

ORIGINAL ARTICLE

## Competition between ryegrass and annual bluegrass due to the establishment time and the shoots and roots strata

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### Abstract

Ryegrass (*Lolium multiflorum* Lam.) is an important forage and suffers negative interference from weeds, like annual bluegrass (*Poa annua* L.). The competition of annual bluegrass with ryegrass can interfere with crop growth and reduce the amount of forage for animal feed. We aimed to evaluate the interference of annual bluegrass in ryegrass crops through the differentiation of niche and establishment. Two experiments were carried out testing different competition strata (shoot, root, and both) and ryegrass establishment with or after annual bluegrass, with increasing populations of annual bluegrass (0, 35, 139, 279, 419 plants m<sup>-2</sup>). The variables were plant height, height length of the root system, stem diameter, and fresh weight of shoot and root systems of ryegrass. The negative interference of growing populations of annual bluegrass occurred when in competition for the shoot and root systems of ryegrass. The fresh weight of shoots and roots were impacted when competing for the same strata. Ryegrass, established after the population of annual bluegrass, showed less competitive capacity. The negative interference of annual bluegrass in ryegrass is greater when the plants fully compete for the resources of the environment, and when the ryegrass establishes after the competitor.

**Keywords:** *Lolium multiflorum*, neighborhood studies, *Poa annua*

## Introduction

Competition between crops and weeds is one of the factors that can limit growth, development and yield (Oliveira Jr. *et al.* 2011). It can be established between different species (interspecific), plants of the same species (intraspecific) or between parts of the same plant (intraplant), and is primarily competition for resources such as light, water, nutrients and CO<sub>2</sub> (Yamauti *et al.* 2011; Gerhards and Schappert 2020). Weeds can also negatively interfere with forage grass production, reducing the biomass production capacity of these species (Annicchiarico and Proietti 2010). In this case, ryegrass (*Lolium multiflorum* Lam.) is one of the forage crops used in the winter growing season under the subtropical conditions present in southern Brazil.

Ryegrass is a winter pasture grass belonging to the Poaceae family. It has a cosmopolitan distribution, an annual development lifecycle, and adapts to almost all types of soil. It is able to tolerate edaphoclimatic conditions of excessive soil humidity and cold temperatures (Correia *et al.* 2013). Therefore, it is widely used in the crop-livestock integration system in southern Brazil, mainly for the production of hay, seeds, nutrient cycling capacity and forage (Mittelmann *et al.* 2010). Weed management is essential to maintain productivity in winter pastures. *Poa annua* L. (annual bluegrass), a grass weed which makes management difficult in ryegrass pastures, stands out among weed species, mainly due to the selectivity of herbicides.

Annual bluegrass is a poacea with an annual development lifecycle, with subspecies and biotypes that can be perennial and it can infest areas of lawns and gardens (Kaminski and Dernoeden 2007; Diehl *et al.* 2021). Also, cases of herbicide resistance have been seen around the world mainly to enolpyruvyl shikimate phosphate synthase (EPSPs), acetolactate synthase (ALS) and acetyl CoA carboxylase (ACCase) inhibitors (Heap 2022). Currently, according to recent reports from farmers in southern Brazil, a rapid occupation and development of annual bluegrass in winter forage crop production areas has been seen. These characteristics allow annual bluegrass to establish itself in winter pasture production areas, which may reduce forage biomass production. Therefore, one of the basic principles for planning weed management practices is the recognition of the competitive potential between species (Garrison *et al.* 2014). Therefore, the dynamics of competition between crops and weeds must be understood, especially in the case of two species of the same family such as annual bluegrass and ryegrass.

Among the methodologies of competition between crop and weed studies, the neighborhood method can be mentioned, where the performance of a target-individual is registered as a function of number, biomass, soil coverage, aggregation or distance from its neighbors (Swanton *et al.* 2015). The target species is cultivated alone or is surrounded by individuals of the neighboring species, whose effect of the neighboring species on the target species is defined by the slope of the regression of the performance of the latter as the number of neighbors increases (Radosevich *et al.* 2007). The advantages of this method include the speed in obtaining the results and no preliminary studies are needed for its realization.

The recognition of interference relationships between ryegrass and annual bluegrass is extremely important in understanding the potential for damage and, consequently, helping in the recommendation of management measures. We hypothesized that competition between the weed and the crop is greater when the two strata (shoot and root) are in competition and when the crop emerges after the weed. Therefore, we aimed to evaluate the interference of annual bluegrass in the ryegrass crop through the differentiation of niche and time of establishment.

## Materials and Methods

The studies were conducted in a greenhouse in the city of Santa Maria, Rio Grande do Sul, from August to November 2019. Two experiments were carried out in a completely randomized design with five replications. The experiments were carried out using the

neighborhood method to evaluate the competition between crops and weeds (Radosevich *et al.* 2007), between annual bluegrass and ryegrass. Ryegrass was considered to be the target species and the population of annual bluegrass was varied by experimental unit. The experimental units consisted of polypropylene pots with a capacity of 8 kg, filled with a sifted red arctic argisole soil, with 1.6% organic matter, 13% clay and texture classified as 4. The fertility was corrected according to the soil analysis and water was supplied daily according to soil moisture. The annual bluegrass plants used in the experiments came from a production area in the city of Dilermando de Aguiar – RS (29°50'26.2" S; 54°01'50.9" W).

The first experiment was carried out to verify the competition potential of the aforementioned species for the shoot and root system resources. Thus, an experiment was carried out in a 3 × 5 factorial scheme, whose A factor tested different strata of competition between annual bluegrass and ryegrass, such as competition in the shoot, competition in the root system and competition in the shoot and root systems. Factor B, in turn, tested different populations of annual bluegrass competing with a single ryegrass plant, whose populations tested were: 0, 2, 8, 16, 24 pot<sup>-1</sup> plants (equivalent to 0, 35, 139, 279, 419 plants · m<sup>-2</sup>). The collected annual bluegrass plants were then transferred to pots 1 day after collection. The ryegrass plants used in the experiment came from seeds and were transplanted to the experimental units at the same time as the weeds. It should be noted that the collected annual bluegrass and ryegrass plants were transplanted to experimental units at the same stage, that is, two leaves, to ensure similar conditions for competition.

To establish the different competition strata in treatments with competition for shoots, the root of the target species (ryegrass) was isolated using a disposable plastic cup containing 0.8 kg of soil with corrected fertility. Thus, competition for soil resources between annual bluegrass and ryegrass plants was prevented, occurring only in the shoot part. To assess competition for the root system, the shoot of ryegrass was isolated for annual bluegrass plants through a polyvinyl chloride (PVC) barrier, allowing the species to compete for soil resources. Additionally, for the shoot and root system competition conditions, no apparatus was included, thus promoting a complete competition for shoot and root systems.

The second experiment was carried out to evaluate the impact of the establishment period of the target species (ryegrass) in relation to annual bluegrass. Thus, the experiment was carried out in a 2 × 5 factorial scheme, in which factor A tested times of ryegrass establishment, whether together or after annual bluegrass; while factor B tested populations of the last species, as mentioned in the previous experiment. To

establish the levels of factor A, different forms of establishment of the ryegrass target species were carried out. Therefore, in the case of the simultaneous establishment of the two species, the methodology followed that mentioned in the first experiment, in which two species that were in the two-leaf development stage were transplanted. To establish ryegrass after annual bluegrass, the weed plants were transplanted into pots and ryegrass was sowed. Thus, the emergence of ryegrass occurred when the annual bluegrass plants had an average of six leaves.

Fifty days after the beginning of the experiments, evaluations were carried out for the variables of shoot height (cm), root length (cm), stem diameter (mm), number of tillers, and fresh weight of shoots and roots (g) of the target species (ryegrass). Plant height and root length assessments were performed with the aid of a ruler graduated in millimeters. The stem diameter was assessed using a digital caliper, while the number of tillers was obtained through direct counting. To evaluate the fresh weight of the shoot and of the root, the plants were collected and the root system was washed. Later they were weighed in a precision scale.

In all studies, the variables were subjected to analysis of variance, normality (Shapiro-Wilk) and homogeneity (O'Neill-Matthews). The levels of factor A were differentiated by calculating the confidence interval of the means, and for factor B, the complementary analysis was performed by quadratic regression (Equation 1):

$$Y = a + bx^2 + cx,$$

where:  $Y$  – the response variable,  $x$  – the population of the annual bluegrass,  $a$  – the intercept,  $b$  and  $c$  – slope parameters of the regression. The maximum point (MP) was also calculated in each regression, as the maximum response point found in the quadratic regression response. For statistical analysis and for graphs, the R software (R Core Team 2020) and the packages ExpDes.pt (Ferreira *et al.* 2014) and ggplot2 were used.

## Results

For the first experiment, a statistical interaction was observed between the factors for the following variables: root length, stem diameter and fresh weight of shoot and root. For the number of tillers, there was only a simple effect for the annual bluegrass population factor, while for the plant height variable, no significance was observed by the analysis of variance (data not shown). For the second experiment, there was an interaction between the factors for all variables. It was considered that, overall, the data adequately fit the quadratic polynomial equation, based on the

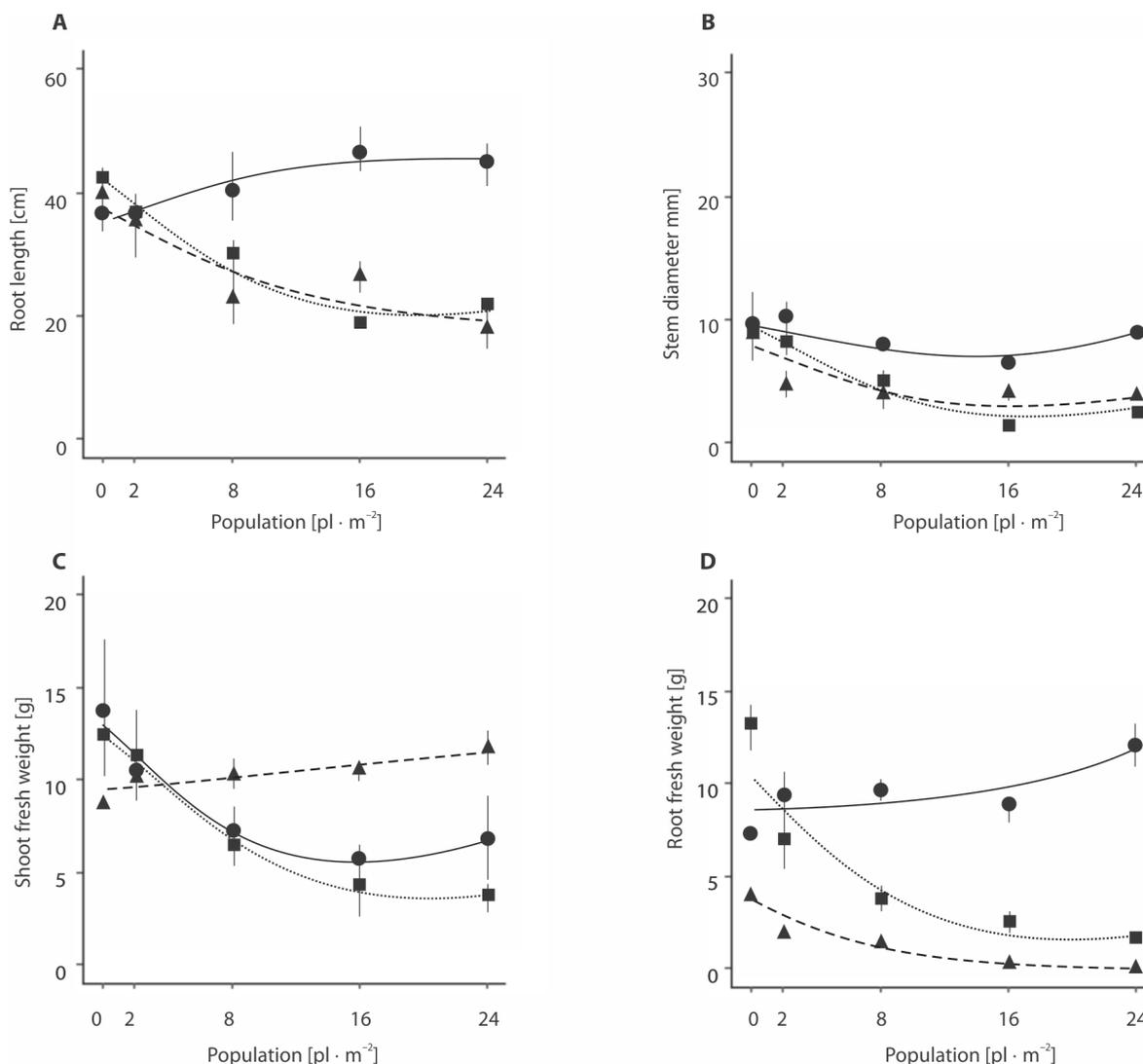
significance of the parameters (data not shown) and on the values of the coefficient of determination ( $R^2$ ) in relation to the other models tested. It is noteworthy that the quadratic behavior of the regressions in each variable is commonly found in neighborhood experiments, showing a peak response in certain populations (Radosevich *et al.* 2007). Therefore, as a measure of comparison, the parameters of the equation related to parameters  $a$ ,  $b$  and  $c$  will be used to differentiate each other, in addition to the maximum response point (MP) which represents the peak of the quadratic regression.

The root length variable stood out, where there was only interaction for this factor level and with parameter  $b$  of  $-2.19$  and a MP of  $21.90$  (Table 1, Fig. 1A). As for the stem diameter variable, the competition of shoot and root systems showed a parameter  $b$  of  $-1.74$  and MP of  $17.41$ , while the other strata of competition in roots and shoots had a parameter  $b$  of  $-1.07$  and  $-0.66$ , with a curve peak of  $17.83$  and  $16.51$ , respectively.

In the comparison of the different ryegrass competition strata with annual bluegrass populations, it can be highlighted that the stem diameter and the fresh weight of roots and shoots were more impacted when the competition between the plants occurred for the root system compared to the competition for the shoot part (Table 1, Fig. 1). This inference is justified by the smaller values of angular coefficient  $b$  for the condition of competition for the root system, and as a function of the slope behavior of the curves.

The reduction in the fresh weight of ryegrass roots when competing with the annual bluegrass root system may be due to the occupation of soil space by the competitor, evidencing the reduction of the root system of the target species (Fig. 1D). However, it is noteworthy that the length of ryegrass roots in all of the strata competition did not show such behavior due to the non-adjustment of regression in the variable. There was a negative effect on shoot growth of plants of ryegrass when the species competed only for shoot strata. In this case, it was found that the fresh weight of roots of ryegrass plants was significantly reduced by an increase in the population of the weed (Fig. 1D).

Another important variable regarding the growth of the shoots of plants in response to the light resource is the number of tillers. However, for this variable, a simple effect of the *Poa annua* plant population factor was observed (Table 2, Fig. 2). The observed behavior followed the reduction in the number of emitted tillers as the competitor's population increased (Radosevich *et al.* 2007), regardless of the competition stratum. It is noteworthy that no negative effects on the fresh weight of shoots of ryegrass were observed when competing with the annual bluegrass root system (Fig. 1D). Likewise, the fresh weight of the root system of ryegrass plants competing for the shoot of the competitor was not negatively affected (Fig. 1D).



**Fig. 1.** Root length (A), stem diameter (B), shoot fresh weight (C), root fresh weight (D) of ryegrass plants competing with the root system (▲), shoot (●) and whole plant (■) of annual bluegrass plant populations

**Table 1.** Quadratic regression parameters and maximum curve point (MP) for root length, number of tillers, stem diameter and shoot and root fresh weight of ryegrass plants in competition for different strata with increasing populations of annual bluegrass

Treatment	<i>a</i>	<i>b</i>	<i>c</i>	<i>R</i> <sup>2</sup>	MP
Root length					
Root competition			ns		
Shoot competition			ns		
SC + RC	43.95	-2.19	0.05	0.96	21.90
Number of tillers					
No interaction	9.7	-0.61	0.01	0.99	30.50
Stem diameter					
Root competition	13.98	-1.07	0.03	0.87	17.83
Shoot competition	13.71	-0.66	0.02	0.58	16.51
SC + RC	18.56	-1.74	0.05	0.87	17.41
Shoot fresh weight					
Root competition	9.99	0.07	>0.01	0.18	35.00
Shoot competition	17.95	-1.62	0.05	0.83	16.20
SC + RC	25.74	-2.83	0.08	0.84	17.69

ns – non-significant adjustment; SC – shoot competition; RC – root competition

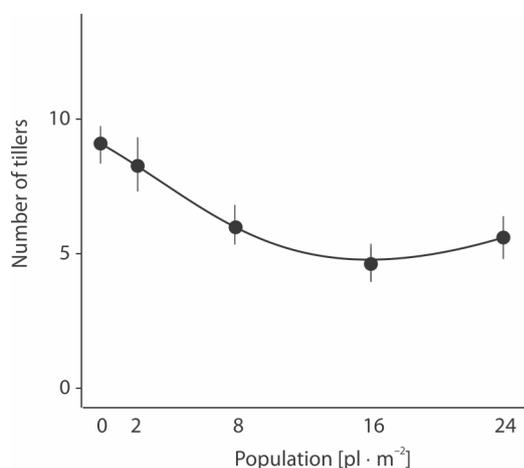
**Table 1.** Quadratic regression parameters and maximum curve point (MP) for root length, number of tillers, stem diameter and shoot and root fresh weight of ryegrass plants in competition for different strata with increasing populations of annual bluegrass – continuation

Treatment	<i>a</i>	<i>b</i>	<i>c</i>	<i>R</i> <sup>2</sup>	MP
Root fresh weight					
Root competition	3.68	-0.32	0.01	0.87	16.00
Shoot competition	8.72	0.02	0.01	0.63	-1.00
SC + RC	16.49	-1.89	0.05	0.74	18.90

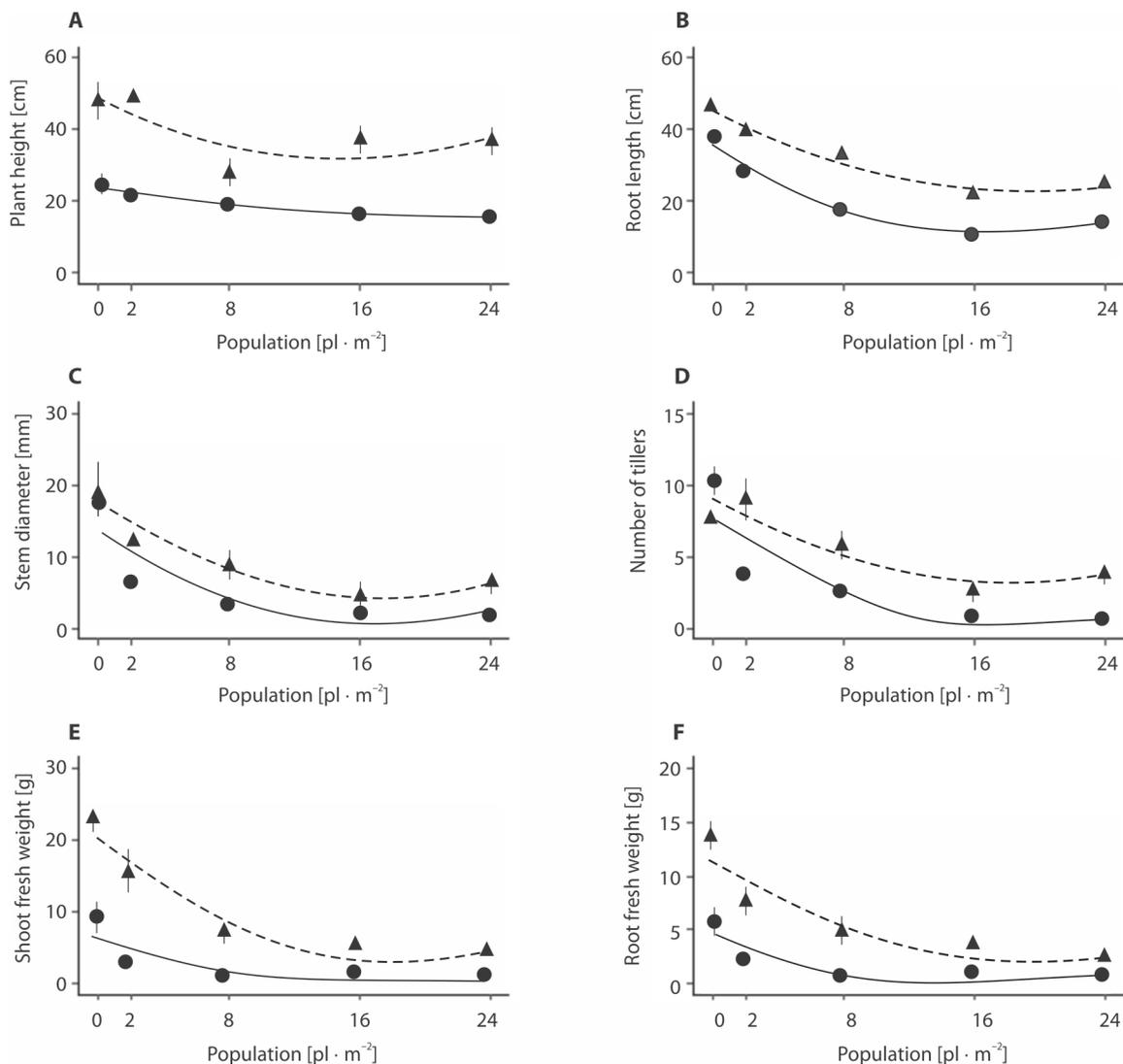
ns – non-significant adjustment; SC – shoot competition; RC – root competition

**Table 2.** Quadratic regression parameters and maximum curve point (MP) for plant height, root length, number of tillers, stem diameter and shoot and root fresh weights of ryegrass plants established at different times and competing with growing populations of annual bluegrass

Treatment (establishment)	<i>a</i>	<i>b</i>	<i>c</i>	<i>R</i> <sup>2</sup>	MP
Plant height					
After	22.28	-0.62	0.01	0.43	31.00
Together	51.16	-2.96	0.1	0.75	14.80
Root length					
After	43.95	-2.26	0.05	0.84	22.60
Together	34.84	-2.99	0.08	0.87	18.69
Number of tillers					
After	7.94	-0.91	0.02	0.73	22.75
Together	10.24	-0.83	0.02	0.47	20.75
Stem diameter					
After	13.62	-1.67	0.04	0.65	20.88
Together	18.56	-1.74	0.05	0.6	17.40
Root fresh weight					
After	6.41	-0.92	0.02	0.53	23.00
Together	25.74	-2.83	0.08	0.85	17.69
Shoot fresh weight					
After	4.51	-0.64	0.02	0.61	16.00
Together	16.49	-1.89	0.05	0.75	18.90

**Fig. 2.** Number of ryegrass plant tillers competing with growing populations of annual bluegrass

For experiment 2, for all variables, there was a harmful effect on the development of ryegrass, when the establishment was after annual bluegrass. This inference was mainly evidenced by the lower values of the angular coefficients and the intercept (Table 2), in addition to significant reductions in the variables analyzed, according to the polynomial regression used (Fig. 3). For the ryegrass height variable (Fig. 3A), we observed a higher intercept value for establishment with the weed (51.16) than after (22.28). Similar behavior was observed for the tiller number variables (Fig. 3C), stem diameter (Fig. 3D), fresh weight of shoots (Fig. 3E) and roots (Fig. 3F). As for the variable of root length, there was a larger intercept for the establishment of ryegrass after annual bluegrass.



**Fig. 3.** Plant height (A), root length (B), stem diameter (C), number of tillers (D) and fresh weight of shoot (E), and roots (F) of ryegrass plants established together (▲) and after (●) annual bluegrass establishment

The MP values for when ryegrass was established with the weed were lower than when established later, except for the variable of fresh weight of the root (Fig. 3F). This indicates that the population of greatest damage was always greater when the ryegrass was established next to the ryegrass than after it. It should be noted that these intercept value variations may be related to the delay in the establishment of the target species, since its emergence took place after the transplanting of the competing species and the sowing of ryegrass in the establishment treatment together.

It was observed that for the number of tillers and fresh weight of shoots and roots of ryegrass of plants established after, from the population of eight plants of annual bluegrass pot<sup>-1</sup>, there were values close to zero and significantly different from the conditions of establishment next to the species. In this sense, when ryegrass emerged in an environment already occupied by annual bluegrass, it did not show enough competitive ability to overcome the weed suppression.

## Discussion

For the first experiment, based on the parameters of the equation found on all variables, it can be considered that for the conditions of competition for the root system and shoots, ryegrass plants had greater competitive damage, given the negative behavior of the angular coefficient and curves, indicating a decrease in tested variables. Overall, the results showed that the competition of annual bluegrass with ryegrass plants was more intense when there was an interaction with the shoot and root systems, as evidenced by the higher values of parameter *b* of the equations. On the other hand, for the stem diameter and fresh weight of shoots and roots these results may be due to the lower absorption of water and nutrients by the ryegrass root system. In most cases, competition in roots generally presents a greater reduction in biomass than competition only in shoots, especially when competition occurs in Poaceae (Kjær *et al.* 2013).

For the shoots strata competition these results can be explained by the variation in incident light in the different plant canopies formed by the increase in the competitor's population, promoting differences in its partitioning and uptake by the target species (Craine and Dybzinski 2013). This phenomenon can change the quality of light available to the canopy, that is, low quality solar radiation is reflected horizontally by the plants and acts as a sign of the presence of neighbors, providing the plant's suitability for future competition (Ballaré and Casal 2000). Thus, both the quality and quantity of light are related as the first environmental resource that modifies the competitive relationships between crops and weeds (Afifi and Swanton 2012), promoting the responses observed in this study for the fresh weight of shoots. Overall, in experiment 1 what we found indicates a compensatory effect on ryegrass growth as a function of the competition stratum, represented by the increase in the response of the variable directly proportional to the increase in the competitor's population and by the positive coefficient  $b$  for such situations.

For experiment 2 in similar work an increase in the growth capacity of *Agrostis capillaris* L. was observed, with no relation to annual bluegrass, due to the increase in the density of the first species (Espevig *et al.* 2014), raising the difficulty of establishment in a space previously occupied by annual bluegrass. These results can be indicative for the management of the species in forage production areas where it occurs. Weed species management practices must be designed to avoid the late establishment of ryegrass in detriment to annual bluegrass growth. It should be noted, however, that the competitive response of annual bluegrass with perennial ryegrass (*Lolium perenne* L.) may have a different effect than that observed in the present study. The increase in perennial ryegrass density in areas with high infestation of annual bluegrass showed less ground cover by the competitor plant (Sousek and Reicher 2019).

Another relevant factor may be the choice of perennial ryegrass genotypes to be used in the field, given that differences were observed between perennial ryegrass cultivars in their ability to suppress the emergence of annual bluegrass plants (Masin and Macolino 2016). This inference may be based on distinct morphological characteristics between different genotypes that affect factors involved in the germination process, such as light penetration in the canopy and soil temperature (Masin and Macolino 2016). These characteristics are highly important for the competitive ability of any crop against weeds such as plant height, deeper and more prolific roots, and tillering capacity (Dass *et al.* 2017).

Another important factor that we must take into account when interpreting the results is the possibility of allelopathic effects in the interaction between these

two species which is a characteristic that determines the dynamics of plant species in different environments (Trezzi *et al.* 2016). However, in a study evaluating ryegrass as a weed, no correlation was found between the release of allelopathic compounds and the competitive capacity of wheat (Worthington *et al.* 2015). Meanwhile, ryegrass was the target species (as a forage crop) and even though it is a species with allelopathic potential, we did not find negative allelopathic interference in the literature with the interaction with annual bluegrass. Furthermore, we believe that because the two species are from the same botanical family and occupy similar niches, competition is the main ecological interaction (Radosevich *et al.* 2007).

Overall, the establishment of ryegrass should be avoided after the weed, given that there are negative implications for the growth of the species, and even when the species emerge consecutively, the competition established between the root system and the aerial part can reduce forage growth. Also, it is of a great importance to know several management strategies, because the control of species of the same family is highly complex. Therefore, this high competition between these species can be explained by the fact that both occupy similar ecophysiological niches, since they are species of the same botanical family.

## Conclusions

All the results we found in the work support our hypothesis.

- 1) The negative interference of annual bluegrass in ryegrass is greater when the plants fully compete for the resources of the environment, that is, for the shoot and root systems. No niche preference was seen for shoot or soil resources.
- 2) There is a detrimental effect on ryegrass growth as well as less competitive ability when it is established after annual bluegrass. This demonstrates the importance of establishing ryegrass without the presence of weeds, especially annual bluegrass.

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