

Fitness of *Echinochloa crusgalli* var. *mitis* biotypes susceptible and resistant to imazapyr + imazapic herbicide

Joice Fernanda Lübke Bonow^{1*}, André Andres³,
Eduardo Nogueira¹, Fabiane Pinto Lamego²

¹Federal University of Pelotas, Pelotas, Brazil

²Brazilian Agricultural Research Corporation, Bagé, Brazil

³Brazilian Agricultural Research Corporation, Pelotas, Brazil

*Corresponding author, email: joicef.agronomia@gmail.com

Abstract

The continuous use of herbicides with the same mechanism of action has caused the selection of resistant weed biotype that may present differences in fitness when compared with susceptible biotype. The objective of the present study was to identify and distinguish the fitness of barnyardgrass biotypes resistant and susceptible to imazapyr + imazapic herbicide under controlled and noncompetitive conditions. For that, there were used barnyardgrass biotype susceptible (ECH1 and ECH38) and resistant (ECH14 and ECH44) to ALS inhibitors, collected at Pelotas/RS and Rio Grande/RS cities, respectively, in experiment installed in a greenhouse in a completely randomized experimental design during the months from October to December 2015. The biotype were evaluated during the period of 15 to 120 days after the emergence related to plant height, foliar area, shoot dry matter mass and root dry matter mass. The results showed insignificant differences between the biotypes during the development of the plants. The susceptible biotype ECH44 showed a higher number of panicles per plant and seeds along with the resistant ECH1. There is variability among *Echinochloa crusgalli* var. *mitis* biotypes resistant and susceptible to ALS inhibitors, indicating an absence in fitness penalty caused by resistance when cultivated without the selection of pressure (herbicide).

Keywords: barnyardgrass, fitness, herbicide resistance, weed control, irrigated rice

Introduction

In irrigated rice cultivation, the intensive use of herbicides inhibiting the enzyme acetolactate synthase (ALS) has favored the selection of biotypes of resistant weeds worldwide; among them, those of barnyard grass (*Echinochloa crusgalli*) (Heap, 2017). The discovery of herbicide-resistant biotypes has called for research initiatives aimed at recognizing the issues surrounding the occurrence of resistance in a plant.

The adaptive or fitness value of a plant is defined as the ability of the plant to grow, develop, and leave fertile offspring (Radosevich et al., 2007). Thus, it is crucial to investigate

changes that may occur associated with the evolution of resistance. In particular, knowledge about the competitive capacity of the resistant biotype, whether or not altered by resistance, is fundamental to understanding the dynamics of the resistant population (Vila-Aiub et al., 2009).

At least three mechanisms can explain costs associated with herbicide resistance in plants: (1) mutation or mutations at the target site, leading to protein production or reduction in herbicide efficiency over the target enzyme; (2) increased energy required for replication of an additional gene upon high expression of the site of action, and (3) pleiotropic effects caused by negative ecological interactions (Vila-Aiub et al.,

Received: 10 May 2017
Accepted: 22 June 2018

2009).

Studies comparing the adaptive value between resistant and susceptible barnyard grass biotype have shown that resistance to the herbicides propanil and clomazone does not alter the growth and competitiveness of the resistant biotype, suggesting that the frequency of alleles for resistance in the populations evaluated will likely remain stable in the absence of herbicide selection (Muthukumar et al., 2011).

In researches that compared the relative growth and seed germination of *Bidens subalternans* (Beggarticks) of susceptible and resistant biotypes to ALS inhibitors, under different temperature conditions, there was no difference in the relative growth rate between the biotypes studied; but the germination rate of the resistant biotype was lower than that of the susceptible population (Lamego et al., 2011). However, adaptability costs may occur depending on the point where the mutation occurred in the gene when this is the mechanism of resistance and the species involved (Yu et al., 2010). Therefore, the knowledge of the bioecological characteristics, as well as the behavior of the resistant and susceptible weed, becomes necessary to establish management strategies aiming at the prevention of resistance.

The present study has as the hypothesis that *Echinochloa crusgalli* var. *mitis* biotypes resistant to ALS inhibitor herbicides have no fitness penalty when grown in the absence of the selection agent (herbicide). Therefore, the objective of the study was to identify and compare the fitness of barnyardgrass biotypes resistant and susceptible to imazapyr + imazapic herbicide under controlled and noncompetitive conditions.

Materials and Methods

The experiment was carried out in a greenhouse at *Embrapa Clima Temperado*, Capão do Leão-RS, from October to December 2015, in a completely randomized experimental design with six replications. The treatments were arranged in a 4 x 6 factorial scheme, where factor A comprised the accessions of barnyardgrass (ECH14, ECH44 - susceptible, ECH1, ECH38 - resistant), and factor B was composed of

evaluation periods (15, 30, 45, 60, 75 and 90 days after emergence (DAE)).

The seeds of biotypes were collected from February to April 2014 in rice fields where the chemical control was not efficiency. To confirm the herbicide resistance, a screening and a dose-response curve experiment were performed to determine the dose of the herbicide imazapyr + imazapic required to control 50% of the population (C50) and reduce 50% of the mass production dry grasses (GR50) in susceptible and resistant biotypes (Bonow et al., 2018).

The resistant biotypes ECH1 and ECH38 come from the municipalities of Pelotas - RS (latitude 31°30'33" S and longitude 52°10'45" W) and Rio Grande - RS (latitude 32°12'57" S and longitude 52°30'09" W), respectively. Likewise, susceptible biotypes ECH14 (latitude 31°29'26" S and longitude 52°10'55" W) and ECH44 (latitude 32°16'54" S and longitude 52°28'20" W) originated in the same municipalities, respectively, however, from regions and areas with no herbicide application history.

For the fitness study, seeds of the biotypes considered as resistant were sown in trays and sprayed with the herbicide imazapyr + imazapic (73.5 + 24.5 g e.a. ha⁻¹); after confirmed the total absence of symptoms, were transplanted to vessels with capacity of 8L, being left to produce seeds for the next generation (F1). During the same period, susceptible biotype plants were also kept in another greenhouse to provide seeds for use in the fitness study.

The experimental units were composed of a potted barnyardgrass plant, with a volumetric capacity of 4L, containing dystrophic Haplosol planosol. The soil analysis showed: pH in water = 5.0; CTC_{pH7} = 7.2 cmol_c dm⁻³; organic matter = 1.5%; clay = 16%; texture = 4; Ca = 4.1 cmol_c dm⁻³; Mg = 1.1 cmol_c dm⁻³; Al = 1.8 cmol_c dm⁻³; P = 6.5 mg dm⁻³ and K = 0.15 cmol_c dm⁻³. The fertility correction was performed according to the recommendations for irrigated rice cultivation (Recomendações Técnicas para a Cultura do Arroz Irrigado, 2012). The barnyardgrass seeds were arranged in the plastic pots in the quantity of 10 pot⁻¹ seeds and, after germination, the shoots were thinned, establishing one plant per pot.

The variables evaluated were: plant height (PHt), leaf area (LA) with a leaf area meter (model LI 3100C), shoot dry mass (APDMM) and root dry mass (DRMM).

The results of the APDMM and LA were used to determine the growth rate (GR), the relative growth rate (RGR), the leaf area rate (LAR), according to Radford (1967). The GR (mg plant⁻¹ day⁻¹) expresses the average growth velocity of the plants during the observation period, is calculated by the formula:

$$GR_{n-1} = (W_2 - W_1) / (t_2 - t_1) + GR_1,$$

where: W2 and W1 are the APDMM of two successive samplings, t2 and t1 are the days elapsed between the two observations and GR1 is the growth rate observed in the previous sample.

In the same way, the relative growth rate (mg day⁻¹) representing the increase in the dry mass of the shoot (APDMM) per unit of time, is calculated by the formula:

$$RGR = (\ln W_2 - \ln W_1) / (T_2 - T_1)$$

where: W2 and W1 are the APDMM of two successive samplings; ln = neperian logarithm; t2 and t1 are the days elapsed between the two observations.

The LAR, in cm² g⁻¹, expressed to the useful area available for photosynthesis, is calculated by the formula:

$$LAR = LA / APDMM$$

The number of panicles (panicles plant⁻¹) was determined at 90 DAE and from there were collected up to 120 DAE to estimate the number of seeds (seeds plant⁻¹), which was assessed performing the counting in any single plant.

The data obtained were analyzed for normality (Shapiro Wilk test) and submitted to variance analysis (p ≤ 0.05). In the case of statistical significance, regression analyzes were performed using SigmaPlot 10.0 (SigmaPlot, 2007) or a comparison between averages using the Duncan test.

For all variables measured, except for

LA, the data were adjusted to the sigmoidal regression model with three parameters according to Schaedler et al. (2013) and Kaspary (2014):

$$y = a / (1 + e^{-(x - x_0)/b})$$

where: y = response variable of interest; e = exponential function; x = days after emergence; e; the values of a, b and x₀ are the non-linear regression parameters of the model with a = difference between the maximum and minimum asymptote; b = slope of the curve; x₀ = days after the emergency corresponding to 50% of the yield of the dependent variable for the maximum asymptote value (a).

For LA, the data were adjusted to the Peak Gaussian regression model with three parameters, according to Lopes et al. (2009):

$$y = a * e^{-0.5[(x - x_0)/b]^2}$$

where: y = response variable of interest; e = exponential function; x = days after emergence; e; the values of a, b and x₀ are the non-linear regression parameters of the model with a = difference between the maximum and minimum points of the variable, x₀ = number of days accumulated after transplantation, and b = slope of the curve.

The 95% confidence intervals for the sample averages were constructed based on the standard deviation for each variable and access at different times and were used to verify the differences between the accessions.

Plants of each biotype were sent to the Herbarium of the Department of Botany of the Federal University of Pelotas to identify the species of *Echinochloa*. The biotypes were identified as *Echinochloa crusgalli* var. *mitis*, which according to Agrasar et al. (2005), although it is not mentioned in the flora of Rio Grande do Sul, var. *mitis* presents the same geographical distribution of the typical variety (var. *crusgalli*), living together. Furthermore, var. *mitis* displays lemons with smaller edges, inflorescences with a lower number of branches and is less pigmented than var. *crusgalli* (Agrasar et al., 2005).

Results and Discussion

The interaction between the access factors and time of assessment for all evaluated variables was verified: plant height (PHT), shoot dry mass (APDMM), root dry mass (DRMM), total dry mass (TDMM), leaf area (LA), leaf area rate (LAR), growth rate (GR), and relative growth rate (RGR). For all the analyzed data there were significant adjustments for the regression curves (Figures 1, 2, 3, 4, 5, 6, 7, 8).

The sigmoidal behavior of plant height between biotypes was similar (Figure 1), considering the exponential coefficient of the equations, and the susceptible ECH14 presented smaller plant height than the resistant biotypes

from the 75 DAE, whereas the susceptible ECH44 of Rio Grande, differed from the others during the evaluation periods. This result possibly arises from differences between biotypes with different places of origin and genetic characteristics of the same; plant height influences the amount of light intercepted by plants and has significant implications for competitive potential (Falster & Westoby, 2003). In studies of adaptive value and competitive ability performed with *Cyperus difformis*, resistant plants susceptible to ALS inhibitor herbicides showed similar behavior and did not differ between them when compared to plant height (Dal Magro et al., 2011).

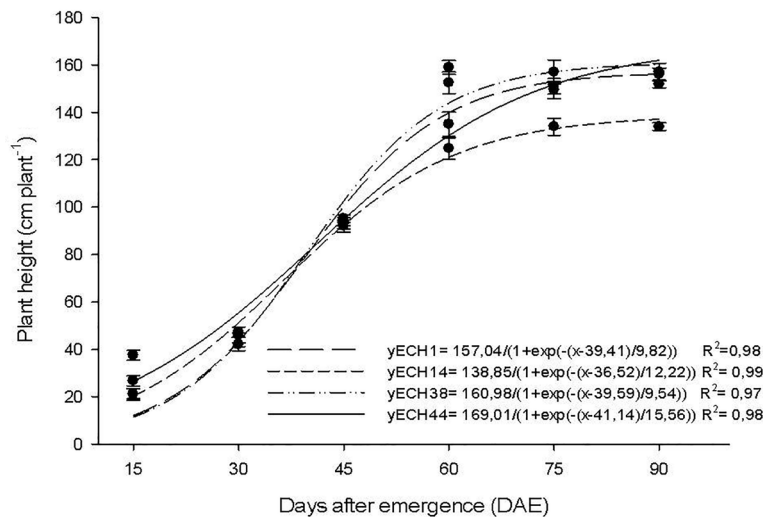


Figure 1. Plant height (cm⁻¹) of *Echinochloa crusgalli* var. *mitis* resistant (ECH1-Pelotas/RS and ECH38-Rio Grande/RS) and susceptible (ECH14-Pelotas/RS e ECH44-Rio Grande/RS) to the herbicide imazapyr + imazapic, evaluated from 15 to 90 days after emergence (DAE). The dots represent the average values of the repeats between biotype and the bars, the respective confidence intervals of the average.

For the LA variable, the initial evaluations at 15 and 30 DAE do not show differences between the biotype; However, between 45 and 60 DAE when the maximum increment of the variable occurred, the resistant biotype of Pelotas (ECH1) and susceptible from Rio Grande (ECH44) stood out in front of the others (Figure 2). The overlap of the confidence interval between Pelotas (susceptible ECH14) and Rio Grande biotype (resistant ECH38), shows that the observed differences occur due to the physiology of each biotype, independent of the resistance caused by the intensive use of ALS inhibitor herbicides. Plants with a higher index of leaf area concentrated in the upper part of the canopy may present advantages over the light

competition since the leaf area is an indicator of great importance, being used to investigate ecological adaptation, competition with other species and effects of the management (Fleck et al., 2007).

Comparing the biotype concerning APDMM, we observed that there was an exponential increase according to the advancement of the evaluation periods, in which we verified that the maximum accumulation occurred at 60 DAE and was maintained until 90 DAE (Figure 3). The collection of the dry matter mass in a smaller possible time demonstrates a greater ability of the plant when in competition (Radosevich et al., 2007).

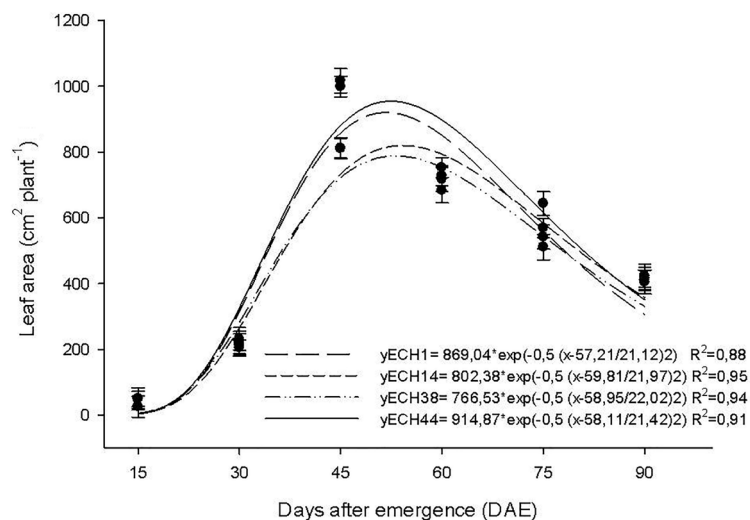


Figure 2. Leaf area ($\text{cm}^2 \text{plant}^{-1}$) of *Echinochloa crusgalli* var. mitis resistant (ECH1-Pelotas/RS and ECH38-Rio Grande/RS) and susceptible (ECH14-Pelotas/RS e ECH44-Rio Grande/RS) to the herbicide imazapyr + imazapic, evaluated from 15 to 90 days after emergence (DAE). The dots represent the average values of the repeats between biotype and the bars, the respective confidence intervals of the average.

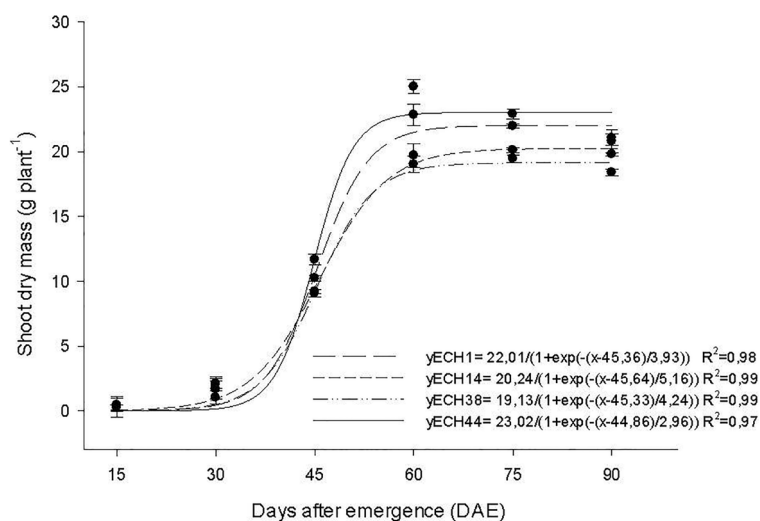


Figure 3. Shoot dry mass (g plant^{-1}) of *Echinochloa crusgalli* var. mitis resistant (ECH1-Pelotas/RS and ECH38-Rio Grande/RS) and susceptible (ECH14-Pelotas/RS e ECH44-Rio Grande/RS) to the herbicide imazapyr + imazapic, evaluated from 15 to 90 days after emergence (DAE). The dots represent the average values of the repeats between biotype and the bars, the respective confidence intervals of the average.

At 60 DAE, ECH44 susceptible biotype produced, on average, $25.8 \text{ g plant}^{-1}$ of APDMM, that is, 31% higher than the susceptible ECH14 of Pelotas, while the resistant presented intermediate values. Also, at the same time, the susceptible ECH44 was superior to the resistant ECH38 from the same site (Rio Grande), and this difference remained until the last evaluation at 90 DAE. For the Pelotas biotypes, the resistant (ECH1) was superior to the susceptible (ECH14) (Figure 3).

Studies with *Echinochloa crusgalli* show

that there are no significant differences in the accumulation of dry matter mass for resistant and susceptible access to ALS inhibitor herbicides, as well as inhibitors of FSII and Carotenoids even when compared to competitive ability (Muthukumar et al. 2011).

The ECH1 (resistant) Pelotas biotype, at 45 DAE, presented the highest DRMM alone, while the susceptible ECH14 presented the lowest, however, at 60 DAE, values were very close. However, it cannot be affirmed that there are significant differences between the

biotype studied since there is an overlap of the confidence intervals (Figure 4). Studies with resistant plants susceptible to the herbicide pyrazosulfuron-ethyl in *Sagittaria montevidensis*, in which there were no differences in the dry mass of the root system between the studied biotypes

(Moura, 2014) displayed similar results. It is worth mentioning that the increase of the root system is directly related to the accumulation of dry mass of the aerial part in the plants, due to the higher demand in photosynthetic activity and carbon fixation (Aguar et al., 2011).

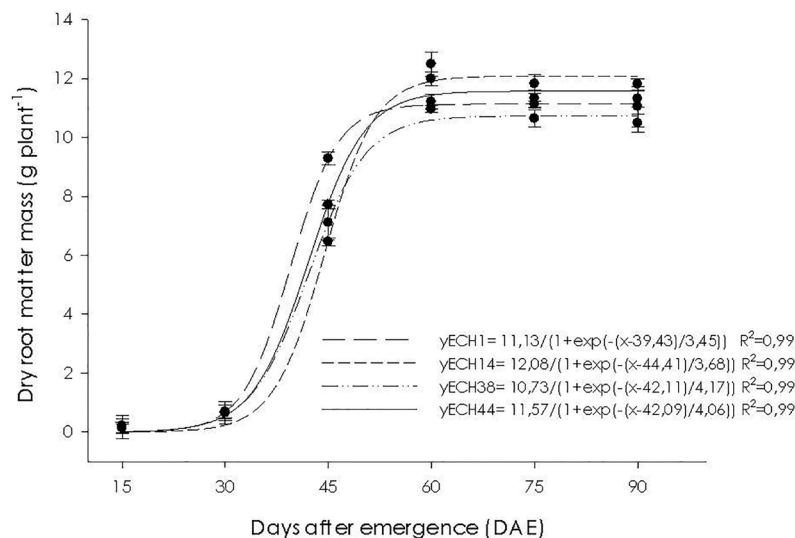


Figure 4. Root dry mass (g plant⁻¹) of *Echinochloa crusgalli* var. *mitis* resistant (ECH1-Pelotas/RS and ECH38-Rio Grande/RS) and susceptible (ECH14-Pelotas/RS e ECH44-Rio Grande/RS) to the herbicide imazapyr + imazapic, evaluated from 15 to 90 days after emergence (DAE). The dots represent the average values of the repetitions between biotype and the bars, the respective confidence intervals of the average.

Studies related to weed resistance to ACCase and ALS inhibitor herbicides, in general, have shown no differences between the growth of different biotypes (Christoffoleti, 2001). The similarity of growth in different biotypes may occur due to the mechanism of weed resistance to ALS inhibitors which is generally related to the mutation and thus does not disadvantage the growth of the resistant plant in the absence of selection pressure (Sathasivan et al., 1990).

The results obtained for the mass of the total dry mass matter (TDMM) variable showed that there is no evidence of differential growth in which any difference in the adaptive value of the biotypes can be highlighted. However, at 60 DAE, the behavior of the curve remained the same as APDMM, where susceptible ECH44 stood out to the others and even to its resistant (ECH38) (Figure 5). Lamego et al. (2011) described identical results, as the authors compared susceptible and resistant biotypes of *Bidens subalternans* resistant to ALS inhibitor herbicides, as well as in studies with *Amaranthus retroflexus* biotypes resistant to ALS inhibitor herbicides that did not find differences in TDMM when compared to the susceptible

biotype (Sibony & Rubin, 2003).

Studies of adaptive value were investigated by several authors, with most of them focusing on the behavior of resistant and susceptible plants and their effect on the accumulation and distribution of nutrients through varying responses as TDMM. The production and partition of dry matter by crops and weeds vary with the species involved (Zanine & Santos, 2004). However, these differences have not been observed in studies with plants resistant to ALS inhibitors.

Regarding the leaf area rate (LAR), there was a difference between biotypes only at 15 DAE, when resistant and susceptible Pelotas biotypes, respectively, ECH1 and ECH14, maintained the same values and in the same way for those from Rio Grande, ECH38, and ECH44 (Figure 6). The difference among biotypes may have occurred due to their origin which, in turn, have high genetic and physiological similarity, which initially present similar development. However, due to the overlap of the confidence intervals, we observed no differences between them. The analogy was possibly due to the

interference occurring from leaves located in the upper part of the canopy on the lower ones during the development of the barnyardgrass plants, besides the formation of reproductive

structures that represent drains and consequently smaller partition of photoassimilates to the leaves in relation to flowers, fruits and seeds, from the 45 DAE.

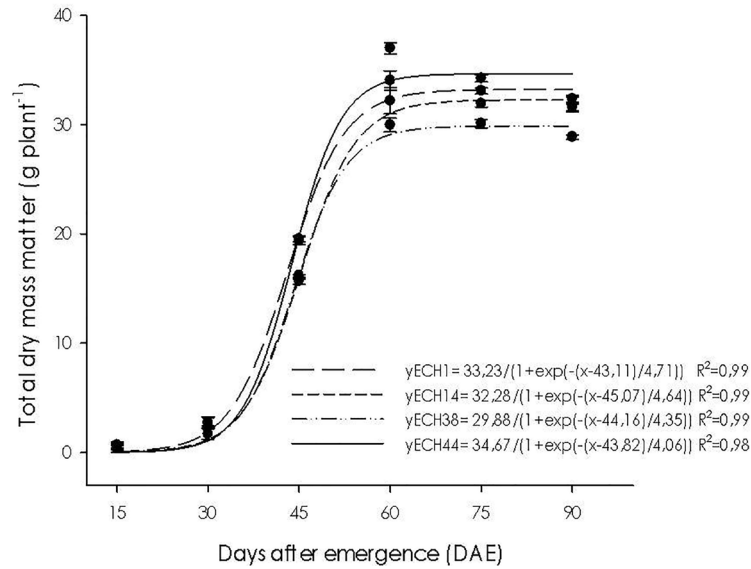


Figure 5. Total dry mass (g plant^{-1}) of *Echinochloa crugalli* var. *mitis* resistant (ECH1-Pelotas/RS and ECH38-Rio Grande/RS) and susceptible (ECH14-Pelotas/RS e ECH44-Rio Grande/RS) to the herbicide imazapyr + imazapic, evaluated from 15 to 90 days after emergence (DAE). The dots represent the average values of the repeats between biotype and the bars, the respective confidence intervals of the average.

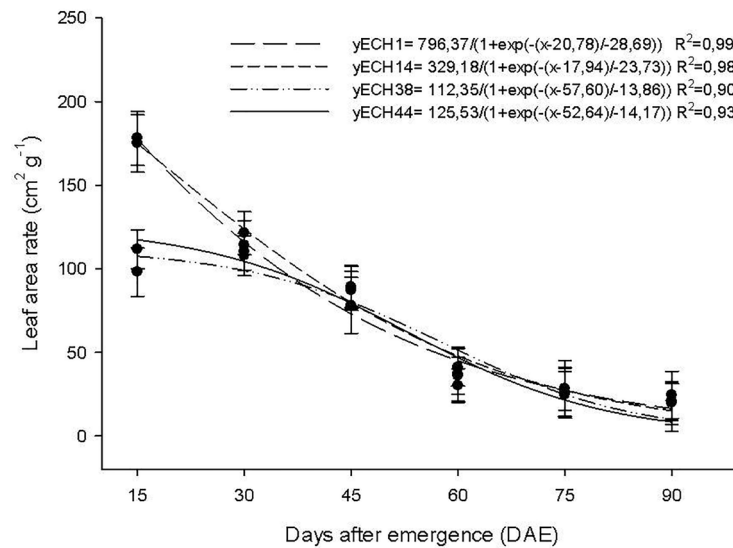


Figure 6. Leaf area rate ($\text{cm}^2 \text{g}^{-1}$) of *Echinochloa crugalli* var. *mitis* resistant (ECH1-Pelotas/RS and ECH38-Rio Grande/RS) and susceptible (ECH14-Pelotas/RS e ECH44-Rio Grande/RS) to the herbicide imazapyr + imazapic, evaluated from 15 to 90 days after emergence (DAE). The dots represent the average values of the repeats between biotype and the bars, the respective confidence intervals of the average.

The accelerated growth in the initial period of the plants is due to the higher amount of photoassimilates being destined to the production and formation of new leaves for higher absorption of the solar radiation (Urchei et al., 2000). In a certain way, the variable

presented a decline throughout the evaluations, being the observed variations possibly due to the differential distribution of photoassimilates to the leaves and other components of the plant and the differences in leaf density. However, the higher the LAR, the higher the interception of the

light energy by the plant and the shading of the others, showing more significant development and competitive potential (Ferreira et al., 2008).

When analyzing the results of the GR, it was possible to determine that the highest value occurred between 30 and 60 DAE and, later, the curves remained stable. There were differences between the biotype tested, where, in general, access susceptible to the Rio Grande had a

GR greater than 10% about the resistant, from 60 DAE; already, for Pelotas biotypes, resistant ECH1 was superior to ECH14, susceptible (Figure 7). However, differences such as these were only found for this variable but also observed in plants of *Fimbristylis miliacea* that showed a growth of the resistant biotype of 13 to 15% higher than the growth of the susceptible biotype (Schaedler et al., 2013).

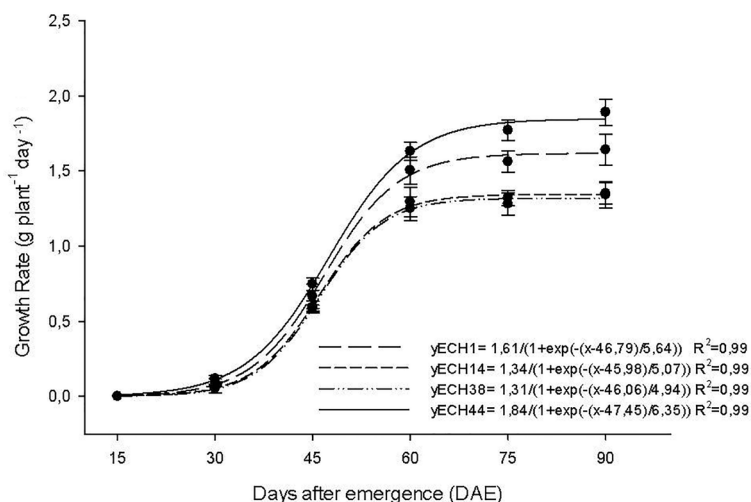


Figure 7. Growth rate ($\text{g plant}^{-1} \text{day}^{-1}$) of *Echinochloa crusgalli* var. *mitis* resistant (ECH1-Pelotas/RS and ECH38-Rio Grande/RS) and susceptible (ECH14-Pelotas/RS e ECH44-Rio Grande/RS) to the herbicide imazapyr + imazapic, evaluated from 15 to 90 days after emergence (DAE). The dots represent the average values of the repeats between biotype and the bars, the respective confidence intervals of the average.

According to Benincasa (2004), the use of growth analyzes is still the most straightforward, fastest and most accurate way to infer the contribution of different physiological processes to plant growth. The GR represents the projection of dry mass accumulation regarding the existing matter difference, as a function of time (Carvalho et al., 2005). In this study, GR was higher than 1 and did not exceed $2 \text{ g plant}^{-1} \text{day}^{-1}$ for all biotypes, demonstrating that resistant and susceptible barnyardgrass plants showed rapid initial development (Figure 7) and are therefore very competitive in relation to other species resistant to ALS inhibitors such as *Cyperus difformis* (Dal Magro et al., 2011).

The evaluation of the relative growth rate displayed similar behavior between the biotypes, varying throughout the cycle and decreasing until the final phase of development. This behavior was due to the lower accumulation of APDMM and decrease of the leaf area useful for photosynthesis, once that reductions in RGR

values at the end of the plant cycle may be related to the decline in the rate of net assimilation. In studies involving other ALS-inhibiting herbicides, the absence of developmental impairment is partly due to the lack of interference of the gene responsible for resistance with the photosynthesis process (Massinga et al., 2005). It is known that weeds with high RGR may have an ecological advantage about the others because they occupy faster a space for their growth (Radosevich et al., 2007).

The RGR is vital for assessing plant growth which depends on the amount of material accumulated gradually and expresses the increase in dry matter mass per unit of initial weight over a period (Carvalho et al., 2005). In general, the maximum growth of the plants associates to the beginning of the reproductive process. In this case, the maximum growth is reflected in the total dry matter accumulation curve as the inflection point (b), being 90 DAE for all biotypes (Figure 8).

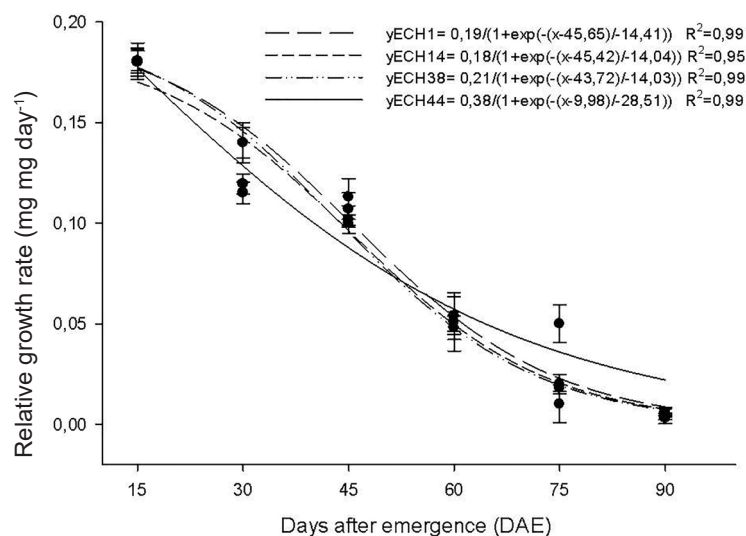


Figure 8. Relative growth rate (mg mg day⁻¹) of *Echinochloa crusgalli* var. mitis resistant (ECH1-Pelotas/RS and ECH38-Rio Grande/RS) and susceptible (ECH14-Pelotas/RS e ECH44-Rio Grande/RS) to the herbicide imazapyr + imazapic, evaluated from 15 to 90 days after emergence (DAE). The dots represent the average values of the repeats between biotype and the bars, the respective confidence intervals of the average.

For the analysis of the variables related to the reproductive stage, we observed no close relationship between the number of panicles and the number of seeds per plant (Table 1). This fact is possibly related to the significant variability between the biotypes and the absence of

adaptive costs observed in the majority of the cases of weeds resistant to the herbicides inhibiting ALS, being one of the main factors that contributes to the rapid evolution and dispersion of the resistant plants worldwide (Li et al., 2013).

Table 1. Number of panicles per plant (NPP) and number of seeds per plant (NSP) of *Echinochloa crusgalli* var. mitis resistant (ECH1-Pelotas/RS and ECH38-Rio Grande/RS) and susceptible (ECH14-Pelotas/RS and ECH44-Rio Grande/RS) to the herbicide imazapyr + imazapic

Access	Number of panicles per plant ¹	Number of seeds per plant ¹
	Pelotas/RS	
ECH1 (Resistant)	9 B	4.099 A
ECH14 (Susceptible)	8 B	2.552 C
	Rio Grande/RS	
ECH38 (Resistant)	8 B	3.707 B
ECH44 (Susceptible)	12 A	4.213 A
C.V. (%)	11,3	12,65

¹ Averages followed by the same letter in columns do not differ by Duncan Test (p≤0,05).

The susceptible biotype ECH44 of Rio Grande presented the highest number of panicles per plant along with the resistant ECH1 of Pelotas; both showed the highest number of plant⁻¹ seeds (4,213 and 4,099, respectively), being superior to their peers in each municipality, resistant and susceptible, respectively. Boody et al. (2012) described similar results in plants of the same genus as *Echinochloa crusgalli* and *Echinochloa phyllopogon*, which present multiple resistance to ALS and ACCase inhibitor herbicides. The authors observed no difference for seed production between susceptible and resistant plants.

Seed production is one of the determining factors in understanding how resistance impacts the life cycle processes of resistant and susceptible weeds (Vila-Aiub et al., 2009) and how we can use this knowledge to reduce dissemination of herbicide-resistant species. It is known that allele frequency over the years for ALS-resistant biotypes is highly dependent on environmental conditions, localities, mixtures between species, and the rate and amount of gene flow of plants located in crops and populations considered wild (Massinga et al., 2005).

The knowledge of the costs of adaptability for a plant is essential, aiming

to establish management strategies that will prevent the reproduction and dissemination of herbicide-resistant species. The biotypes evaluated in this study demonstrated opposite patterns of response in general, while the Rio Grande susceptible biotype (ECH44) showed superiority to the other biotypes and, even to the resistant one (ECH38) in Pelotas, resistant ECH1 was highlighted in most of the variables evaluated when compared to the susceptible ECH14, from the same municipality. In addition to the morphophysiological assessed variables, seed production per plant confirmed this pattern of response, with ECH44 and ECH1 producing the highest amounts of plant-1 seeds. Thus, in the Rio Grande, susceptible biotype seems more adapted while in Pelotas, the resistant stands out against the susceptible in the absence of the selection agent. In this context, it is possible to conclude that the differences occur solely due to the inherent genetic variability of the biotypes, with no relation to resistance to ALS inhibitors.

From the practical point of view, less adaptability due to the resistant biotype, in field conditions, favors the dissemination of the susceptible that, being more competitive, occupies the ecological niche. The results of the present study agree with those previously published articles where resistance to ALS inhibitor herbicides did not result in fitness penalty by resistant plants when cultivated in the absence of the selection agent. The lack of fitness penalty suggests that the frequencies of resistant alleles in these populations will likely remain stable in the absence of the herbicide (selection agent).

Conclusion

There is variability between biotypes of *Echinochloa crusgalli* var. *mitis* resistant and susceptible to ALS inhibitor herbicides. We observed no fitness penalty among them due to resistance when cultivated in the absence of the selection agent (herbicide).

Acknowledgments

To Prof. Raquel Ludtke of the Department of Botany of the Federal University of Pelotas, for assistance in identifying the biotypes, confirmed as *Echinochloa crusgalli* var. *mitis*.

To CAPES for funding the scholarship for the first author.

References

- Agrasar, Z.R., Steibel, P.E., Troiani, H.E. 2005. *Manual Ilustrado de Las Gramíneas de La Provincia de La Pampa*. Universidad nacional de La Pampa: Córdoba de Río Cuarto/Universidad Nacional de Río Cuarto, Santa Rosa, Argentina. 359p.
- Aguiar, F.A., Kanashiro, S., Tavares, A., Rodrigues, T. 2011. Growth of Brazil wood seedlings (*Caesalpinia echinata* Lam.), submitted to five levels of shading. *Revista Ceres* 58: 193-205.
- Benincasa, M.M.P. 2004. *Plant growth analysis: basics*. Funep, Jaboticabal, Brazil. 41p.
- Boddy, L.G., Streibig, C.J., Yamasue, Y., Fischer, A.J. 2012. Biomass, Fecundity, and Interference Ability of Multiple Herbicide-Resistant and Susceptible Late Watergrass (*Echinochloa phyllopogon*). *Weed Science* 60: 401-410.
- Bonow, J.F.L., Lamego, F.P., Andres, A., Avila, L.A., Teló, G.M., Egewarth, K. 2018. Resistance of *Echinochloa crusgalli* var. *mitis* to imazapyr+imazapic herbicide and alternative control in irrigated rice. *Planta Daninha* 36: e018168627.
- Carvalho, S.J.P., Moreira, M.S., Nicolai, M., Ovejero, R.F.L., Christoffoleti, P.J., Medeiros, D. 2005. Growth and development of camalote weed. *Bragantia* 64: 591-600.
- Christoffoleti, P.J. 2001. Comparative analysis of black-tip weed plant growth (*Bidens pilosa*) resistant and susceptible to ALS inhibitor herbicides. *Planta Daninha* 19: 75-83.
- Dal Magro, T., Haedler, C.E., Fontana, L.C., Agostinetto, D., Vargas, L. 2011. Competitive ability among *Cyperus difformis* L. biotypes resistant or susceptible to ALS inhibitor herbicides and of these with irrigated rice. *Bragantia* 70: 294-301.
- Falster, D.S., Westoby, M. 2003. Leaf size and angle vary widely across species: What consequences for light interception? *New Phytologist* 158: 509-525.
- Ferreira, E.A., Concenço, G., Silva, A.A., Reis, M.R., Vargas, L., Viana, R.G., Guimarães, A.A., Galon, L. 2008. Competitive potential of ryegrass biotypes (*Lolium multiflorum*). *Planta Daninha* 26: 261-269.
- Fleck, N.G., Lamego, F.P., Schaedler, C.E., Ferreira, F.B. 2007. Response of soybean cultivars to competing cultivar simulator of infestation of competing plants. *Scientia Agraria* 8: 213-218.

- HEAP: *International Survey of Herbicide resistant Weeds*. 2017. <http://www.weedscience.org/>< Accessed on: February 20th 2017>
- Kaspary, T.E. 2014. *Biological and physiological characterization of flax-leaf fleabane (Conyza bonariensis L.) resistant to herbicide glyphosate*. 99p. Dissertation (Master in Agronomy: Agriculture and environment) - Federal University of Santa Maria, Frederico Westphalen, Brazil.
- Lamego, F.P., Vidal, R.A., Burgos, N.R. 2011. Competitiveness of ALS inhibitor resistant and susceptible biotypes of greater beggarticks (*Bidens subalternans*). *Planta Daninha* 29: 457-464.
- Li, M., Yu, Q., Han, H., Vila-aiub, M., Powles, S.B. 2013. ALS herbicide resistance mutations in *Raphanus raphanistrum*: evaluation of pleiotropic effects on vegetative growth and ALS activity. *Pest Management Science* 69: 689-695.
- Lopes, J.P., Machado, E.C., Deuber, R., Machado, R.S. 2009. Analysis of growth and gas exchange in no-tillage and conventional corn. *Bragantia* 68: 839-848.
- Massinga, R.A., Kassim, A.K., Paul, S.A., Jerry, F.M. 2005. Relative fitness of imazamox-resistant common sunflower and prairie sunflower. *Weed Science* 53: 166-174.
- Moura, D. 2014. *Resistance to herbicides and management alternatives of Sagittaria montevidensis biotypes in irrigated rice*. 93p. Dissertation (Master in Herbology) - Federal University of Pelotas, Pelotas, Brazil.
- Muthukumar, V.B., Jason K., Prashant, J., Kenneth, S. 2011. Does Resistance to Propanil or Clomazone Alter the Growth and Competitive Abilities of Barnyard grass (*Echinochloa crus-galli*)? *Weed Science* 59: 353-358.
- Radford, P.J. 1967. Growth analysis formulae – their use and abuse. *Crop Science* 7: 171-175.
- Radosevich, S.R., Holt, J., Ghera, C. 2007. *Ecology of weeds and invasive plants: Relationship to agriculture and natural resource management*. John Wiley & Sons, New York, EUA. 454p.
- Technical meeting of irrigated rice crop. 2012. Technical recommendations of the research for the South of Brazil. Eduardo Rodrigues Hickel – Epagri/ Itajaí Experimental Station, Itajaí, Brazil. 179p.
- Sathasivan, L.G., Haughn, G.W., Murai, N. 1990. Nucleotide sequence of a mutant acetolactate synthase gene from an imidazolinone-resistant *Arabidopsis thaliana* var. Columbia. *Nucleic Acids Research* 18: p. 2188.
- Schaedler, C.E., Noldin, J.A., Agostinetto, D., Dal magro, T., Fontana, L.C. 2013. Germination and growth of *Fimbristylis miliacea* biotypes resistant and susceptible to acetolactate synthase-inhibiting herbicides. *Planta Daninha* 31: 687-694.
- Sibony, M., Rubin, B. 2003. The ecological fitness of ALS-resistant *Amaranthus retroflexus* and multiple-resistant *Amaranthus blitoides*. *Weed Research* 43: 40-47.
- Sigmaplot – *Scientific Graphing Software*. Version 10.0, 2007.
- Urchei, M.A., Rodrigues, J.D., Stone, L. F. 2000. Growth analysis of two common bean cultivars under irrigation, in no-tillage and conventional tillage. *Brazilian Agricultural Research* 35: 497-506.
- Vila- Aiub, M.M., Neve, P., Powles, S.B. 2009. Fitness costs associated with evolved herbicide resistance alleles in plants. *New Phytologist* 184: 751-767.
- Yu, Q., Han, H., Vila-aiub, M.M. 2010. AHAS herbicide resistance endowing mutations: effect on AHAS functionality and plant growth. *Journal of Experimental Botany* 61: 3925-3934.
- Zanine, A.M., Santos, E.D. 2004. Competition among plant species - a review. *Plant Physiology* 11: 103-122.