Viability of Italian ryegrass (*Lolium multiflorum*) seeds under application of synthetic auxin herbicides

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ABSTRACT

Italian ryegrass (*Lolium multiflorum*) is one of the main weeds in the wheat crop, and the application of synthetic auxin herbicides has the potential to reduce seed viability, therefore, restricting the input into the seed bank. The objective was to evaluate the effects of synthetic auxin herbicides applied at different growth stages on germination, emergence and initial growth of Italian ryegrass. The herbicides treatments were 2,4-D, 2,4-D+picloram and dicamba applied at four different growth stages of Italian ryegrass in field and seeds quality evaluated in laboratory and greenhouse studies. In both studies, the herbicides did not reduce Italian ryegrass seed germination or emergence regardless of application growth stages. Under laboratory conditions, the application of synthetic auxin herbicides increased vigor and germination speed by up to 64 and 47%, respectively, applied in advanced growth stages. The greenhouse test evidenced an increase in vigor by the herbicides application, but only 2,4-D increased the emergence speed and plant dry matter when applied in early plant stage. The synthetic auxin herbicides do not alter germination or emergence. Progenies treated with herbicides had greater seed vigor.

Keywords: vigor; emergence; germination; 2,4-D; picloram; dicamba.

INTRODUCTION

Italian ryegrass (*Lolium multiflorum*) is one of the main weeds in the wheat crop, and the critical period of interference prevention is between 11 and 21 days after crop emergence, with a potential yield reduction of up to 59% (Galon *et al.*, 2019). Similarly, failure to manage desiccation cause detrimental effects in soybean establishment (Oliveira *et al.*, 2014).

The development of crops resistant to synthetic auxin herbicides allows these herbicides to be applied in desiccation and post-emergence management. Similarly, 2,4-D is recommended in preplanting and post-emergence applications in wheat. Thus, Italian ryegrass can invariably be affected by these herbicides over a long period throughout the plant cycle.

Although Italian ryegrass is a species tolerant to synthetic auxin herbicides, its application on some species of the Poacea family has the potential to reduce germination and seed viability (Boutin *et al.*, 2014; Rinella *et al.*, 2013). Italian ryegrass plants have a high seed production capacity of up to 45,000 seeds m^{-2} (Bararpour *et al.*, 2017); therefore, management strategies aimed at reducing the seed bank of weeds in the soil are important to mitigate the effects of interference on crops (Schaeffer *et al.*, 2020).

Non-chemical approaches, through the mechanical destruction of Italian ryegrass seeds during harvest, have been proposed as a weed management strategy in the final crop cycle, in order to restrict the increment of the seed bank, which, in the case of ryegrass, can be more than 96% (Walsh *et al.*, 2018); however, this technology is not yet accessible to all producers. In this case, weed management strategies with selective and non-selective herbicides to make ryegrass seeds unfeasible has been a more accepted and practiced action aimed at reducing weed infestations (Schaeffer *et al.*, 2020).

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Thus, the application of synthetic auxin herbicides on Italian ryegrass plants can reduce the physiological quality of seeds and the initial growth of progeny plants. The aim of this study was to evaluate the effects of synthetic auxin herbicides applied at different growth stages on the germination, emergence, and initial growth of Italian ryegrass.

MATERIAL AND METHODS

A field trial was conducted during 2018, and laboratory and greenhouse trials during 2019 at Universidade Federal de Santa Maria, campus Frederico Westphalen RS. The soil was a Typical Dystrophic Red Latosol with a clay content of 60%, 3.2% organic matter, and pH 6.2. The mean temperatures and rainfall are described in Figure 1.

Field procedure

The field procedure was composed of the combination of the application of 2,4-D (806 g ha^{-1}), 2,4-D + picloram (406 g ha^{-1} + 103 g ha^{-1}), and dicamba (480 g ha^{-1}) at four growth stages of Italian ryegrass: tillering (21 – Zadoks scale), elongation (31), panicle emission (59), and flowering (65). A control not treated with herbicide was included. Plots were 1.5 × 1.5 m and randomly allocated in the field with four repetitions. The Italian ryegrass plants had natural infestation.

At the determined growth stages, the plants and stem were marked for seed collection and herbicides later applied with a CO_2-pressurized back-pack sprayer outfitted with a spray boom with two 11003 flat-fan nozzles spaced 50 cm apart and calibrated to deliver 150L ha^{-1}. At the time of herbicide application, the temperatures were 23, 25, 22, and 28 °C, and the relative humidity was 48, 52, 42, and 58% at growth stages of 21, 31, 59, and 65, respectively. The seeds were collected at the final cycle and stored in paper bags in a refrigerator at a temperature of 5 ºC for 70 days.

After the storage period, the seeds were separated manually and subjected to asepsis and disinfection through exposure to 70% alcohol for 5 minutes and 2% sodium hypochlorite for 20 minutes (Galvan *et al.*, 2015). Afterwards, they were washed in running water. Seeds were exposed to 0.2% potassium nitrate for 4 hours to break dormancy (Brasil, 2009).

Seed viability tests

Two trials were carried out to test seed viability in the laboratory and the greenhouse. The design used was completely randomized with four replications according to the treatments performed in the field procedure. In the laboratory, the experimental units were gerbox containing germitest paper moistened in a 2.5 weight portion of water/paper, in which 25 ryegrass seeds were distributed equidistantly (Galvan *et al.*, 2015). The gerbox were kept in a BOD-type chamber with a temperature of 25/15ºC and a photoperiod of 12:12 hours light/dark for 14 days (Brasil, 2009).

In the greenhouse, the experimental units were pots with a capacity of 5.5 L filled with PlantMax® substrate. In this trial, 16 ryegrass seeds were sown per pot and later covered with a thin layer of substrate. Irrigation was performed daily, and the mean temperature was 17.3 °C, ranging between 9 and 28ºC.

In both trials, seed germination and emergence were evaluated daily for up to 14 days, considering the protrusion of the radicle and coleoptile and/or the emergence of normal seedlings. The seed germination speed index (GSI) and emergence (ESI) were determined by a daily seed count. Seed vigor and germination/emergence percentage were estimated by counting normal seedlings on the seventh and fourteenth days after the installation of the tests, respectively, and expressed as a percentage. In the greenhouse trial, the plant dry matter

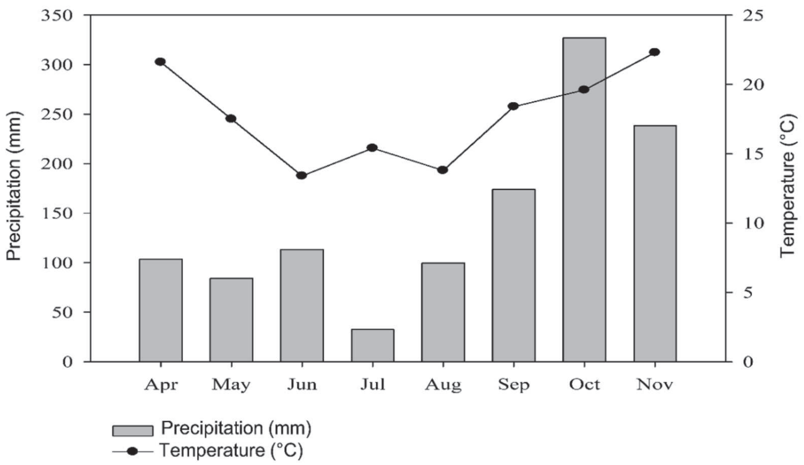

Figure 1: Average temperatures and accumulated rainfall during field experiment.

(DM) were determined 28 days after sowing. The aboveground plants were cut and placed in paper bags, which were placed in a forced ventilation oven at a temperature of 65ºC for 72 hours and subsequently weighed on a precision scale. Seedling DM was expressed in mg pot^{-1}.

Statistical analysis

The data were tested for normality and homogeneity of variances, which required emergency data transformations, but these did not improve the normality of the data. The data were submitted to ANOVA with $p < 0.05$ for the main effects and $p < 0.07$ for the interactions. Herbicides and, growth stages factors, and interactions were compared using the Scott Knott test ($p < 0.05$), and the Dunnett test ($p < 0.05$) was used to compare treatments with the untreated control. Germination and emergence data accumulated as a function of time were analyzed using the four-parameter nonlinear logistic regression model.

$$y = y0 + a/(1 + (x/x0)^b)$$

Where *y* is variable response, *a* and *y0* are the maximum and minimum asymptotes, respectively, *x* is the number of days, *x0* indicates the number of days for which the mean y value is between the values of *a* and *y0*, and *b* indicates the slope of the curve close to *x0*. The number of days taken to reach the accumulated 50% (t50) of ryegrass germination/emergence was estimated by the logistic equation.

Potential correlations between seed quality parameters (vigor, ESI, and emergence) and DM accumulation as a function of the application of synthetic auxin herbicides at different growth stages were investigated using path analysis for the greenhouse trial. First, a correlation matrix was obtained for each set of characteristics, in which the degree of multicollinearity was tested and verified by the Variance Inflation Factor (VIF) and the relation of condition numbers (CN) between the eigenvalues, which presented the values of VIF = 2 and CN = 73. Standardized regression coefficients were estimated for a single model analyzed for each herbicide. Analyses were performed using the RBio software.

RESULTS AND DISCUSSION

Laboratory test

Herbicides applied at different growth stages did not produce any symptoms, visible changes in development, or significant delays in seed maturation in the field.

The application of auxinic herbicides did not cause harmful effects on germination, vigor, and the germination speed index (GSI) of Italian ryegrass seeds, regardless of the growth stage of application (Table 1). While germination was not influenced by any of the factors tested, seed vigor was increased when the herbicides were applied in late growth stages. The application of 2,4-D at stages 31, 59, and 65 increased seed vigor compared with stage 21 and the control. The application of 2,4-D+picloram at stage 65 caused a significant increase in vigor in relation to the control, but it did not differ in stages 21 and 59. The application of dicamba in stages 59 and 65 increased seed vigor compared to untreated control. In general, the synthetic auxin herbicides applied in late growth stages of Italian ryegrass increased the seeds vigor between 40% and 64%.

From growth stage 31 onwards, application of 2,4-D caused the largest increases in ryegrass vigor among the herbicides, but similar to the effects of dicamba applied at growth stage 59 (Table 1). The vigor results are similar to those of t50 and GSI, in which 2,4-D and dicamba accelerate t50 in more than 2 days when applied from stages 31 and 59, respectively (Figure 2). Although the application of 2,4-D+picloram did not show differences between the growth stages and the untreated control, the t50 was reduced by 1 day in relation to the control. These results corroborate those found by Bobadilla *et al.* (2020), who verified that the application of 2,4-D and dicamba in the boot and anthesis stage of *Lolium perene* increased the germination speed of the progeny seeds.

Greenhouse test

The application of synthetic auxins in the different growth stages of Italian ryegrass has no effect on seedling emergence in the greenhouse (Table 2). However, the application of 2,4-D and 2,4-D+picloram generated an increase in seed vigor when applied in the initial growth stage, with a decrease in seed vigor through the cycle plant. The application of dicamba in late growth stages (59 and 65) increased seed vigor in relation to the control; however, it did not differ between the other growth stages.

The application of 2,4-D+picloram and dicamba at stages 59 and 21, respectively, caused less vigor in the seeds between the growth stages (Table 2). The ESI response to the application of herbicides at different growth stages was similar to vigor, but only the application of 2,4-D at stage 21 differed from the control. Applications of 2,4-D alone or mixed with picloram at stage 21 reduced ryegrass t50 by up to 1.5 days (Table 2; Figure 3). However, the application of 2,4-D+picloram at stage 59 increased the t50 by more than two days compared with the control. The application of dicamba reduced the t50 when applied in the more advanced growth stages, by about a day.

Some contrasting results were found between laboratory and greenhouse studies. Thus, it can be deduced that the effects of the interaction of herbicides, growth stages and the environment can change the dynamics of seed establishment (Cechin *et al.*, 2021). For example, application of 2,4-D at stage 21 did not change vigor and GSI compared with the control in the laboratory study. However, in the greenhouse, there was an increase in vigor and ESI in relation to the control and application in the other stages (Tables 1 and 2; Figures 2 and 3). Another case observed was regarding the vigor response in relation to 2,4-D+picloram in the greenhouse, which was greater than the control when applied at stage 21, and in the laboratory, it was greater when applied at stage 65. Contrasting results were also found by Ball (2014), who found differences in the reduction efficiency of *Bromus tectorum* seeds by the application of synthetic auxins in the different studies conducted. In this sense, further studies are needed to assess the responses of progeny under the effects of herbicides under different environmental conditions.

The applications of 2,4-D+picloram or dicamba did not influence the DM of Italian ryegrass plants, regardless of the growth stage of application (Table 3). However, the DM response in relation to the application of 2,4-D was dependent on the growth stage. While the effects of applying 2,4-D in stages 31 and 65 did not change the DM compared to the control, an increase in DM of 40% when applied in stage 59 and a reduction of 47% when applied in stage 21 was verified, in relation to the control.

Path analysis of seed quality parameters in the DM accumulation of Italian ryegrass seedlings indicated different responses for each herbicide, especially regarding

Table 1: Effects of application of synthetic auxin herbicides at different growth stages of Italian ryegrass on germination (%), vigor (%), time required (days) for germination of 50% of seeds (t50) and germination speed index (GSI)

Growth stage[1]	Germination (%)			Vigor (%)		
	2,4-D	2,4-D + picloram	Dicamba	2,4-D	2,4-D + picloram	Dicamba
Control	94	94	94	54	54	54
21	ns 93	ns 93	ns 90	ns 62 Ab	ns 66 Aa	ns 52 Ab
31	ns 96	ns 88	ns 95	*80 Aa	ns 52 Cb	ns 67 Ba
59	ns 96	ns 94	ns 96	*85 Aa	ns 67 Ba	*82 Aa
65	ns 99	ns 92	ns 94	*89 Aa	*76 Ba	*75 Ba
VC (%)		1.8			3.2	
		t50			GSI	
Control	6.7 (0.43)	6.7 (0.43)	6.7 (0.43)	19	19	19
21	5.8 (0.31)	6.0 (0.11)	6.9 (0.33)	ns 21 Ab	ns 21 Aa	ns 19 Ab
31	4.5 (0.14)	6.8 (0.23)	5.8 (0.15)	*27 Aa	ns 19 Ba	ns 21 Bb
59	4.6 (0.08)	5.7 (0.19)	4.4 (0.10)	*28 Aa	ns 22 Ba	*28 Aa
65	4.6 (0.06)	5.4 (0.12)	5.0 (0.13)	*28 Aa	ns 23 Ba	*24 Ba
VC (%)					7.55	

[1]Tillering (21 – Zadoks scale), elongation (31), panicle emission (59) and flowering (65). Averages followed by capital letters compare herbicides within each growth stage; and lowercase letters compare growth stages for each herbicide by the Scott-Knott test ($p > 0.05$). * or ns indicates difference or not differences between treatments and control without herbicide application by Dunnett's test ($p > 0.05$). Values of t50 obtained by the logistic equation $y = y0 + a/(1 + (x/x0)^b)$. VC and values in parentheses indicate variation coefficient and standard error, respectively.

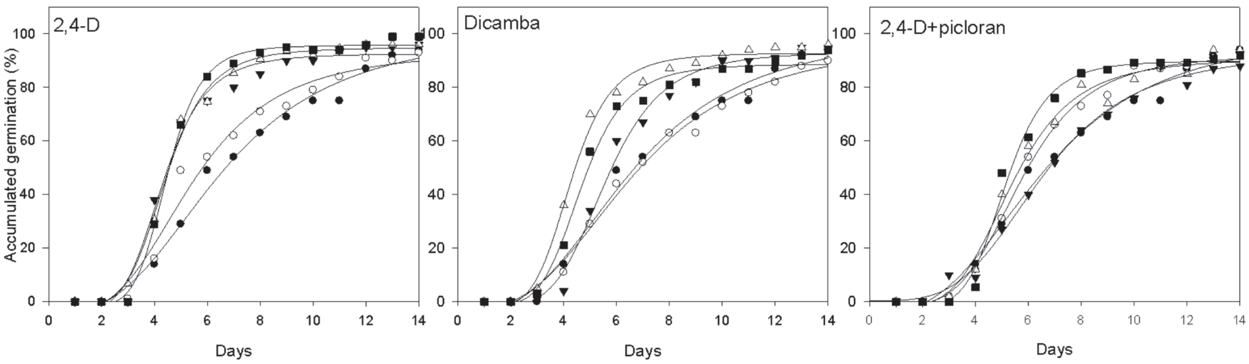

Figure 2: Effects of application of synthetic auxin herbicides at different growth stages of Italian ryegrass on accumulated germination. (●) control, (○) Tillering (▼), elongation (31), (▲) panicle emission (59) and (■) flowering (65). Numbers in parentheses represent Italian ryegrass growth stage on the Zadoks scale.

direct effects on DM (Table 4). The emergence and vigor seed were strongly correlated with ESI, but there was no direct or indirect relationship between emergence and DM for any herbicide. The variables ESI and vigor had a higher positive correlation with the DM of ryegrass seedlings, with ESI being more important for 2,4-D and vigor for 2,4-D+picloram. There was no direct and significant correlation between seed quality and DM for dicamba application at different growth stages.

The results found in this study indicate that synthetic auxin herbicides applied at different growth stages of Italian ryegrass can positively impact vigor, germination/emergence speed, and DM accumulation in plants. These results are similar to those found with the use of wheat seed treatment with auxins before sowing (seed priming), which can increase viability, standardize emergence, and modulate seedling growth processes (Karimi *et al.,* 2021; Kosakiska *et al.*, 2021; Rhaman *et al.*, 2021).

According to literature reports, the effects of the application of synthetic auxin herbicides on the physiological quality of the progeny seeds depend on the species, growth stage, herbicide, and study environment. In Poacea's species, such as the *Lolium perene*, Bobadilla *et al*. (2020) did not verify deleterious effects of the application of 2,4-D and dicamba on seed viability in either field or greenhouse studies, but when aminopyralide was applied in the anthesis, it reduced seed viability by up to 55%, while the anticipation of application in boot caused a

Table 2: Effects of application of synthetic auxin herbicides at different growth stages of Italian ryegrass on emergence (%), vigor (%), time required for emergence of 50% of seeds (t50) and emergence speed index (ESI)

Growth stage[1]	Emergence (%)			Vigor (%)		
	2,4-D	2,4-D + picloram	Dicamba	2,4-D	2,4-D + picloram	Dicamba
Control	70	70	70	42	42	42
21	ns 89	ns 81	ns 71	*78 Aa	*79 Aa	ns 50 Ba
31	ns 81	ns 67	ns 70	ns 67 Aa	ns 56 Ab	ns 43 Aa
59	ns 75	ns 67	ns 83	ns 48 Ab	ns 28 Bc	*67 Aa
65	ns 77	ns 67	ns 77	ns 50 Ab	ns 56 Ab	*65 Aa
VC		2.3			3.6	
		t50			ESI	
Control	7.4 (0,07)	7.4 (0.07)	7.4 (0.07)	8	8	8
21	5.9 (0,05)	6.1 (0.06)	7.3 (0.11)	*13 Aa	ns 12 Aa	ns 9 Ba
31	6.4 (0,10)	6.6 (0.10)	7.6 (0.13)	ns 11 Aa	ns 10 Ab	ns 8 Aa
59	7.6 (0,27)	9.6 (0.33)	6.4 (0.13)	ns 9 Bb	ns 7 Bb	ns 12 Aa
65	7.5 (0,32)	6.8 (0.05)	6.5 (0.07)	ns 9 Ab	ns 9 Ab	ns 11 Aa
VC					17.6	

[1]Tillering (21 - Zadoks Scale), elongation (31), panicle emission (59) and flowering (65). Averages followed by capital letters compare herbicides within each growth stage; and lowercase letters compare growth stages for each herbicide by the Scott-Knott test (p > 0.05). * or ns indicates difference or not differences between treatments and control without herbicide application by Dunnett's test (p > 0.05). VC and values in parentheses indicate variation coefficient and standard error, respectively.

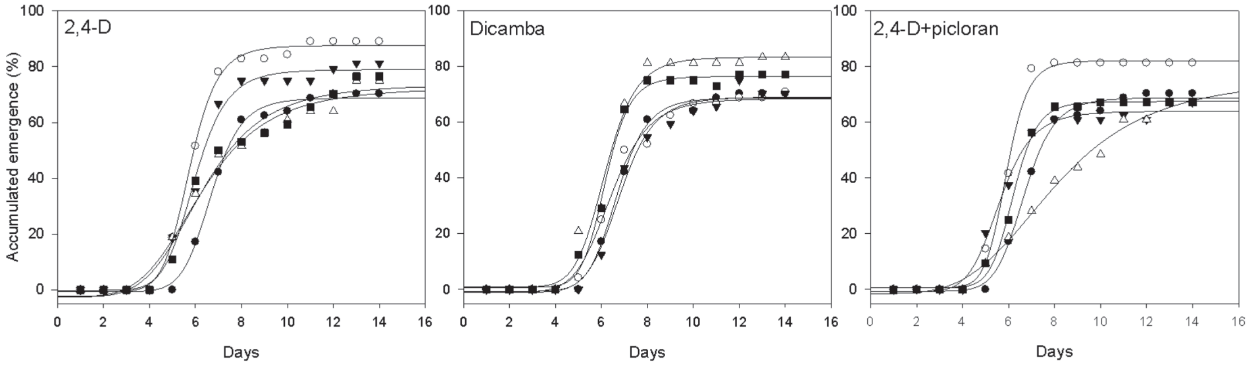

Figure 3: Effects of application of synthetic auxin herbicides at different Italian ryegrass growth stages of accumulated emergence. (●) Control, (○) tillering (21), (▼) elongation (31), (▲) panicle emission (59) and (■) flowering (65). Numbers in parentheses represent Italian ryegrass growth stage on the Zadoks scale.

reduction of 46%. Similarly, the application of 2,4-D and growth regulators such as ethephon and trinexapac-ethyl in the inflorescence emergence stage did not affect the viability, vigor, or germination of *Lolium multiflorum* seeds (Schaeffer *et al.,* 2020). According to the authors, when 2,4-D, ethephon and trinexapac-ethyl are applied at the seed development stage, it increases the viability of *Lolium multiflorum* seeds.

Under field conditions, the application of aminopyralid and picloram from the beginning of elongation stage until the spike emergence of *Bromus japonicus* reduced seed germination by more than 95%, while the application of aminopyralid or aminocyclopyrachlor at the beginning of panicle emergence of *Bromus tectorum* reduced germination by 100% (Rinella *et al.*, 2010a; Ball, 2014). Similar results in the greenhouse were found for application of dicamba and picloram, while 2,4-D had less consistent effects on *Bromus japonicus* germination reduction (Rinella *et al.*, 2010b). While greenhouse studies indicated that picloram was not effective in reducing seed viability in *Bromus tectorum* (Rinella *et al.,* 2013).

The difference in results between hormonal herbicides may be due to the difference between auxin receptors that are involved in the mechanism that affects seed viability (Bobadilla *et al.,* 2020). More studies must be carried out with other hormonal herbicides to verify the applicability in reducing the viability of ryegrass seeds and to enable the use of this method on commercial crops.

Non-selective herbicides, such as glyphosate, clethodim, iodosulfuron, paraquat, and glufosinate, show important results in reducing the viability, vigor, germination, and seed production of *Lolium multiflorum* and therefore have the potential to reduce the increase in the soil seed bank (Schaeffer *et al.,* 2020; Ulguim *et al.,* 2019). However, in Brazil, there are reports of ryegrass biotypes that are resistant to EPSPs, ALS, and ACCase inhibiting herbicides, as well as the ban on the use of paraquat, causing chemical control alternatives to be reduced (Heap, 2021). Thus, strategies that reduce the quantity and viability of herbicide-resistant weed seeds are a way to reduce the impact of resistance on crops (Bararpour *et al.,* 2020).

The greenhouse test revealed that the application of 2,4-D or 2,4-D+picloram in the tillering stage or dicamba in the panicle emission stage to flowering can increase the vigor and speed of emergence, which can result in plants with greater accumulation of initial DM, which may outcome in competitive advantages in relation to crops. In this sense, Schaeffer *et al.* (2020) recommend the use of non-selective herbicides to reduce the seeds production that will reach the soil and reduce the physiological quality of these seeds.

From the point of view of ryegrass management, the higher speed of plant establishment caused by the application of synthetic auxins can be beneficial for management with pre- or post-emergence herbicides by shortening the emergence window and by standardizing

Table 3: Effects of application of synthetic auxin herbicides at different growth stages of Italian ryegrass on dry matter at 28 days after sowing

Growth stage[1]	**Dry mass (mg pot^{-1})**		
	2,4-D	**2,4-D + picloram**	**Dicamba**
Control	1398	1398	1398
21	*1961 Aa	^{ns} 2032 Aa	^{ns} 948 Ba
31	^{ns}1061 Bc	^{ns} 1546 Aa	^{ns} 1081 Ba
59	*740 Ac	^{ns} 928 Ab	^{ns} 913 Aa
65	^{ns}1462 Ab	^{ns} 2082 Aa	^{ns} 1507 Aa
VC (%)		29.8	

[1]Tillering (21 – Zadoks scale), elongation (31), panicle emission (59) and flowering (65). Averages followed by capital letters compare herbicides within each growth stage; and lowercase letters compare growth stages for each herbicide by the Scott-Knott test ($p > 0.05$). * or ^{ns} indicates difference or not differences between treatments and control without herbicide application by Dunnett's test ($p > 0.05$). VC indicate variation coefficient.

Table 4: Standardized regression coefficients for path analysis of the contribution of vigor, ESI and emergence of Italian ryegrass seeds on dry matter subjected to application of synthetic auxin herbicides at different growth stages

Herbicides	**Standardized**		**Regression Coefficients**	
	Eme → ESI	**Vigor → DM**	**Vigor → ESI**	**ESI → DM**
2,4-D	0.74*	0.05*	0.97*	0.52*
2,4-D+picloram	0.77*	0.92*	0.97*	0.07*
Dicamba	0.90*	- 0.37^{ns}	0.90*	0.98^{ns}

* or ^{ns} indicates significance or not significance of standardized regression coefficients ($p < 0.05$), respectively. Eme= emergence at 14 days after sowing, ESI= emergency speed index, DM= dry matter at 28 days after sowing.

the plants. It is noteworthy that the effects of herbicides on the dormancy of these seeds were not evaluated, and in this sense, new studies should be conducted to assess the impacts of herbicides on the dormancy of ryegrass seeds.

CONCLUSION

The application of herbicides from the synthetic auxin group does not cause harmful effects on the germination or emergence of Italian ryegrass seeds, regardless of the growth stage of application.

Increases in the vigor and speed of establishment of Italian ryegrass seedlings are correlated with greater accumulation of initial plant dry matter and are dependent on the herbicide and application growth stage.

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