4).

Quinclorac provided no control (0%) for the CA07 biotype, and other quinclorac-resistant biotypes have also been found through escapes in crops in southern Brazil in 2001 in Tubarão-SC and

Table 4 - Control (%) of two biotypes of barnyardgrass (CA01 and CA07) at 7, 14, and 28 days after application (DAA).

Treatment	---Control (%)---					
	---7DAA---		---14DAA---		---28DAA---	
	CA01	CA07	CA01	CA07	CA01	CA07
Control (POS)	0.00 dA	0.00 dA	0.00 eA	0.00 dA	0.00 eA	0.00 eA
Penoxsulam	1.25 dA	0.00 dA	5.50 dB	1.25 dA	6.25 eB	4.50 eB
Imazapyr + Imazapic	1.00 dA	5.75 cB	4.50 dB	15.50 cC	5.00 eB	26.75 cC
Bispyribac-sodium	11.25 cB	0.00 dA	37.50 cC	0.00 dA	41.50 cC	0.00 eA
Propanil	8.75 cB	1.25 dA	20.00 cC	9.25 cB	27.00 dC	12.75 dB
Quinclorac	22.50 bB	0.00 dA	69.50 bC	0.00 dA	78.00 bC	0.00 eA
Florpyrauxifen-benzyl	1.25 dA	13.25 bB	7.00 dB	73.25 bC	7.5 B	87 bC
Control (PRE)	0.00 dA	0.00 dA	0.00 eA	0.00 dA	0.00 eA	0.00 eA
Pendimethalin	98.75 aA	98.00 aA	100 aA	100 aA	100 aA	100 aA
Clomazone	99.50 aA	98.50 aA	100 aA	100 aA	100 aA	100 aA

Lowercase letters in the same column and uppercase letters in the same row do not differ from each other by Tukey's test with a 5% probability.

Araranguá-SC (HEAP, 2022). Since then, cases of *Echinochloa* spp. resistance to this herbicide has increased. Similar to our findings, TRAVLOS et al. (2020) reported that penoxsulam shows low efficacy in five of the six accessions of *Echinochloa colona*, as found in a previous study where three biotypes of *E. crus-galli* were confirmed as resistant to the herbicide. This scenario demonstrated that alternative herbicides are not effective for both biotypes, indicating that the advance of resistance to other herbicides should be studied more thoroughly, as it probably has already compromised other mechanisms of action.

As shown in figure 1, the shoot dry matter (SDM) of the CA01 biotype was reduced by the application of penoxsulam, imazapyr + imazapic, and florpyrauxifen-benzyl. SDM of both biotypes (CA01 and CA07) was completely reduced after the application of the pre-emergence herbicides clomazone and pendimethalin. For the CA07 biotype, florpyrauxifen-benzyl provided the greatest reduction in SDM. In line with our study, TRAVLOS et al. (2020) found that the application of florpyrauxifen-benzyl resulted in a 60 to 66% reduction in SDM of five *E. colona* accessions at 7 DAA and 73 to 76% at 21 DAA. According to PINTO et al. (2008), in some cases, even at low populations, control measures that eliminate up to 99% of infestation do not prevent losses in yield for irrigated rice.

CONCLUSION

In the preliminary assay, the biotypes CA02, CA05, and CA07 showed suspected resistance and were selected for the dose-response assays. In the dose-response assay, only the biotype CA07 had cross-resistance confirmed in both generations.

The level of resistance was higher for the herbicides belonging to the aryloxyphenoxypropionate chemical group (cyhalofop-p-butyl and quizalofop-p-ethyl), with FR greater than 7.0. For the cyclohexanedione chemical group (profoxydim), the resistance level was low, with FR less than 5.0.

Post-emergence control alternatives were not effective in controlling the biotypes CA01 and CA07, and only the herbicides clomazone and pendimethalin, applied pre-emergence, provided 100% control for both biotypes.

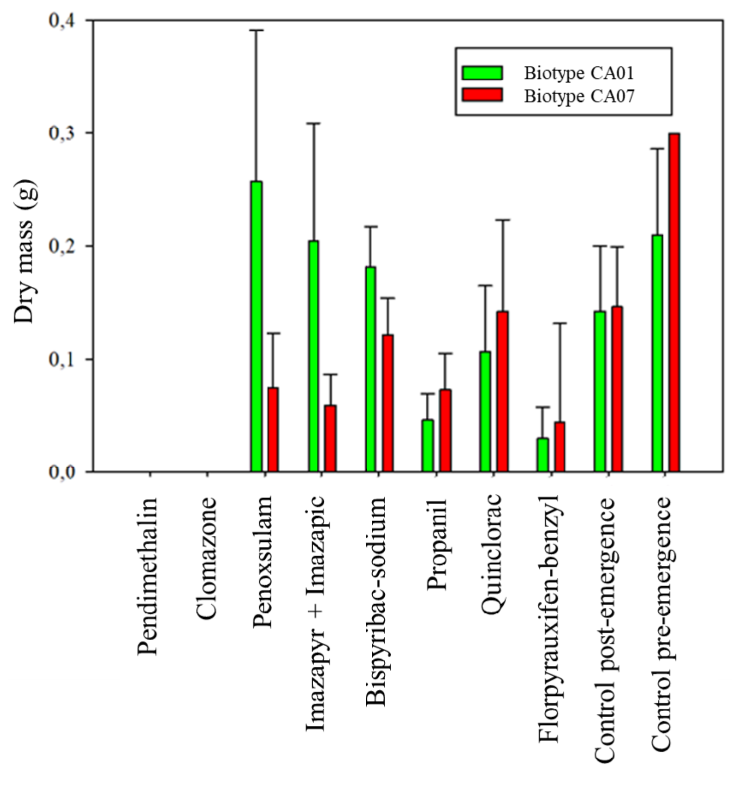

Figure 1 - Shoot dry mass (g) for biotypes CA01 and CA07 at 28 days after treatment application.

ACKNOWLEDGMENTS

This study was financed by Universidade do Estado de Santa Catarina (UDESC), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001".

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest. The funding sponsors did not play a role in the study design, data collection, analysis, interpretation, writing of the manuscript, or decision to publish the results.

AUTHORS' CONTRIBUTIONS

Conceptualization of the manuscript and development of the methodology: MLS, AMON, NG, FNS and AMON; data collection and curation: MLS, MPS and LFFFPS; data analysis: MLS and AMON; data interpretation: MLS and AMON; funding acquisition and resources: AMON; project administration: MLS and AMON; supervision: NG and AMON; writing the original draft of the manuscript: MLS; writing, review and editing: AMON and NG; all authors critically revised the manuscript and approved of the final version.

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