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Research Article

Resistance detection of blackjack to ALS inhibitors by *in vitro* plant growth method

Daniela Tessaro[a], Daiane Benneman[a], Lais T. Perboni[a], Jéssica R. Garcia[a], Marlon M. Teixeira[a], Dirceu Agostinetto[a]*

[a] Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas-RS, Brasil.

INFORMATION ARTICLE

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***Corresponding author:**
<agostinetto.d@gmail.com>

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The authors declare that there is no conflict of interest regarding the publication of this manuscript.

HIGHLIGHTS

- Early detection of resistance assists in the fast decision making on the use of control methods.
- The in vitro plant growth method allows faster verification of resistance.
- The in vitro culture method allows the detection of blackjack biotypes resistant.

ABSTRACT

Background: The resistance of weeds to herbicides is a problem that has been increasingly studied because of its great importance in agriculture and rapid growth of this phenomenon worldwide. The quick detection of resistant plants is extremely important for resistance management. Weed resistance to herbicides can be detected through field, greenhouse and laboratory tests. A fast way of detecting resistance is using tissue culture method, where is possible to use resistant plant clones and obtain results faster than whole-plant method in greenhouse.
Objective: The aims of the research were to detect the resistance of blackjack to herbicides inhibitors of the acetolactate synthase (ALS), addition in vitro cultivation; determine the herbicide concentration of imazethapyr to 95% control of the susceptible plant population; evaluate explants growth; and, determine the herbicide concentration added to the culture medium where is possible to distinguish a resistant from a susceptible plant.
Methods: Experiments were carried out in greenhouse and tissue culture laboratory. Screening was performed to select resistant and susceptible biotypes, tests for specie establishment and dose response curves in vitro were made.
Results: The herbicide concentration added to the culture medium that provided 95% susceptible biotype control and efficiently differentiated susceptible biotype from resistant one was 0.6µM of imazethapyr.
Conclusions: This method helps recommendations of weed management and provides a quick decision to alternative control of this specie, thus avoiding major damage to the crops.

1 INTRODUCTION

Weeds, present in agricultural systems, interfere with the development of crops, causing great economic damage if not controlled at the right time. Weed control has been carried out primarily through the use of herbicides, due to lower cost and greater efficiency when compared to other control methods. However, due to the intensive use of herbicides, the selection of resistant weed biotypes has been

observed (Gazziero et al., 2009). In the world, there are about 500 biotypes with resistance to herbicides, of which 50 occur in Brazil (Heap, 2019). This plant, belonging to the Asteraceae family, has distribution worldwide, being one of the most competitive weeds in agricultural production systems (Santos and Cury, 2011).

Among the weeds that have resistance problems, the blackjack (*Bidens pilosa*) stands out. This plant, belonging to the Asteraceae family, has distribution worldwide, being one of the most competitive weeds in agricultural production systems (Santos and Cury, 2011). The blackjack has a high production of seeds, which are easily dispersed over long distances. The species is found throughout the national territory, but is concentrated in agricultural areas in the Center-South region (Santos and Cury, 2011). The species has resistant biotypes to four herbicides mechanisms of action in the world, they are inhibitors of the enzymes such as acetolactate synthase (ALS), enolpyruvyl shikimate phosphate synthase (EPSP) and photosystems (FS) I and II. In Brazil, there are resistant biotypes to FSII and ALS inhibitors (Heap, 2019), which makes their chemical control difficult.

The most important prevention and control measure of resistant biotypes is the rotation of the mechanism of action, in order to reestablish effective chemical control. This procedure depends on confirming the presence of herbicide-resistant weeds in the field (Matzenbacher et al., 2013). When there are suspected cases of weeds resistance in the field, scientific evidence of this resistance can be done through field experiments, in greenhouse and also in the laboratory.

Early detection of blackjack biotypes resistance assists in the fast decision making regarding the use of control methods seeking to avoid the spread of resistance to adjacent areas. With the *in vitro* plant growth method, there is greater practicality and quickness in verifying the resistance of biotypes, results can be obtained in up to ten days, in contrast to traditional greenhouse methods, where, if the observation of the field leak is considered, obtaining the seed of the suspect biotype the results can reach to150 days.

There is a need to conduct researches to develop fast resistance detection tests, as well as to improve existing methods, so that they are faster and that resistance can be detected even in the same growing season. The objectives of this study were: to detect blackjack biotypes resistant and susceptible to ALS in the Planalto region (RS); determine the best culture medium for the *in vitro* regeneration of blackjack; determine the concentration of the imazethapyr herbicide necessary for 95% control of the susceptible biotype population; evaluate the growth of resistant and susceptible explants subjected to different concentrations of imazethapyr herbicide; and determining the concentration of imazethapyr herbicide added to the medium in which it is possible to distinguish susceptible from resistant plants.

2 Material and Methods

Three experiments were carried out to obtain the herbicide concentration necessary for the blackjack resistance test in *in vitro* cultivation: the first consisted of collecting seeds and selecting the resistant and susceptible biotypes to be used in the test; the second consisted in the selection of the culture medium for the *in vitro* regeneration of blackjack; and the third consisted of dose-response curves tests of susceptible and resistant blackjack biotypes to imazethapyr herbicide in *in vitro* cultivation.

2.1 Blackjack biotypes selection susceptible and resistant to ALS

Seeds of blackjack plants that survived the applications of ALS-inhibiting herbicides were collected from crops in the Northern region of the state of Rio Grande do Sul. Each sampled point, identified by geodetic coordinates using the Global Positioning System (GPS), corresponded to seeds from a plant, identified by technicians as plants that survived the application of ALS-inhibiting herbicides. Thirty four seeds samples were collected in the municipalities of Passo Fundo and Coxilha, which were cleaned, identified and stored in a cold chamber (15 ºC). For resistance verification and the choice of susceptible and resistant biotypes for *in vitro* tests, an experiment was carried out in a greenhouse.

The experimental design was completely randomized, with five replications. Four seeds of each biotype were sown in trays with a volumetric capacity of 8 L and spacing between rows of 10 cm, totaling three biotypes per tray. Organic substrate and soil classified as Gleyic Luvic Planosol (IUSS Working Group WRB, 2015) were used.

When the blackjack plants were in the three to four leaf development stage, the imazethapyr herbicide was applied, at the recommended dose of 1 L ha^{-1} (100 g a.i. ha^{-1}) (Agrofit, 2018). A

backpack sprayer was used in the herbicides application, pressurized with CO_2, equipped with fan-type nozzles and 110.015 tips, spaced 50 cm apart, with a constant pressure of 1 bar (1.0197 kgf cm^{-2}), regulated to a spray volume equivalent to 120 L ha^{-1}.

The control variable was assessed visually at 28 days after treatments application (DAT), the biotypes were identified according to the response to the herbicides as susceptible or resistant, adopting a binary scale, where zero (0) represented the death of the plants (susceptible) and one (1) the absence of symptoms (resistant). The resistant plants that survived were grown in a greenhouse and later established *in vitro*. The data obtained were analyzed using descriptive statistics.

2.2 Selection of the culture medium for *in vitro* regeneration of blackjack

The experiment was carried out in a tissue culture laboratory to determine the best culture medium for the *in vitro* regeneration of blackjack. The treatments were arranged in a factorial scheme, with 20 repetitions, in which factor A tested the culture medium: 100% MS (Murashige and Skoog, 1962) with sucrose (30 g L^{-1}) and myo-inositol (0.1 g L^{-1}) and 50% MS (1/2MS) with sucrose (15 g L^{-1}) and myo-inositol (0.05 g L^{-1}); and factor B consisted of the absence and addition of benzylaminopurine growth regulator (BAP) at 1 mg L^{-1}. The pH of the two medium was adjusted to 5.8 before adding agar (7 g L^{-1}); subsequently, they were poured into test tubes (10 mL $tube^{-1}$). The medium was sterilized in an autoclave at 121 °C and 1.5 atm for 20 minutes, cooled at room temperature until solidification and inoculated with one blackjack explant per tube (aseptic nodal segment, 1 cm long), in a laminar flow chamber. Then, the material was taken to a growth chamber, with a photoperiod of 16/8 hours of light/dark and a temperature of 24 °C during the entire period of the experiment.

The variables analyzed at 14 days after the experiment installation were: length of the aerial part and root of the explants (cm) and growth percentage, obeying the evaluation scale of 0, 50 and 100%. The value of 100% was attributed to the plants that showed maximum growth. Contaminated explants were not counted. The data obtained were analyzed for normality (Shapiro-Wilk test) and subsequently subjected to analysis of variance ($p \leq 0.05$). In case of significance, the effects of culture medium and BAP regulator were analyzed using the t test ($p \leq 0.05$).

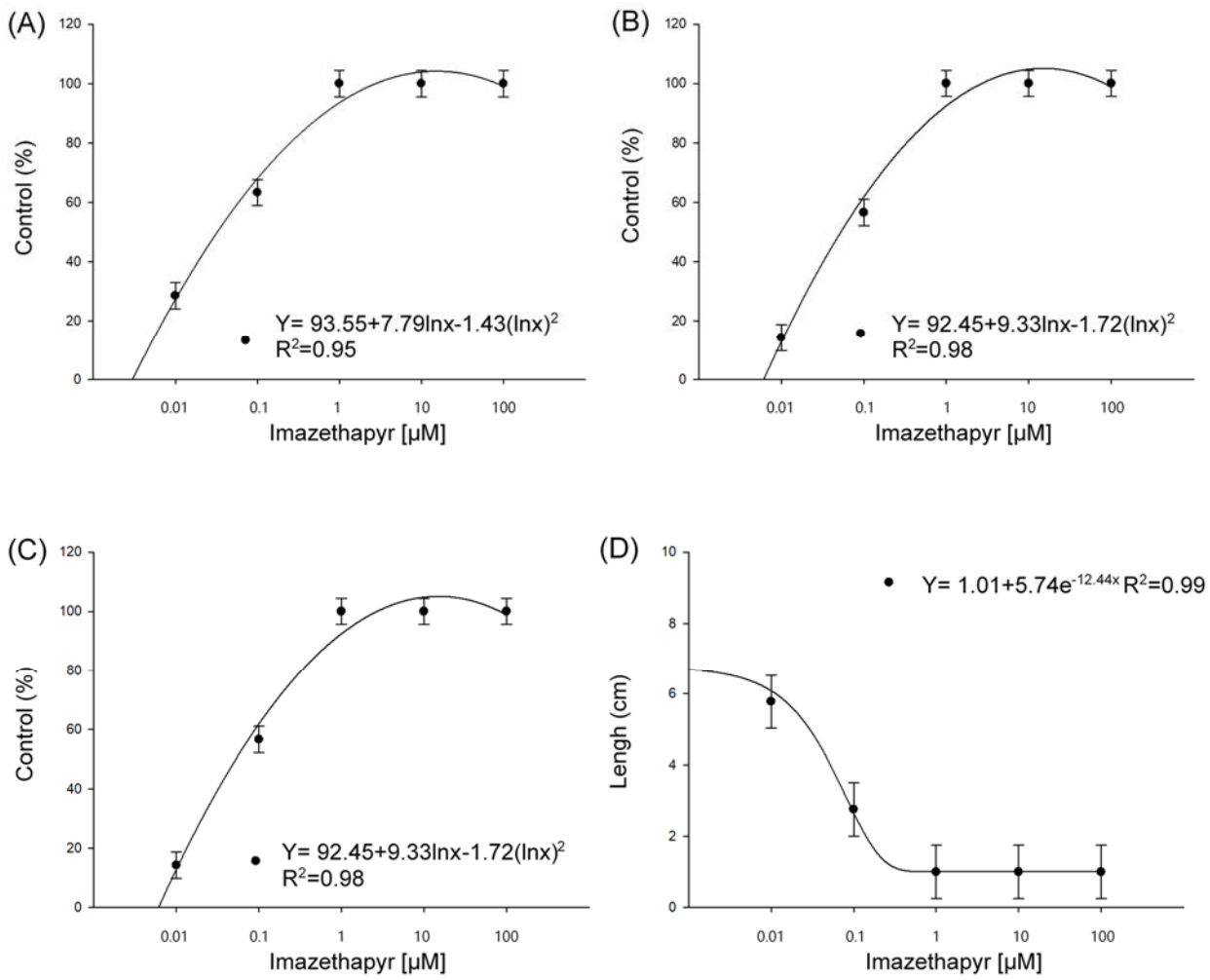
2.3 *In vitro* dose-response curve for susceptible and resistant blackjack biotypes

The tests were carried out with material from the biotypes tested in the selection in which a susceptible and a resistant biotype were chosen, coming from nearby locations. From these biotypes, eight nodal segments per plant were collected, which were disinfected for *in vitro* establishment. Disinfestation was performed by washing the explants in 70% alcohol and detergent for one minute, immersion and agitation in 1.5% sodium hypochlorite solution, for 10 minutes, and triple washing with autoclaved and distilled water. The explants established were multiplied, in a total of nine subcultures, to produce plant material for the dose-response curves.

The culture medium used was MS 50% (1/2MS) and its preparation, in all curves for blackjack, followed the one described in the medium selection experiment. For the addition of the concentrations of imazethapyr herbicide to the medium, stock solutions were prepared in concentrations of 1,000 and 100 µM, which were filtered and aliquoted, and were added to the hot sterilized medium, according to the treatment. Then, the medium was poured into test tubes (10 ml $tube^{-1}$).

In the first test, the choice of concentrations for the dose-response curve of the susceptible biotype followed a logarithmic scale of 0.01; 0.1; 1.0; 10; 100 µM; and control without application, corresponding to zero concentration (0). In the second test, the amplitude of the concentrations was reduced according to the results observed in the susceptible biotype curve, described above. Thus, an exponential scale curve was constructed, in the following concentrations: 0; 0.1; 0.2; 0.4; 0.8; and 1.6 µM, where again only the susceptible biotype was subjected to these concentrations. With the results of this second curve, it was possible to obtain the concentration required to control 95% of explants from susceptible plants and, thus, build a new curve with both resistant and susceptible biotypes, where the following concentrations were tested: 0; 0.15; 0.3; 0.6; 1.2; 2.4; 4.8; and 9.6 µM.

The tubes were inoculated with 1 cm nodal segment per tube (Figure 1), in a laminar flow chamber. Subsequently, the material was taken to a growth chamber, with a photoperiod of 16/8 hours of light/dark and a temperature of 24 °C during the entire experiment period. The variables analyzed were the percentage of control at 7, 14 and 21 days after inoculation (DAI) and the length of explants (cm) in the first and second tests. In the third test, control



Error bars correspond to the 95% confidence interval of error probability.

Figure 1 - Visual control (%) of blackjack explants susceptible to imazethapyr, due to different concentrations of the herbicide, at 7 (A), 14 (B) and 21 (C) days after the inoculation of treatments (DAI) and length of the explants aerial part (D) at 21 DAI.

assessments were made at 5 and 10 DAI, in order to shorten the test time, and the length of the explants was presented as growth percentage compared to the control. Dead explants were considered to be those that did not show normal development and no sprouting, differing from the control (zero concentration). The tubes that presented contaminated explants (2.5%) were discarded, not being counted in the variables.

Data were submitted to analysis of variance ($p \leq 0.05$) and, in case of significance, analyzed by logarithmic and exponential regression in the first two tests. For the third test, the biotype factor was compared by t test ($p \leq 0.05$), and the concentration factor, by sigmoidal regression of the logistic type, as follows: $y = a / [1 + (x / x0) b]$, where: y = percentage of control; x = concentration of the herbicide; and a, x0 and b = parameters of the equation, where "a" is the difference between the maximum and minimum points of the curve, "x0" is the concentration that provides 50% of the variable response and "b" is the slope of the curve. The resistance factor (RF) was calculated by the R/S ratio, which corresponds to the division of C50 (concentration required for 50% control) of the resistant biotype by C50 of the susceptible biotype.

3 Results and Discussion

The results and discussion will be presented following the sequence of activities presented in material and methods.

3.1 Blackjack biotypes selection susceptible and resistant to ALS

The analysis of the data showed that, from 34 tested biotypes, 12 (35%) presented resistance to imazethapyr. It was found that the dose of 100 g a.i. ha^{-1} controlled 65% of the biotypes with suspected resistance, applied at the stage of three to four leaves (Table 1). Twelve resistant biotypes were identified, which survived after the application of the

Table 1 - Geographic localization and blackjack biotypes response to imazethapyr application at 28 days after treatment (DAT)

Biotype	County	Coordinates		Imazethapyr response
		Latitude	Longitude	
P 01	Passo Fundo	28° 13' 39''	52° 24' 10''	0(1)
P 02	Passo Fundo	28° 13' 39''	52° 24' 10''	0
P 03	Passo Fundo	28° 13' 39''	52° 24' 10''	0
P 04	Passo Fundo	28° 13' 39''	52° 24' 10''	0
P 05	Passo Fundo	28° 13' 39''	52° 24' 10''	0
P 06	Passo Fundo	28° 13' 41''	52° 24' 46''	0
P 07	Passo Fundo	28° 13' 41''	52° 24' 46''	0
P 08	Passo Fundo	28° 13' 41''	52° 24' 46''	0
P 09	Passo Fundo	28° 13' 41''	52° 24' 46''	0
P 10	Passo Fundo	28° 13' 41''	52° 24' 46''	0
P 11	Passo Fundo	28° 13' 41''	52° 24' 46''	1
P 12	Passo Fundo	28° 13' 40''	52° 24' 35''	1
P 13	Passo Fundo	28° 13' 40''	52° 24' 35''	0
P 14	Passo Fundo	28° 13' 40''	52° 24' 35''	0
P 15	Passo Fundo	28° 13' 40''	52° 24' 35''	1
P 16	Passo Fundo	28° 13' 40''	52° 24' 35''	0
P 17	Coxilha	28° 10' 05''	52° 20' 56''	0
P 18	Coxilha	28° 10' 05''	52° 20' 56''	0
P 19	Coxilha	28° 10' 05''	52° 20' 56''	0
P 20	Coxilha	28° 10' 05''	52° 20' 56''	0
P 21	Coxilha	28° 10' 05''	52° 20' 56''	0
P 22	Coxilha	28° 11' 09''	52° 19' 30''	0
P 23	Coxilha	28° 11' 09''	52° 19' 30''	1
P 24	Coxilha	28° 11' 09''	52° 19' 30''	1
P 25	Coxilha	28° 11' 09''	52° 19' 30''	1
P 26	Coxilha	28° 11' 09''	52° 19' 30''	0
P 27	Coxilha	28° 11' 09''	52° 19' 30''	0
P 28	Passo Fundo	28° 14' 12''	52° 24' 16''	1
P 29	Passo Fundo	28° 14' 12''	52° 24' 16''	1
P 30	Passo Fundo	28° 14' 12''	52° 24' 16''	1
P 31	Passo Fundo	28° 14' 12''	52° 24' 16''	1
P 32	Passo Fundo	28° 14' 12''	52° 24' 16''	1
P 33	Passo Fundo	28° 14' 12''	52° 24' 16''	1
P 34	Passo Fundo	28° 13' 50''	52° 24' 15''	0

(1) 0 = susceptible or 1 = resistant.

herbicide, from which the biotype P31 was selected as resistant biotype and used in the following experiments. The other biotypes were considered susceptible, as they had no survivors after the treatment was applied; the biotype P34 was chosen as susceptible to be used in the *in vitro* experiment because it presents collection region closer to that of the resistant biotype.

3.2 Selection of culture medium for *in vitro* regeneration of blackjack

There was no interaction between the factors of culture medium and growth regulator for the variables growth percentage and root length or simple effect for the factor culture medium (data not shown). A simple effect of growth regulator was observed only for the variable length of the explant. It was found that the longest segment length occurred in the medium without the hormone presence (Table 2). Similar responses were observed for blackjack in micropropagation using the BAP regulator, where the authors observed a 33.3% decrease in the sprouting percentage of nodal segments in the

Table 2 - Length of blackjack explants evaluated at 14 days after inoculation in the culture medium

Treatment	Length of the explant (cm)
Without BAP	1.84 A
With BAP	1.47 B
CV (%)	44.01

Means followed by different capital letters in the column differ from each other using the t test (p≤0.05).

regulator presence (Santos, 2015). As there was no significance among the medium, the use of the 50% MS medium was defined for the following experiment, since the development of the explants was similar with less use of reagents.

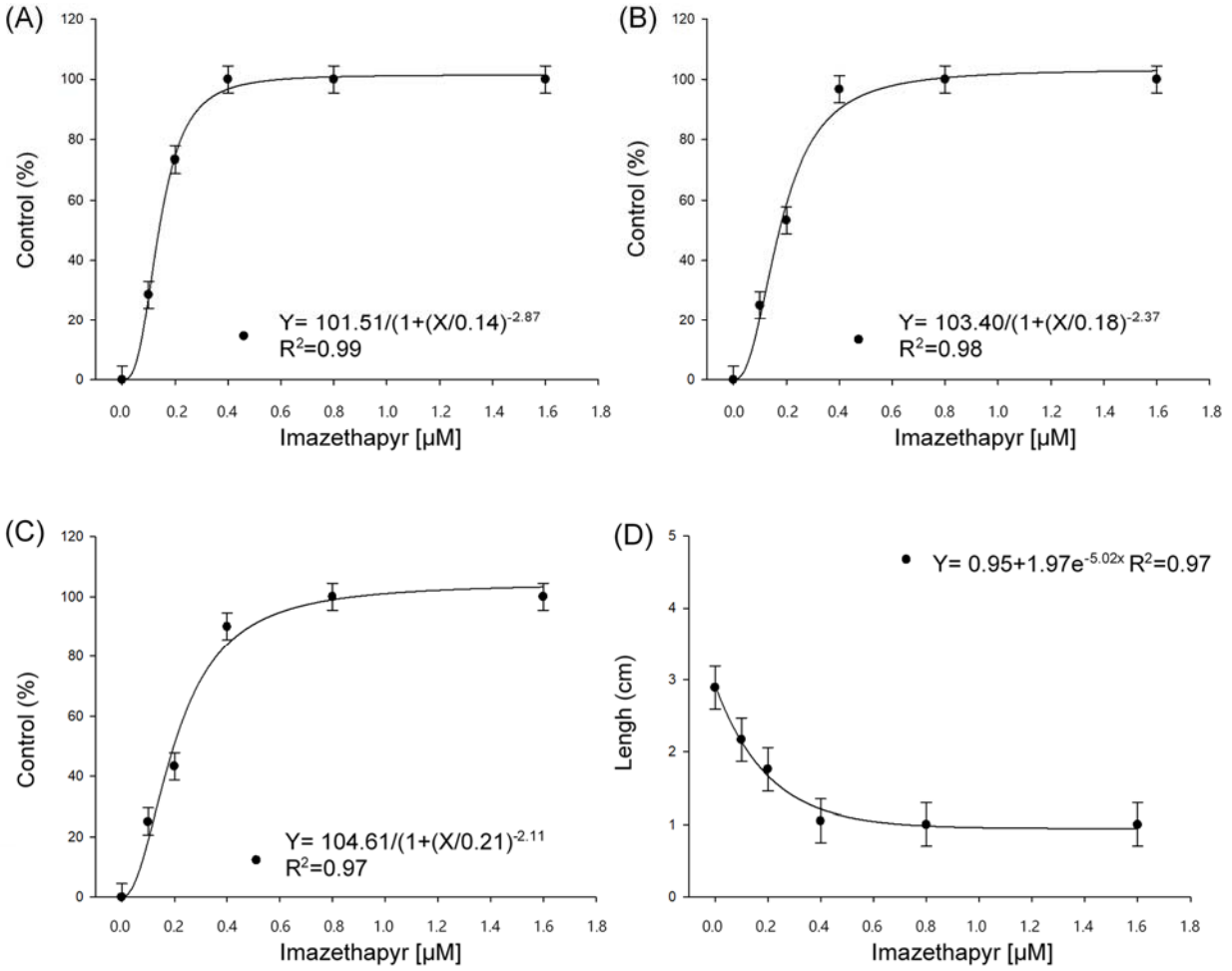
3.3 *In vitro* dose-response curve for susceptible and resistant blackjack biotypes

In the first test, for the susceptible blackjack control variable, the second order logarithmic regression model was adjusted, with coefficient of determination (R^2) values from 0.95 to 0.98 (Figure 1A, B and C), while for the length of the explants, the model that adjusted was the exponential of three parameters, with an R^2 value of 0.99 (Figure 1D).

Concentrations above 1 µM resulted in the control of 100% of explants, regardless of the time of evaluation, directly affecting plant growth, which indicates that these concentrations were very high. On the other hand, with concentrations below 0.1 µM, the observed control average was very low; the concentration of 0.01 µM reached a control value of 14.3%, and the concentration of 0.1 µM, a control of 56.6% (Figure 1). Thus, it was decided to create a new curve in the second test, in the concentration range between 0.1 and 1 µM, in order to find the concentration for blackjack effective control.

In the second test, also performed only with the susceptible biotype, the model that adjusted the control curve was the sigmoidal regression of the logistic type, with R^2 values from 0.97 to 0.99 (Figure 2A, B and C); for the length of the explants, the exponential model was adjusted, with an R^2 value of 0.97 (Figure 2D).

Control values greater than 90% were found at concentrations of 0.4, 0.8 and 1.6 µM, in all periods of evaluation, which did not differ from each other by the confidence interval (Figure 2A, B and C). In the same concentration range, there was explants growth inhibition, resulting in an exponential drop in length (Figure 2D). The concentrations of 0.1 and 0.2 µM did not reach values greater than 25% and 75%, respectively, in all periods of evaluation (Figure 2A, B and C). Using the equation values obtained in the



Error bars correspond to the 95% confidence interval of error probability.

Figure 2 - Visual control (%) of blackjack explants susceptible to imazethapyr, due to different concentrations of the herbicide, at 7 (A), 14 (B) and 21 (C) days after the inoculation of treatments (DAI) and length of the explants aerial part (D) at 21 DAI.

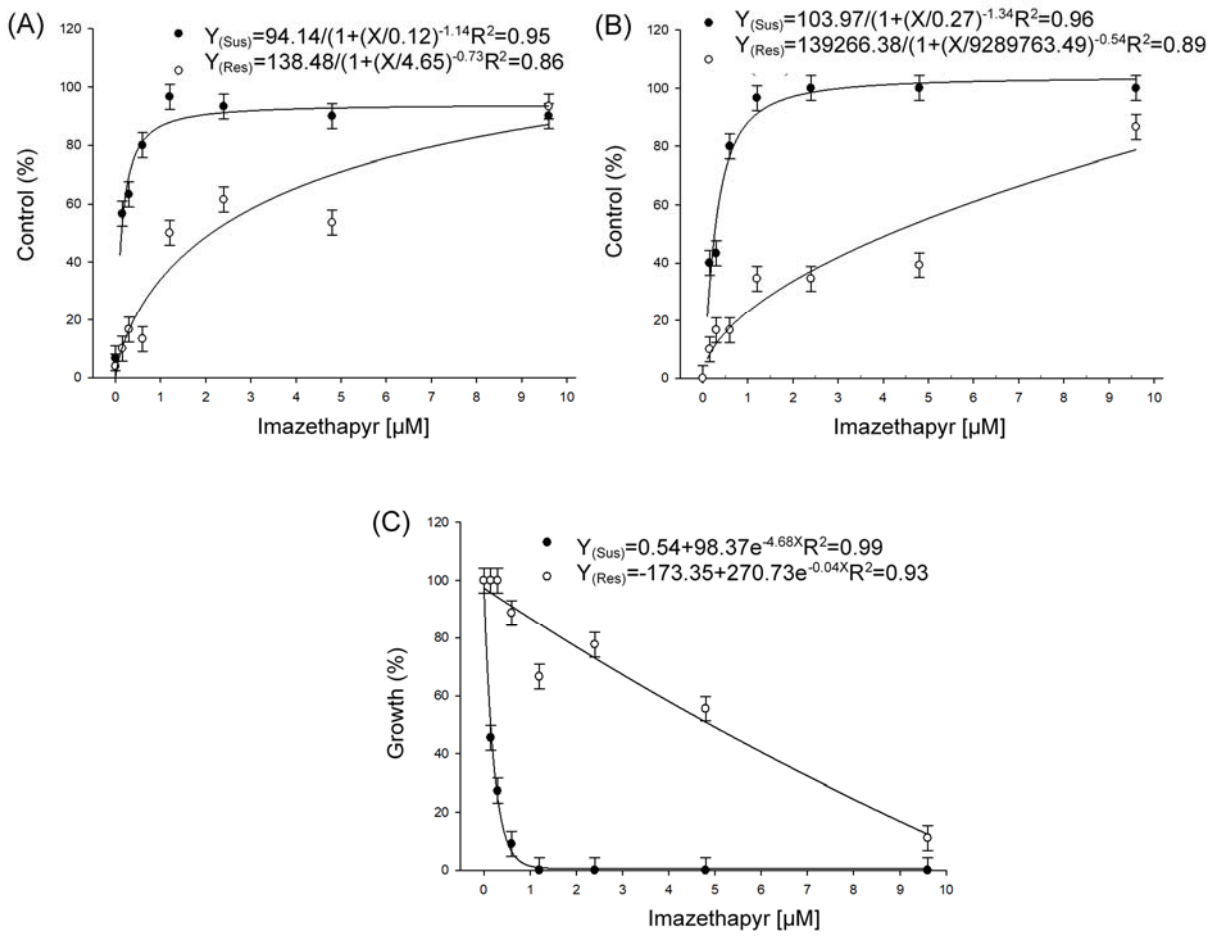
second evaluation period, it was possible to establish the concentration required for 95% control of the susceptible biotype, which was 0.6 µM.

The differences in control between the second and third assessment periods were minimal, showing that the response can be given in the second season, reducing the test time. Therefore, the concentration determined to be the efficient concentration was 0.6 µM, which was tested together with the other concentrations for the two biotypes in the third test of dose-response curve.

In the third test, there was an interaction among biotype factors and herbicide concentrations for all variables. The logistic regression model that was adjusted to the explants control was the sigmoidal one, in both periods of evaluation. The values of R^2 varied from 0.95 to 0.96 and from 0.86 to 0.89, for the susceptible and resistant biotypes, respectively (Figure 3A and B). As for the explants growth, the exponential model of three parameters was adjusted, with R^2 values of 0.93 and 0.99 (Figure 3C), showing a satisfactory adjustment of the data to the models.

The equations built with the control values, in response to the increasing concentrations of the imazethapyr herbicide, prove that the susceptible and resistant blackjack biotypes respond differently to the herbicide in *in vitro* cultivation, in the two evaluation periods.

At 5 DAI, for the susceptible biotype, a control greater than 93% was observed in the range of concentrations from 1.2 to 9.6 µM, which did not differ from each other by the confidence interval. For the resistant biotype, the control values were not greater than 16% at concentrations of 0.15, 0.3 and 0.6 µM, not differing from each other and also not differing from the control. Even in the first period, it was observed by the equation parameters that the herbicide concentration of 0.13 µM was sufficient to obtain control of 50% of the susceptible biotype, whereas, for the same control level of the resistant



Error bars correspond to the 95% confidence interval of error probability.

Figure 3 - Visual control (%) of blackjack explants susceptible (Sus) and resistant (Res) to imazethapyr, in different concentrations of the herbicide, at 5 (A), 10 (B) days after inoculation of treatments (DAI) and explants aerial part growth (C) at 10 DAI.

biotype, the required concentration was 2.13 µM of imazethapyr, which corresponds to three times the efficient concentration (Figure 3A).

At 10 DAI, for the susceptible biotype, a control greater than 96% was observed in the concentration range between 1.2 and 9.6 µM, with no difference between them by the confidence interval. For the resistant biotype, the control was not higher than 16% at concentrations of 0.15, 0.3 and 0.6 µM, not differing from each other (Figure 3B). For this same biotype, in the range of concentrations from 1.2 to 4.8 µM, the maximum control observed was 39%, and only in the highest concentration of the test (9.6 µM) the control reached 86%, differing from the others treatments. In the second period, 0.26 µM of imazethapyr was required for 50% control of the susceptible biotype, while for the resistant it was necessary 3.9 µM, that is, about six times the efficient concentration for the susceptible (Figure 3B).

As for the growth variable, there was a decrease according to the increase in the herbicide concentrations applied, and it was observed for the susceptible biotype the highest average in the control (100%), differing from the treatments. Concentrations between 1.2 and 9.6 µM showed interrupted growth (0%), not differing for this biotype. In the resistant biotype, the same inhibition in growth was not observed, with a gradual decrease according to the increase in concentrations; in the first three there was no significant difference (0, 0.15 and 0.3 µM), and only the highest concentration (9.6 µM) showed a decrease, with only 11.1% of growth (Figure 3C).

The C50 for the susceptible biotype (P34) was 0.13 and 0.26 µM in the first and second evaluations, respectively; and for the resistant biotype (P31), it was 2.1 and 3.9 µM. Thus, the RF was higher than ten (16.4 in the first evaluation and 15 in the second), proving the resistance of the P31 biotype to imazethapyr. In dose-response curve studies with blackjack biotypes susceptible and resistant to imazethapyr, a resistance factor value of 27.03 was observed for the resistant biotype studied (Monquero et al., 2000). High resistance factor is common for

species resistant to ALS inhibitors, since the most common resistance mechanism in these cases is the enzyme mutation (Han et al., 2012), which leads to the need to use high doses of the herbicide to control resistant plants (Lamego et al., 2011; Beckie et al., 2012).

Differences observed in the control between the first and the second assessment can be caused by the time required for the herbicide action and the appearance of symptoms. ALS-inhibiting herbicides can take up to two months to cause complete plant death in the field; the activity of ALS *in vitro* can be inhibited in minutes with nanomolar concentrations of herbicide (Coob and Reade, 2010), however under conditions of *in vitro* cultivation the time required for the plant death is unknown. When it comes to productivity losses in the field due to interaction with weeds, the five days between one evaluation period and the other represent a big difference in decision making for control. The coexistence period between the crop and the weeds can estimate the level of damage caused by the competition; the longer the period in which the crop interacts with the weed, the greater the damage caused to the grain yield (Silva et al., 2009; Furtado et al., 2012).

Considering the weed's coexistence time with the crop, it becomes of great importance that the resistance test response by *in vitro* cultivation can be obtained at 5 DAI. Another extremely important factor is the time available for a decision making to carry out an alternative control based on the result of the test. Ten days to obtain the response can be a very prolonged period, which allows the resistant plant to continue growing and reach a development stage in which it can no longer be controlled with other herbicides; thus, it survives to new attempts of control, reproducing itself, increasing the seed bank and increasing the resistant population of the area.

The second evaluation period provided better visibility of the herbicide symptoms. However, the t test showed that even in the first evaluation period, a difference between susceptible and resistant biotype can be observed even in the lowest concentration used, where it is possible to discriminate the biotypes with only 0.15 µM of imazethapyr, but with control of the susceptible biotype lower than 80% (Table 3).

For discrimination among biotypes with control of at least 80% of the susceptible biotype, the concentration of 0.6 µM, considered efficient, it was better discriminated of the differential behavior among biotypes, as it resulted in high control efficiency of the susceptible biotype and control levels very low of the resistant biotype. In addition, this value of 80% control of the susceptible biotype is considered effective control of blackjack with imazethapyr for soybean culture. In quick resistance tests via leaf immersion, with biotypes of *Euphorbia heterophylla* resistant to imazethapyr, similar control levels were observed; the susceptible biotype showed 96% control, while the resistant biotype had only 16% control, ten days after the treatments application (Trezzi et al., 2011).

Some resistance mechanisms are the result of the difficulty in absorbing the product, due to leaf characteristics, such as roughness, hairiness and chemical composition of epicuticular wax (Sanchotene et al., 2008). However, in the resistance test developed in this study, in which the herbicide is not supplied via spray drops, but directly in the plant's vascular system, the resistance mechanism becomes essential for its efficiency. In other words, a weed species that has a resistance mechanism by reducing absorption and thus survives a field spraying may not show resistance in the *in vitro* test, since in this case the absorption occurs directly via the vascular system, as a result of the excision of the explant.

Table 3 - Visual control (%) of blackjack explants susceptible (Sus) and resistant (Res) to imazethapyr, in different concentrations of the herbicide, at 5 and 10 (DAI) and explants aerial part growth at 10 DAI

Concentration (µM)	Control (%)				Growth (%)	
	5 DAI(1)		10 DAI(1)		Sus	Res
	Sus	Res	Sus	Res		
0.0	6.7 ns	3.9	0.0 ns	0.0	100.0 ns	100.0
0.15	56.7 *	10.0	40.0 *	10.0	45.5 *	100.0
0.3	63.3 *	16.7	43.3 *	16.7	27.3 *	100.0
0.6	80.0 *	13.3	80.0 *	16.7	9.1 *	88.8
1.2	96.7 *	50.0	96.7 *	34.6	0.0 *	66.6
2.4	93.3 *	61.5	100.0 *	34.6	0.0 *	77.7
4.8	90.0 *	53.6	100.0 *	39.3	0.0 *	55.5
9.6	90.0 ns	93.3	100.0 ns	86.7	0.0 *	11.1
CV (%)	47.5		53.2		12.7	

(1) Days after inoculation; ns not significant and * significant by the t test ($p \leq 0.05$).

The movement of the herbicide from the culture medium to the plants during this test does not interfere with the same barriers and conditions imposed in field situations, until it reaches its place of action. Thus, a resistant plant under normal conditions in the field may not show resistance in the *in vitro* test if its resistance mechanism is related to the absorption and decreased translocation of the herbicide. Attention should be paid to the herbicide action mechanism and the resistance mechanism that the plant presents for the application of the resistance test, as its efficiency is directly related to systemic herbicides and to cases of resistance that do not involve low absorption and compartmentalization of the herbicide, preventing its translocation.

Studies related to the absorption and translocation of herbicide molecules in *in vitro* cultivation and resistance tests using these methods are still limited. So, researches become useful so that other herbicides can be applied to the test, identify the concentrations to be used with different species and, thus, expand its application. This information is essential so that alternatives for blackjack handling can be planned quickly in order to avoid the spread of resistant biotypes.

4 Conclusions

The *in vitro* culture method allows the detection of blackjack biotypes resistant to ALS-inhibiting herbicides. The concentration required for satisfactory control of susceptible blackjack plants grown *in vitro* is 0.6 µM of imazethapyr, it is possible in this concentration, to efficiently discriminate susceptible and resistant biotypes.

5 Contributions

DT: Conducting experiments, analysis and writing. DB, LTP, JRG, and MT: Assistance in conducting the experimente. DA: Creator of research, guidance in driving and writing.

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7 References

Agrofit. Sistema de agrotóxicos fitossanitários. [acesso em: 26 mar. 2018]. Disponível em: <http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>.

Beckie H, Warwick SI, Sauder CA, Kelln GM, Lozinski C. Acetolactate Synthase Inhibitor–resistant false cleavers (*Galium spurium*) in Western Canada. Weed Technol. 2012;26:151-5.

Coob A, Reade J. Herbicides and plant physiology. 2nd. ed. Newport: Wiley-Blackwell; 2010.

Furtado GF, Sousa JRM, Sousa Jr. JR, Lacerda RRA, Souza AS. Períodos de interferência de plantas daninhas na cultura do girassol. Rev Verde. 2012;7:12-7.

Gazziero DLP, Christoffoleti PJ, Vargas L, Kruse ND, Galli AJB, Trezzi MM. Critérios para relatos oficiais estatísticos de biótipos de plantas daninhas resistentes a herbicidas. In: Agostinetto D, Vargas L, editores. Resistência de plantas daninhas a herbicidas no Brasil. Passo Fundo: Berthier; 2009. p.91-101.

Han H, Yu Q, Purba E, Li M, Walsh M, Friesen S, et al. A novel amino acid substitution Ala-122-Tyr in ALS confers high-level and broad resistance across ALS-inhibiting herbicides. Pest Manag Sci. 2012;68:1164-70.

Heap I. International survey of herbicide resistant weeds. [acesso em: 16 out. 2019]. Disponível em: http://www.weedscience.org.

IUSS Working Group WRB. (2015). World reference base for soil resources 2014 International soil classification system for naming soils and creating legends for soil maps Update 2015 World Soil Resources Reports. Rome: World Reference Base for Soil Resources.

Lamego FP, Vidal RA, Burgos NR. Competitiveness of ALS inhibitors resistant and susceptible biotypes of greater beggarticks (*Bidens subalternans*). Planta Daninha. 2011;29:457-64.

Matzenbacher FO, Kalsing A, Menezes VG, Barcelos JAN, Merotto Jr. A. Rapid diagnosis of resistance to imidazolinone herbicides in barnyardgrass (*Echinochloa crus-galli*) and control of resistant biotypes with alternative herbicides. Planta Daninha. 2013;31:645-56.

Monquero PA, Christoffoleti PJ, Dias CTS. Resistência de plantas daninhas aos herbicidas inibidores da ALS na cultura da soja (*Glycine max*). Planta Daninha. 2000;18:419:25.

Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 1962;15:473-97.

Sanchotene DM, Dornelles SHB, Perini FL, Gonçalves RA, Estevo AP. Manejo químico de plantas daninhas tolerantes ao glyphosate na cultura da soja. Rev Fac Zoot Vet Agron. 2008;15:77-84.

Santos DD. Controle de qualidade, análise fitoquímica e atividade antimicrobiana de *Bidens pilosa* Linnaeus (Asteraceae) [dissertação]. Santa Maria: Universidade Federal de Santa Maria; 2015.

Santos JB, Cury JP. Picão-preto: uma planta daninha especial em solos tropicais. Planta Daninha. 2011;29:1159-71.

Silva AF, Concenço G, Aspiazú I, Ferreira EA, Galon L, Coelho ATCP, et al. Interferência de plantas daninhas em diferentes densidades no crescimento da soja. Planta Daninha. 2009;27:75-84.

Trezzi MM, Xavier E, Vidal RA, Portes EDS, Vottri M, Lamego FP. Teste rápido de imersão foliar de Euphorbia heterophylla para confirmação de resistência a herbicidas inibidores da PROTOX e da ALS. Planta Daninha. 2011;29:901-12.