ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 10, pp.39600-39606, October, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EFFECT OF PRE-EMERGENT HERBICIDES ON THE GERMINATION AND INITIAL GROWTH OF *TRIFOLIUM REPENS* L.

Geison Rodrigo Aisenberg[1], Davi Silva Dalberto[2], Felipe Koch[1], Ivan Ricardo Carvalho[1,*], Gustavo Henrique Demari[1], Vinicius Jardel Szareski[1], Maicon Nardino[1], Velci Queiróz de Souza[3], Tiago Pedó[1], Tiago Zanatta Aumonde[1], Dirceu Agostinetto[1]

[1]Federal University of Pelotas
[2]Federal University of Amapá
[3]Federal University of Pampa

ARTICLE INFO

Article History:
Received 10th July, 2016
Received in revised form
15th August, 2016
Accepted 08th September, 2016
Published online 30th October, 2016

Key words:
White clover,
Physiological quality of seeds,
Germination,
Seedling growth,
Doses

ABSTRACT

The aim of this study was to evaluate the effect of the application of pre-emergent herbicide doses on the physiology of white clover seeds and the seedlings growth. The herbicides tested were atrazine, s-metolachlor and diclosulam and the application was performed in doses of 0 %, 50 %, 100 % and 200 % of the maximum registered dose of each product. Two tests were performed. The first one was carried out in laboratory, where germination, first germination count and germination speed index were evaluated. In the second test, performed in greenhouse conditions, the emergence and the length of shoots and roots of white clover seedlings were analyzed. In the test performed in laboratory, the most severe damages were caused by the use of s-metolachlor, resulting in reductions in the total germination, first germination count and germination speed index. In the experiment performed in a greenhouse, all herbicides reduced the growth of white clover seedlings; however, the application of atrazine caused most of the negative effects, reducing the emergence and the length of shoots and roots of the seedlings. The herbicides atrazine, s-metolachlor and diclosulam negatively affected the physiological performance of white clover seeds, as well as the seedling growth of this species.

Citation: ***Geison Rodrigo Aisenberg, Davi Silva Dalberto, Felipe Koch, Ivan Ricardo Carvalho, Gustavo Henrique Demari, Vinicius Jardel Szareski, Maicon Nardino, Velci Queiróz de Souza, Tiago Pedó, Tiago Zanatta Aumonde, Dirceu Agostinetto, 2016***. "Effect of pre-emergent herbicides on the germination and initial growth of *Trifolium repens* L.", *International Journal of Current Research*, 8, (10), 39600-39606.

INTRODUCTION

The use of pasture in the production of cattle in Brazil presents an unquestionable importance since it can provide low-cost production in this management system (Santos *et al.*, 2009). Temperate climate pastures are relevant to the supply of fodder for the cattle during the winter months, where the main fodder species of temperate climate used in the state of RS are black wheat (*Avena strigosa* Schreb.) and ryegrass (*Lolium multiforum* Lam.), representatives of Poaceae; and white clover (*Trifolium repens* L.), birdsfoot trefoil (*Lotus corniculatus* L.) and red clover (*Trifolium pratense* L.), representatives of Fabaceae (Carvalho *et al.*, 2010). Due to its high fodder production and nutrient value (Dall'agnol *et al.*, 1982; Soegaard, 1994), white clover stands out as the most important legume sown with Poaceae species in temperate grasslands (Frame and Newbould, 1986).

***Corresponding author: Ivan Ricardo Carvalho**
Federal University of Pelotas

The main problem that can be found related to the quality of seeds of fodder species is due to the presence of other species, cultivated or non-cultivated, which occur in production fields and end up being harvested along with the fodder crop grown (Silva *et al.*, 2011), which is a limiting factor on the productivity of the culture (Inia, 1993). Many seeds from unwanted species present physical characteristics of size, shape and weight similar to the fodder species planted, making the elimination after harvest difficult and sometimes impossible (Carambula, 1981). Regarding the elimination of seeds of unwanted species in the breeding unit, often there is no physical difference between the seed components. Therefore, it is essential to eliminate the weeds that produce these harmful species in the seed production field. Diverse practices of cleanliness are suggested for seed production fields, such as manual methods, fodder management, grazing and use of chemical control (Formoso, 2011; Carambula, 1981). Chemical control of weeds is a method that aims to eliminate species of unwanted plants in cultivation fields (Machado *et al.*, 2013), with attractive costs and significant efficiency (Agostinetto *et al*., 2009). The utilization of herbicides in agriculture for the control of weeds besides providing increased crop productivity (Agostinetto *et al*., 2009; Correia and Durigan, 2010), has also allowed the increase in cultivation areas, by providing the development of a relatively simple production system (Fleck *et al*., 2006). There is no record of herbicide use for weed control in the cultivation of white clover in Brazil, where there is too little information available regarding this topic. However, according to some studies, this culture can present considerable tolerance to the herbicides bentazon, bentazon + imazethapyr and 2.4-D (Machado *et al*., 2013). Moreover, the use of herbicides with low selectivity in white clover can cause phytointoxication to this culture, affecting negatively the fodder offer due, among other aspects, to the growth reduction and the decrease of the biological fixation of nitrogen (Machado *et al*., 2013). Based on the above, the aim of this study was to evaluate the effect of the application of different pre-emergent herbicide doses on the physiology of white clover seeds and the seedlings growth.

MATERIALS AND METHODS

Two tests were performed in greenhouse conditions at the Plant Science Department and the Laboratory of Didactic Analysis of Seeds, both at the Agronomy College Eliseu Maciel, Federal University of Pelotas, on 2013. White clover seeds (*T. repens* L.), cultivar Zapican, used in the experiments were originally from South Embrapa Cattle & Sheep Research Centre, Bagé – Rio Grande do Sul, Brazil, from a single batch of seeds. The experimental design used was entirely randomized, with six replications and treatments disposed in a factorial scheme 3 x 4, where the factor A tested herbicides and the factor B evaluated the doses (0, 50, 100 and 200 % of the maximum registered dose of each herbicide). The dose of 100% represents the maximum registered dose in Brazil. Data referring to the herbicides used in the treatments for the first and second experiment are presented in Table 1.

For the first test, performed in laboratory, 50 seeds per replication were put on blotting paper, placed on transparent plasticboxes (11x11x3cm) and moistened with 12 mL of the herbicide solutions. These final solutions were obtained through the dilution of a stock solution of 5g L^{-1} (atrazine and s-metolachlor) and 1g L^{-1} (diclosulam). Controls were maintained by moistening the blotting paper with 12 mL of distilled water. The variables evaluated were: germination, first germination count and germination speed index. Germination was performed in BOD chambers, at a temperature of 25 °C, under a photoperiod of 12 hours. Germination count was performed according to the Rules for Seed Analysis, 12 days after sowing (Brasil, 2009). First germination count was performed concomitantly with the germination test, on the fourth day after incubation, evaluating the percentage of normal seedlings (Brasil, 2009). Germination speed index was obtained from the daily counting at the same time that the number of germinated seedlings, considering as germinated the seeds with approximately 2 mm of root protrusion (Rehman *et al*., 1996). Calculation of the germination speed was performed according to Maguire (1962).

On the second test, seeds were sown in a greenhouse to perform the emergence test. After sowing the seeds, herbicides were applied, using a CO_{2}- pressurized backpack sprayer, equipped with an application bar with four range nozzles with a distance of 0.5 m from each other. The sprayer was calibrated with a pressure of 30 psi, using a spraying solution volume of 200 L ha^{-1}. The variables evaluated were: seedling emergence, length of shoots and roots.

Seedling emergence was performed in six replications with 50 seeds for each treatment, sown in perforated polyethylene trays, with volumetric capacity of 25 liters (0,25 m width x 0,50 m length x 0,20 m height), filled with substrate. On the 21st day after sowing, the final count of the emerged plants was performed. Length of shoots and roots were obtained from four samples of 10 seedlings, at the end of the emergence test. Shoot length was obtained from the measure of the distance between the insertion of the basal portion of the primary root to the shoot apex, whereas the root length was determined from the measure of the distance between the apical and basal parts of the primary root. Results were expressed in centimeters of shoot $plant^{-1}$ or root $plant^{-1}$.

A zero dose treatment (absence of application) was used for the three herbicides tested, for all the variables evaluated, in both experiments. The data obtained for all variables, expressed in average values per treatment, were analyzed for normality and homoscedasticity and later submitted to analysis of variance by F test ($p \leq 0.05$), and when the effect of herbicides was significant, they were compared by the Duncan test ($p \leq 0.05$). The effect of doses was compared by polynomial regression analysis.

RESULTS AND DISCUSSION

It was verified interaction between the factors studied for all the variables evaluated, except for the shoot length, evidencing different responses among molecules, which can be attributed to the different mechanisms of action of herbicides. Effect of herbicide and dose occurred for the variable shoot length. No significant difference was evidenced among herbicides in the 0 % dose of herbicides for seed germination (Table 2). The germination of white clover seeds was reduced when using the herbicide s-metolachlor in relation to the herbicides atrazine or diclosulam. This reduction was in the order of 20 % and 22 % for the dose of 50 %; 68 % and 67 % for the dose of 100 %; and 88 % and 87 % for the dose of 200 %, regarding the use of herbicides diclosulam and atrazine, respectively.

In all the herbicide doses tested, for the variable first germination count (FGC), the herbicides atrazine and diclosulam did not show significant difference among them, being only differentiated from the herbicide s-metolachlor on the doses of 50, 100 and 200 % (Table 2). The use of s-metolachlor at 50 % dose caused a reduction in the number of normal plants in FGC, in relation to diclosulam and atrazine, where the reduction was of 35 % and 42 %, respectively. The use of the herbicide s-metolachlor on the 100 % dose reduced the number of normal plants in FGC in 68 % and 70 % in comparison to the herbicides atrazine and diclosulam, respectively. The use of s-metolachlor on the 200 % dose reduced FGC in 57 % and 59 % in relation to atrazine and diclosulam, respectively. When evaluating the germination speed index (GSI), except on the zero dose of the herbicides tested, it was verified that in each dose evaluated, the lowest

Table 1. Commercial name, concentration, active ingredients and herbicide doses used in the treatments in the first and second experiment. FAEM/UFPel. Capão do Leão, 2013

First Experiment						
Commercial name	Concentration	Active ingredients	Doses used (%)			
			0	50	100[1]	200
Atrazina Nortox 500 SC®	500 g L^{-1}	Atrazine	0.00[2]	126.04	252.08	504.17
Dual gold®	960 g L^{-1}	S-metolachlor	0.00	96.80	193.60	387.20
Spider 840 WG®	840 g Kg^{-1}	Diclosulam	0.00	0.31	0.62	1.24
Second Experiment						
Commercial name	Concentration	Active ingredients	Doses used (%)			
			0	50	100[1]	200
Atrazina Nortox 500 SC®	500 g L^{-1}	Atrazine	0.0[3]	1.25	2.5	5.0
Dual gold®	960 g L^{-1}	S-metolachlor	0.0	0.96	1.92	3.84
Spider 840 WG®	840 g Kg^{-1}	Diclosulam	0.0	0.013	0.025	0.05

[1]Maximum dose of registration in Brazil; [2]Concentration of active ingredient in milligram per liter (ml L^{-1} or mg L^{-1}); [3]Kilogram of active ingredients per hectare (Kg ha^{-1}).

Table 2. Germination, first germination count and white clover seed germination speed index subjected to application of different doses of herbicides atrazine, s-metolachlor and diclosulam. FAEM/UFPel. Capão do Leão, 2013

Dose (%)	Atrazine	S-metolachlor	Diclosulam
	Germination (%)		
0	84.0 a[1]	84.0 a	84.0 a
50	81.0 a	63.0 b	79.0 a
100	72.0 a	24.0 b	73.0 a
200	74.0 b	9.0 c	80.0 a
C.V. (%)	6.47	10.70	5.66
	First germination count (%)		
0	52.0 a[1]	52.0 a	52.0 a
50	65.0 a	38.0 b	58.0 a
100	50.0 a	16.0 b	54.0 a
200	56.0 a	6.0 b	57.0 a
C.V. (%)	14.14	19.79	12.41
	Germination speed index		
0	19.6 a[1]	19.6 a	19.6 a
50	25.0 a	18.3 b	23.9 a
100	21.5 a	14.0 b	22.6 a
200	24.2 a	10.3 b	25.2 a
C.V. (%)	11.70	8.94	8.25

[1]Measures followed by the same letter in the line do not differ statistically among them by the Duncan test ($p \leq 0.05$).

Table 3. Emergence, aerial part and root length of white clover seedlings, subjected to the application of different doses of herbicides atrazine, s-metolachlor and diclosulam. FAEM/UFPel. Capão do Leão, 2013

Dose (%)	Atrazine	S-metolachlor	Diclosulam
	Emergence (%)		
0	78.0 a[1]	78.0 a	78.0 a
50	0.0 c	16.0 b	55.0 a
100	0.0 c	12.0 b	64.0 a
200	0.0 c	13.0 b	60.0 a
C.V. (%)	10.00	29.84	14.13
	Aerial part length (cm $plant^{-1}$)		
0	4.3 a[1]	4.3 a	4.3 a
50	0.0 c	0.2 b	0.3 a
100	0.0 c	0.2 b	0.3 a
200	0.0 b	0.2 a	0.3 a
C.V. (%)	16.65	14.60	14.97
	Root length (cm $plant^{-1}$)		
0	14.1 a[1]	14.1 a	14.1 a
50	0.0 c	6.7 a	2.3 b
100	0.0 c	5.1 a	2.5 b
200	0.0 c	5.2 a	2.6 b
C.V. (%)	16.35	20.35	24.04

[1]Means followed by the same letter in line do not differ statistically among them by the Duncan test ($p \leq 0.05$).

value of GSI was obtained when the herbicide s-metolachlor was used, in comparison to the herbicides atrazine and diclosulam (Table 2). The use of the herbicide s-metolachlor on the 50 % dose reduced GSI in 27 % and 30 %, in relation to the herbicides atrazine and diclosulam, respectively. The GSI of the herbicide s-metolachlor on the 100 % dose differed statistically from the other herbicides on the same dose, which was 35 % inferior to that obtained with the use of atrazine and 38 % inferior to that obtained on the seeds where the herbicide diclosulam was used. With the raise in the dose of herbicides to

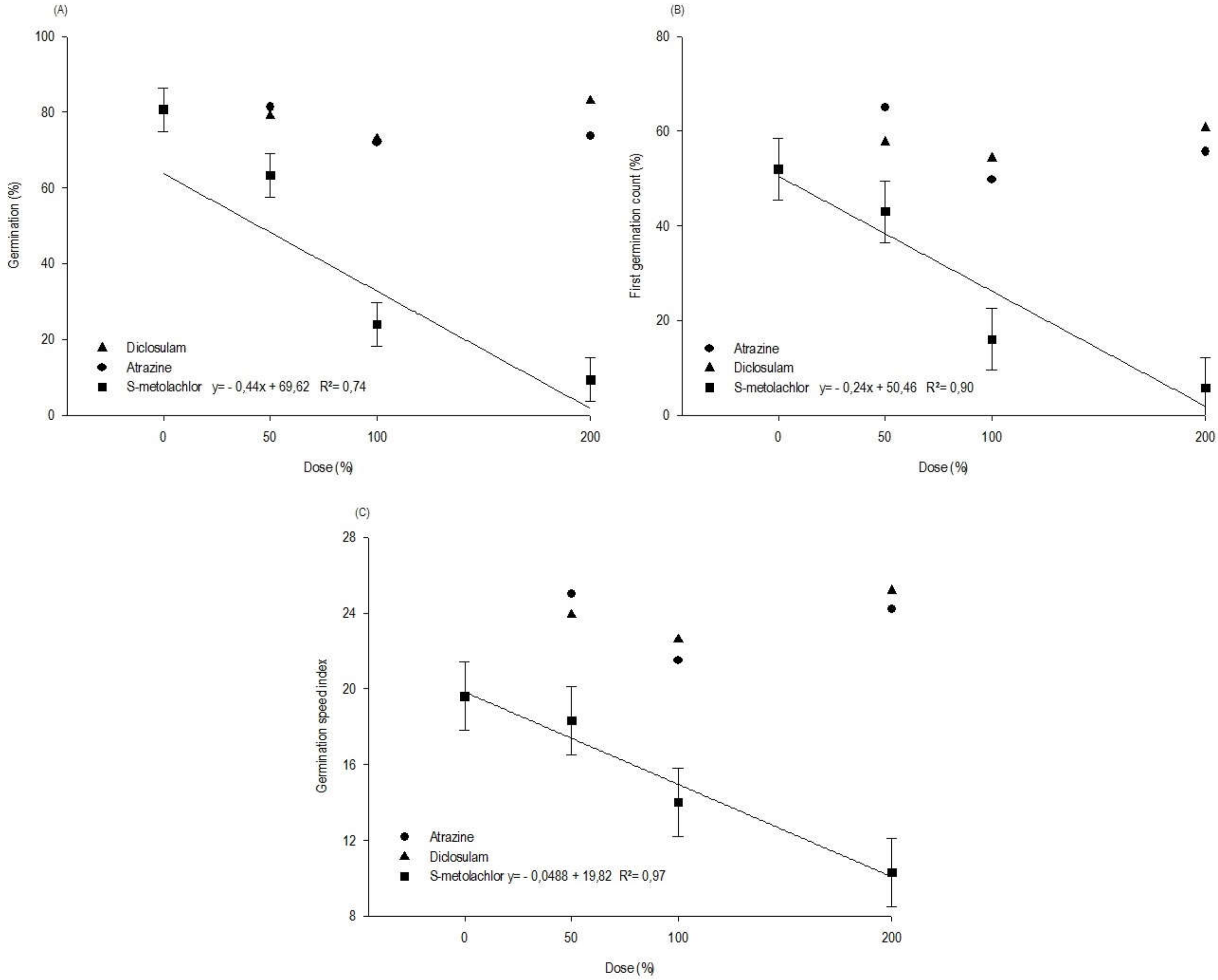

Figure 1. Germination (A), first germination count (B) and germination speed index (C) of white clover seeds under the effect of different doses of herbicides atrazine, s-metolachlor and diclosulam. FAEM/UFPel. Capão do Leão, 2013. *Points represent the mean of treatment and bars represent confidence intervals for dose effect in the herbicide

200 %, it was verified that the use of the herbicide s-metolachlor reduced the GSI of seedlings in 57 % and 59 %, in relation to the herbicides atrazine and diclosulam, respectively.

GSI values coming from the use of the herbicides atrazine and diclosulam were superior to those verified with the use of the herbicide s-metolachlor. The higher values of GSI for the herbicide atrazine may have occurred due to the symptoms and to the death of the plant, caused by this group of herbicides. This situation begins at the moment in which there is a block of the electron flow (Roman *et al*., 2007), therefore, there is a need for the plant to perform photosynthesis, so death can occur. Values of white clover seed germination when s-metolachlor herbicide was used were adjusted to the decreasing exponential equation, whereas values of germination for the herbicides atrazine and diclosulam did not present adjustment (Figure 1A). While evaluating the effect of the increase in the doses for each herbicide on seed germination, it was verified that the germination of the white clover seeds under the effect of the herbicide s-metolachlor on the dose of 200 % was reduced in 89 % when compared to the absence of the same herbicide.

The herbicide s-metolachlor belongs to the chemical group chloroacetanilide and acts on the terminal bud of plants, inhibiting growth on them, where their absorption happens through the coleoptiles in the monocots and through the hypocotyls in the dicotyledons (Syngenta, 2015). Germination reduction, caused by the increase in the dose of the herbicide s-metolachlor, may have possibly occurred due to the fact that it can inhibit the activity of β-ketoacyl-CoA synthase. This, in turn, is involved in the synthesis of fatty acids and lipids (Böger *et al*., 2000), which are formed by structural components of membranes and cell walls, reducing the growth of the embryonic axis and consequently slowing germination. A reduction in the germination of white clover seeds occurred due to the increase in the dose of the herbicide atrazine, which changed from 84 % in the zero dose to 74 % in the 200 % dose, representing a reduction of 12 % (Figure 1A). Atrazine blocks photosystem II electron transport, and the synthesis of ATP and NADPH in the chloroplast is impaired, hindering the synthesis of carbohydrates (Romanet *et al*., 2007) and favoring oxidative stress (Takano *et al*., 2008), which may have decreased the formation of normal seedlings at higher doses of the herbicide.

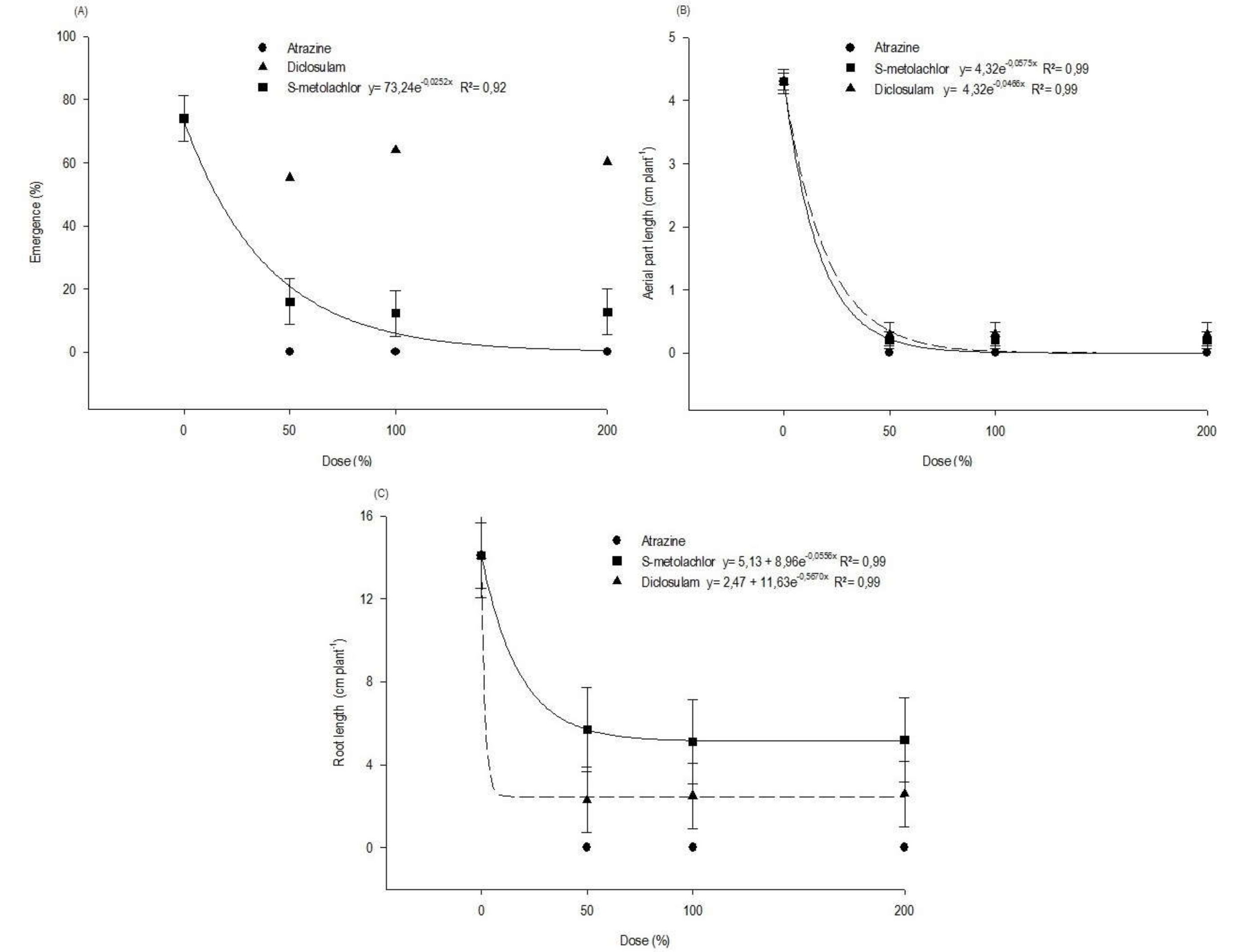

Figure 2. Emergence (A), aerial part length (B) and root (C) of white clover seedlings grown in greenhouse under the effect of herbicides atrazine, s-metolachlor and diclosulam. FAEM/UFPel. Capão do Leão, 2013. *Points represent the mean of treatment and bars represent confidence intervals for dose effect in the herbicide

On the first germination count (FGC), it was concluded that the values reached with the use of the herbicide s-metolachlor presented a decreasing exponential tendency, which did not occur with the values obtained when the herbicides atrazine and diclosulam were used (Figure 1B). The use of the highest dose of the herbicide s-metolachlor reduced in 89 % the number of normal plants on the first count of the germination test, when compared to the zero dose of the same herbicide.

On FGC, the use of the herbicides atrazine and diclosulam did not reduce the number of normal plants even with the increase of the dose (Figure 1B). The non-decrease in the number of normal seedlings on FGC when using the herbicide atrazine was possibly due to the fact that the symptoms of the application of the inhibitors of PS II, as it is for atrazine, and the death of the plant because of its application initiate at the moment when the block of the electron flow occurs (Roman *et al*., 2007), therefore, the plant needs to be performing photosynthesis. A reduction in the germination speed index (GSI) occurred with the increase of the dose of the herbicide s-metolachlor, where this changed from 19.6 on the zero dose, to 10.3 on the 200 % dose of the herbicide, which corresponds to a reduction of 47 % in the number of seedlings that initiated the germination process (Figure 1C). The GSI values, when using the herbicide s-metolachlor, djusted to the decreasing exponential model, whereas the GSI values coming from the use of atrazine and diclosulam did not adjust to this model (Figure 1C). Comparing the zero dose to the 200 % dose of the herbicide s-metolachlor, it was observed a reduction of 47 % in the number of seedlings that initiated the germination process. Regarding plant emergence, in the rest of the doses, the values observed in this variable differed in virtue of the use of different herbicides, where s-metolachlor provided intermediate values, whereas diclosulam and atrazine provided the highest and lowest values of seedling emergence on 50 %, 100 % and 200 %, respectively (Table 3). Thus, it is verified that diclosulam did not significantly affect the emergence, while atrazine reduced the emergence from the lowest dose used, and s-metolachlor inhibited seedling emergence in 100 % from the lowest dose.

The herbicide atrazine was the one that caused the highest reduction in the white clover seedling emergence, when compared to the other herbicide molecules tested, causing the death of 100 % of seedlings, regardless of the dose tested

(Table 3). Atrazine is applied in the pre-emergence stage, it is absorbed by the soil and reaches the leaves. It acts by inhibiting the transport of electrons in the photosynthetic electron transport chain by binding permanently in the place where Q_B reduction occurs, preventing Q_A re-oxidation (Takano *et al*., 2008). This process leads to photo-inhibition and photo-oxidation of the photosystem II (Ramel *et al*., 2009), increasing the production of oxygen reactive species, which leads to oxidative stress and damages to the cell membranes (Hugie *et al*., 2008). Furthermore, when the process of photosynthetic electron transfer is interrupted, the synthesis of ATP and NADPH in the chloroplast is impaired, hindering carbon reduction and the synthesis of other compounds, leading to plant death (Roman *et al.*, 2007).

Evaluating the effect of the increase in the dose of each herbicide on the emergence of the plants, it was found that the values of this variable were adjusted to the decreasing exponential model when using the herbicide s-metolachlor, which did not occur for the values of the herbicides atrazine and diclosulam (Figure 2A). For the herbicide atrazine, the increase from the zero dose to the 50 % dose reduced the emergence of white clover plants in 100 %, as well as for the other doses. For the herbicide s-metolachlor, the increase from the zero dose to the 200 % dose reduced seedling emergence in 84 %. For the herbicide diclosulam, the increase from the dose of 50 % to 200 % caused a reduction of 23 % in the white clover seedling emergence. On the doses of 50 %, 100 % and 200 %, the highest values of shoot length were verified when using the herbicide diclosulam, and on the same doses, the lowest values of shoot length of white clover seedlings were verified when the herbicide atrazine was applied. Shoot length, when using the herbicides diclosulam and s-metolachlor on the 200 % dose, did not present a statistically significant difference, however, with the same dose, the use of atrazine caused a reduction of 100% in this variable when compared to the other two herbicides evaluated. On the doses of 50 %, 100 % and 200 %, the use of the herbicide s-metolachlor provided the highest values of root length of white clover seedlings, whereas with the same doses, it was verified that the application of the herbicide diclosulam caused the lowest values of seedling length (Table 3). On the doses of 100 % and 200 %, the use of the herbicide diclosulam reduced in 50 % the values of root length in comparison to the use of s-metolachlor, on the same doses. On the doses of 50, 100 and 200 %, the application of the herbicide atrazine reduced in 100 % the values of the white clover root length when compared to the herbicides s-metolachlor and diclosulam.

The lowest growth of shoots in relation to the root zone in plants cultivated in the presence of s-metolachlor is due to the fact that the herbicide, which inhibits the meristems division, is absorbed mainly by the hypocotyl, affecting the apical meristem of the shoots with higher intensity than on the roots. On the other hand, for the plants that underwent the diclosulam treatment, their roots had lower growth by this inhibitory action on the acetolactate synthase (Johnson *et al*., 2012). This, in turn, is responsible for the synthesis of valine (branched-chain amino acids), leucine and isoleucine, which are essential for the cell wall expansion (Baumberger *et al*., 2001) and the process of intracellular signaling (Forsthoefel *et al*., 2005; Schnabel *et al*., 2005). The shoot length values of the white clover seedlings, when applications of the herbicides diclosulam and s-metolachlor were performed, adjusted to the decreasing exponential model, these being obtained with a high coefficient of determination (R^2=0.99), whereas the same values did not adjust to the model when applying atrazine (Figure 2B).

Increased doses of the herbicides s-metolachlor and diclosulam reduced the shoot length of white clover seedlings (Figure 2B). Considering the exponential value of the model, it was verified that the herbicide s-metolachlor reduced more intensely the variable when compared to diclosulam. The values of root length, when the herbicides diclosulam and s-metolachlor were applied, adjusted to the decreasing exponential model, where a high determination coefficient was observed (R^2=0.99), whereas the values of this variable did not adapt to this model when the herbicide atrazine was applied (Figure 2C). The application of the herbicide s-metolachlor on the 200 % dose reduced in 63 % the root length of white clover seedlings, in relation to the 0 % dose of the same herbicide. The use of the herbicide diclosulam on the 200 % dose caused a reduction of 82 % in the root length of white clover seedlings, in relation to the 0 % dose of the same herbicide. Regarding the exponential value of the model, it was verified that the use of diclosulam reduced more intensely the root length, when compared to s-metolachlor. On the test preformed in laboratory, the most severe damages were caused by the use of the herbicide s-metolachlor, due to the fact that it affected negatively the physiological quality of seeds, reducing the values of germination, first germination count and germination speed index. On the test performed in a greenhouse, all herbicides affected white clover seedlings; however, the application of atrazine provided most of the negative effects on these variables, causing a reduction in the emergence, shoot length and root length of white clover seedlings. Thus, most of the severe effects observed for atrazine in the test performed in greenhouse conditions can possibly be related to the activation of the photosynthetic process on seedlings of the species in this environment, and consequently, to the interruption of the electron flow among photosystems II and I in this stage, leading to a negative effect on seedling development.

Conclusions

The herbicide atrazine does not affect the physiological performance of white clover seeds; nonetheless, it affects negatively the growth of plants. S-metolachlor affects negatively the physiological performance of white clover seeds as well as seedling growth. Diclosulam does not affect the physiological performance of white clover seeds; however, it affects the roots and shoots of plants. The use of the herbicides atrazine, diclosulam and s-metolachlor is not feasible in this culture.

REFERENCES

Agostinetto, D.,Tironi S.P., Galon L., Dal Magro T**.** 2009. Desempenho de formulações e doses de glyphosate em soja transgênica. Revista *Trópica – Ciências Agrárias e Biológicas*, 3(2): 35-41.

Baumberger N., Ringli C., Keller B. 2001. The chimeric leucine-rich repeat/extensin cell wall protein LRX1 is required for root hair morphogenesis in Arabidopsis thaliana. *Genes & Development*, 15(9): 1128-1139.

Böger P., Matthes B.,Schmalfuss J. 2000. Towards the primary target of chloroacetamides–new findings pave the way. *Pest Management Science,* 56(6): 497-508.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. 2009. Regras para análise de sementes. *Brasília*: Mapa/ACS, 399 p.

Carambula M. 1981. Producción de semillas de plantas forrajeras. Ed. Hemisfério Sul. Uruguai, 518 p.

Carvalho P.C.F., Santos D.T Dos. ., Gonçalves E.N., Moraes a de., Nabinger C. 2010. Forrageiras de Clima Temperado. In: Fonseca DM da, Martuscello JA. Plantas Forrageiras. Viçosa: UFV, v. 1, pp. 494 - 537.

Correia n.m., Durigan j.c., 2010. Controle de plantas daninhas na cultura de soja resistente ao glyphosate. *Bragantia*, 69(2): 319-327.

Dall'agnol M., Paim N.R., Riboldi J. 1982. Cultivares e progênies de policruzamento de trevo branco consorciadas com gramíneas. *Pesquisa Agropecuária Brasileira,* 17(11): 1591-1598.

Fleck N.G., Bianchi M.A., Rizzardi M.A.,, Agostinetto D. 2006. Interferência de *Raphanus sativus* sobre cultivares de soja durante a fase vegetativa de desenvolvimento da cultura. Planta Daninha, 24(3): 425-434.

Formoso F. 2011. Producción de Semillas de Especies Forrajeras. Ed. INIA Serie Técnica N° 190. Montevidéu, Uruguai. 234 p.

Forsthoefel n.R., Cutler K., Port M.D., Yamamoto T., Vernon D.M., 2005. PIRLs: a novel class of plant intracellular leucine-rich repeat proteins. *Plant and Cell Physiology*, 46(6): 913-922.

Frame J., Newbould P**.** 1986. Agronomy of white clover. *Advances in Agronomy*, 40: 1-88.

Hugie J.A., Bollero G.A., Tranel P.J., Riechers D.E. 2008. Defining therate requirements for synergism between mesotrione and atrazine in red root pig weed (Amaranthus retroflexus). *Weed Science*, 56: 265–270.

INIA - Instituto Nacional de Investigación Agropecuaria. 1993. Producción de semillas forrajeras – tecnologias en uso. Montevideo – Uruguay: INIA, 40 p.

Johnson T.C., Mann R.K., Schmitzer P.R., Gast R.E. 2012. Acetohydroxyacid synthase inhibiting triazolopyrimidine herbicides. In: Lamberth C, DINGENS J. Bioactive Heterocyclic Compound Classes: Agrochemicals, 1ª Ed., Weinheim, Wiley-VCH, pp. 51-60.

Machado D., Lustosa S.B.C., Baldissera T.C., Turok J.D.N., Machado M., Watzlawick L.F., De Mendonça C.G; Pelissari A. 2013. Seletividade de herbicidas em trevo branco no estádio fenológico de expansão do primeiro trifólio. *Ciência Rural,* 43(12): 2132-2138.

Maguire J.D. 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Science,* 2: 176-177.

Peske S.T; Labbé L.M.B. 2012. Beneficiamento de Sementes. IN: Peske ST, Villela FA, Meneghello GE [Eds.]. Sementes: Fundamentos Científicos e Tecnológicos. Ed. Universitária UFPel, 3rd. Ed., Pelotas-RS.

Ramel F., Sulmon C., Bogard M., Couée I., Gouesbet G. 2009. Differential patterns of reactive oxygen species and antioxidative mechanisms during atrazine injury and sucrose-induced tolerance in Arabidopsis thaliana plantlets. *BMC Plant Biology,* 9(28): 28-45.

Rehman S., Harris P.J.C., Bourne W.F., Wilkin J. 1996. The effect of sodium chloride on germination and the potassium and calcium contents of Acacia seeds. *Seed Science and Technology,* 25(1): 45-57.

Roman E.S; Beckie H., Vargas L., Hall L., Rizzardi M.A.,, Wolf T.M**.** 2007. Como funcionam os herbicidas: da biologia à aplicação. Passo Fundo: Gráfica Editora Berthier, 160 p.

Santos M.E.R., Fonseca D.M da; Euclides V.P.B., Júnior J.I.R., Júnior D do N., Moreira l de M. 2009. Produção de bovinos em pastagens de capim-braquiária diferidas. *Revista Brasileira de Zootecnia,* 38(4): 635-642.

Schnabel E., Journet E.P., Carvalho-Niebel F.D.E., Duc G., Frugoli J**.** 2005. The *Medicago truncatula* SUNN gene encodes a CLV1-like leucine-rich repeat receptor kinase that regulates nodule number and root length. *Plant Molecular Biology,* 58(6): 809-822.

Silva G.M., Maia M.B., Maia M.S. 2011. Qualidade de Sementes Forrageiras de Clima Temperado. Bagé - RS. Embrapa Pecuária Sul, 36 p. Documentos, 119.

Takano A., Takahashi R., Suzuki H., Noguchi T. 2008. Herbicide effect on the hydrogen-bonding interaction of the primary quinone electron acceptor Q_A in photosystem II as studied by fourier transform infrared spectroscopy. *Photosynthesis Research,* 98(1-3): 159-167.

Soegaard K. 1994. Agronomy of white clover. Proceedings of the 15th General Meeting of the European Grassland Federation, Wageningen, The Netherlands. Proceedings…Wageningen Pers, pp. 515-524.

Syngenta – Syngenta Proteção de Cultivos Ltda**.** 2015. Acessado em 10 de Julho de 2015. Disponível em: http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Herbicidas/DUALGOLD.pdf

****