PLANTA DANINHA

SBCPD
SOCIEDADE BRASILEIRA DA
CIÊNCIA DAS PLANTAS DANINHAS
<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Fitness Cost and Competitive Ability to Different Ploidy Levels in Ryegrass Genotypes

Custo Adaptativo e Habilidade Competitiva de Genótipos de Azevém com Diferentes Níveis de Ploidia

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ABSTRACT - The intergenotypic competition of tetraploid ryegrass with natural diploid population may be a tool to reduce the frequency of resistant individuals in an area. This study aimed to identify and compare the phenological development, fitness cost, and competitive ability between diploid and tetraploid ryegrass genotypes. Genotypes were grown in pots, and the morphological variables of genotype growth were assessed every 15 days up to 120 days after emergence to evaluate the fitness cost. Phenological development and seed yield components were measured in a single time together with the fitness cost. Competitive ability was determined in a replacement-series experiment with proportions of tetraploid and diploid ryegrass, in which the number of tillers, plant height, leaf area, and shoot dry weight were assessed at 50 days after emergence. The results of fitness cost showed that the number of tillers, leaf area, root dry weight, and the number of seeds were higher for tetraploid ryegrass, which presented a higher competitive ability than the diploid genotype regardless of the tested proportions. Tetraploid ryegrass may be useful for reducing the frequency of herbicide-resistant diploid ryegrass because of its higher competitive potential.

Keywords: *Lolium multiflorum*, competition, diploid, tetraploid, integrated weed management.

RESUMO - A competição intergenotípica de azevém tetraploide com a população natural diploide pode ser uma ferramenta para reduzir a frequência de indivíduos resistentes em uma área. O objetivo deste estudo foi identificar e comparar o desenvolvimento fenológico, custo adaptativo e habilidade competitiva entre genótipos de azevém diploide e tetraploide. Para avaliar o custo adaptativo, os genótipos foram cultivados em vasos, e as variáveis morfológicas de crescimento dos genótipos, avaliadas a cada 15 dias até os 120 dias após a emergência. O desenvolvimento fenológico e os componentes de produtividade das sementes foram mensurados em época única, em conjunto com o custo adaptativo. A habilidade competitiva foi determinada em experimento de série de substituição com proporções de azevém tetraploide e diploide, em que o número de afilhos, estatura, área foliar e massa seca da parte aérea foram avaliados aos 50 dias após a emergência. Os resultados de custo adaptativo demonstraram que o número de afilhos, a área foliar, a massa seca de raiz e o número de sementes foram superiores para o azevém tetraploide e que este apresenta maior habilidade competitiva comparativamente ao diploide, independente das proporções testadas. O azevém tetraploide, por apresentar maior potencial competitivo, pode ser útil para redução da frequência de azevém diploide resistente a herbicidas.

Palavras-chave: *Lolium multiflorum*, competição, diploide, tetraploide, manejo integrado de plantas daninhas.

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Received: June 27, 2017
Approved: November 28, 2017

Planta Daninha 2019; v37:e019181921

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Doi: 10.1590/S0100-83582019370100115

INTRODUCTION

Lolium multiflorum (ryegrass) is the main winter grazing crop that composes the agricultural systems in the South region of Brazil due to its bromatological qualities, low production costs, and natural potential of soil seed bank replenishment (Aguinaga et al., 2008). This species is considered an important weed of winter cereals crops because of its persistence in the seed bank and heterogeneous germination flows, which hinder the adoption of crop rotation and efficient tools to weed management, interfering with the crops development and leading to crops yield losses (Tironi et al., 2014).

Chemical control is considered the main management tool for ryegrass due to its practicality, efficiency, and lower cost when compared to other management tools (Côrrea et al., 2014). However, continued and repeated use of herbicides with the same site of action has led to the selection of resistant biotypes. Currently, in Brazil, there are ryegrass biotypes with simple and multiple resistance to 5-enolpyruvyl-3-phosphate synthase (EPSPs), acetyl-coenzyme A carboxylase (ACCase), and acetolactate synthase (ALS) (Heap, 2017) herbicides inhibitors. The resistance to these sites of action hinders the adoption of chemical management due to the lack of selective post-emergence herbicides to winter cereals. Thus, it is necessary to search for management alternatives that may contribute to reducing the ryegrass seed bank and its adverse effects on crops.

The increasing use of tetraploid ryegrass as a forage crop may be an alternative to herbicide-resistant diploid ryegrass since tetraploid cultivars are more aggressive and have a higher ability to occupy the ecological niche than diploid ryegrass (Gilliland et al. 2011). Moreover, tetraploid ryegrass has a longer phenological development, higher dry weight production, and better bromatological quality compared to diploid species (Gilliland et al., 2007). Fitness cost and competitive ability can be changed with chromosome duplication due to the alteration of gene expression patterns of genes responsible for cell growth, development and biosynthesis of secondary metabolites of stress response (Zhou et al. al., 2015).

The comparison between ryegrass genotypes through fitness cost although not originated from the same place of origin, is a valuable tool to understand the growth, survival, and seeds yield of genotypes as a function of the use of environmental resources. Moreover, studies of competitive ability between genotypes of different ploidy levels allow developing management strategies to avoid the spread of herbicide resistance ryegrass biotypes as a function of differences between growth and phenological development of the desired genotype. This study aimed to identify and compare the phenological development, fitness cost, and competitive ability between diploid and tetraploid ryegrass genotypes.

MATERIAL AND METHODS

The experiments were carried out from May to December 2016 in a greenhouse of the Center of Herbology (CEHERB/FAEM/UFPel). The treatments were arranged in a completely randomized design with four replications. Each experimental unit consisted of plastic pots filled with a sandy loam textured Red-Yellow Argisol belonging to the mapping unit of Pelotas, RS, with pH and fertility previously corrected according to soil analysis based on the recommendations of fertilization and liming of cold season grass forages (CQFS RS/SC, 2004). Ryegrass genotypes used in the experiments were diploid (wild type) from Passo Fundo (geographical coordinates $28^{o}13'49''$ S and $52^{o}24'23''$ W), and tetraploid (cv. INIA TITAN®).

Phenological development of genotypes

The Bleiholder et al. (1991) scale was used in order to determine the time interval necessary for ryegrass genotypes to reach the phenological stages, as follows: sowing (SO), emergence (EM), first leaf through coleoptile (FC), tillering (TI), elongation (EL), heading (HE), flowering (FL), watery grain (WG), milky grain (MG), dough grain (DG), and fully ripe (FR). Each predetermined phenological stage was considered when 50% or more of the plants had the same phenotypic characteristics.

Fitness cost

In this experiment, each experimental unit was composed of a ryegrass plant placed in a polyethylene pot with a diameter of 23 cm and a volume of 6 dm^3. Treatments were arranged in a factorial scheme (2 × 8), in which factor A consisted of diploid (wild type) and tetraploid (cv. INIA TITAN®) ryegrass genotypes and factor B included eight collection times, i.e., at 15, 30, 45, 60, 75, 90, 105, and 120 days after emergence (DAE).

The analyzed growth variables at each time were plant height (PH), number of tillers (NT), leaf area (LA), shoot dry weight (SDW), root dry weight (RDW), leaf area index (LAI), leaf area ratio (LAR), and absolute growth rate (AGR). LA was determined with a leaf area meter (LICOR 3100C), while PH was measured using a millimeter ruler from the ground level to the plant apex, with the leaf blade extended. SDW and RDW were obtained by drying shoot and root samples, respectively, in a forced air circulation oven at 60 °C for 72 hours and weighing on an analytical balance, with values expressed as g per plant. LAI expresses the ratio between the total leaf area per unit soil area, indicating the available surface for interception and absorption of light. LAR, in $cm^2\ g^{-1}$, represents the ratio of LA and SDW, showing the leaf area available for photosynthesis obtained by the equation $LAR = (LA_1 + LA_2)/(SDW_1 + SDW_2)$, where LA_1 and LA_2 and SDW_1 and SDW_2 are, respectively, LA and SDW variation between two collection intervals. AGR, in g day^{-1}, measures the average growth rate in a given time, obtained by the equation: $AGR = (SDW_2 - SDW_1)/(T_1 - T_2)$, where SDW_1 and SDW_2 are SDW variation in two consecutive samples taken at times T_1 and T_2 (Magalhães, 1979).

Yield components assessed at physiological maturity were the number of spikes per plant (NSP), number of seeds per spike (NSS), and number of spikelets per spike (SS), obtained by counting in ten spikes harvested at random; number of filled seeds per spikelet (NFS) and number of seeds per plant (NS), obtained by counting filled seeds in ten spikes multiplied by NSP; seed weight per spike (SWS), obtained by determining the average seed weight assessed in ten spikes; and one thousand-seed weight (TSW), estimated by measuring the weight of eight subsamples of 100 seeds.

The data were analyzed for normality (Shapiro-Wilk test) and then submitted to analysis of variance ($p \leq 0.05$). In the case of statistical significance, a regression analysis was performed for the factor assessment times for all variables using the non-linear sigmoidal model: $y = a/(1 + e^{(-(x-x0)/b)})$, where y is the response variable, x are the days after emergence, and a, $x0$, and b are equation parameters, in which a is the difference between the maximum and minimum points of the curve, $x0$ are the days that provide 50% of response of the variable, and b is the curve slope. For yield components of genotypes, the means were compared for each response variable using the t-test ($p \leq 0.05$).

Competitive ability

For this study, additive and replacement-series experiments were carried out, in which each experimental unit was composed of pots with a volume of 4 dm^3 and diameter of 19 cm.

Monoculture (additive series) of ryegrass genotypes (diploid and tetraploid) were set up in increasing populations of 1, 2, 4, 8, 16, 32, 64, and 128 plants per pot (equivalent to 35, 70, 140, 280, 560, 1120, 2240, and 4480 plants m^{-2}). SDW was measured at 50 DAE, in which the data were analyzed by the reciprocal production method to provide the moment when SDW per unit area (g m^{-2}) becomes constant and not dependent of the population. The results in the average of genotypes showed that SDW was constant and not dependent of the population in 1693 plants m^{-2}, equivalent to 48 plants pot^{-1} (data not shown).

A replacement-series experiment was carried out from the population obtained per pot in additive series, which the genotypes were maintained under monoculture or associated with variable proportions. The used mixtures were 100:0 (tetraploid ryegrass monoculture), 75:25, 50:50, 25:75, and 0:100 (diploid ryegrass monoculture). Genotypes were previously sown in trays and equidistantly transplanted at 4 DAE, with the assessment of LA, PH, NT, and SDW of all plants of the experimental unit at 50 DAE.

The graphical analysis methodology proposed for replacement-series experiments was used in the analysis of LA, PH, NT, and SDW (Roush et al., 1989; Cousens, 1991; Radosevich et al., 2007). It consists of constructing diagrams based on relative yield (RY) and relative yield total (RYT) for each proportion and variable-response. RY of each genotype was calculated dividing by the mean of association by the mean of monoculture, and RYT was obtained by adding up RYs in the respective proportions of plants (Hoffman and Buhler, 2002). In this case, the competition effects are verified based on theoretical straight lines drawn between the maximum and minimum RY (100 to 0%) and between 100% points for RYT. If RY is equal to a theoretical straight line, it shows the absence of interference between genotypes.

On the other hand, if RY results in a concave line, it is characterized a loss in growth of one or both genotypes; if RY presents as a convex line, it shows a benefit in the growth of one or both genotypes. When RYT shows a line equal to the theoretical straight line, there is a competition for the same resources; when RYT is higher than the theoretical straight line, forming a convex line, there is no competition for resources of the niche; and when RYT is lower than the theoretical straight line, forming a concave line, an antagonism occurs, i.e., the growth of both genotypes was impaired (Cousens, 1991). Resources refer to consumable environmental factors that interfere with plant growth, such as radiation, CO_2, water, nutrients, and oxygen (Radosevich et al., 2007).

Competitive ratio (CR), relative crowding coefficient (K), and aggressivity (A) were calculated in the proportion of 50% of plants of each genotype (Hoffman and Buhler, 2002). CR represents the comparative growth between genotypes, K indicates the relative dominance of one genotype over another, and A shows the most competitive genotype (Cousens, 1991), in which tetraploid genotype (a) is more competitive than diploid (b), when $CR > 1$, $Ka > Kb$ and $C > 0$, and vice versa.

To perform the statistical analysis of the relative yield, the difference was initially calculated for RY values, i.e., the relative yield differences (RYD), obtained in proportions of 25, 50, and 75% of plants in relation to values belonging to the hypothetical straight lines in the respective proportions. Subsequently, the t-test was performed at 5% probability to verify differences in the RYD, RYT, CR, K, and A indices (Roush et al., 1989; Hoffman and Buhler, 2002). The null hypothesis ($Ho = 0$) to test the differences of RYD and C and for RYT and CR were considered equal to 1 ($Ho = 1$); for K, the means of differences between Ka and Kb were considered null [$Ho = (Ka – Kb) = 0$]. The standard used to consider whether RY and RYT curves were different from theoretical straight lines was that, in at least two proportions, a significant difference occurred by the t-test (Bianchi et al., 2006).

The mean results obtained for the variables LA, DW, PH, and NT were evaluated for normality (Shapiro-Wilk test) and then submitted to analysis of variance ($p \leq 0.05$). When statistical significance was found for LA, DW, PH, and NT, means of treatments were compared by the Dunnett test ($p \leq 0.05$), with monoculture being the standard treatment.

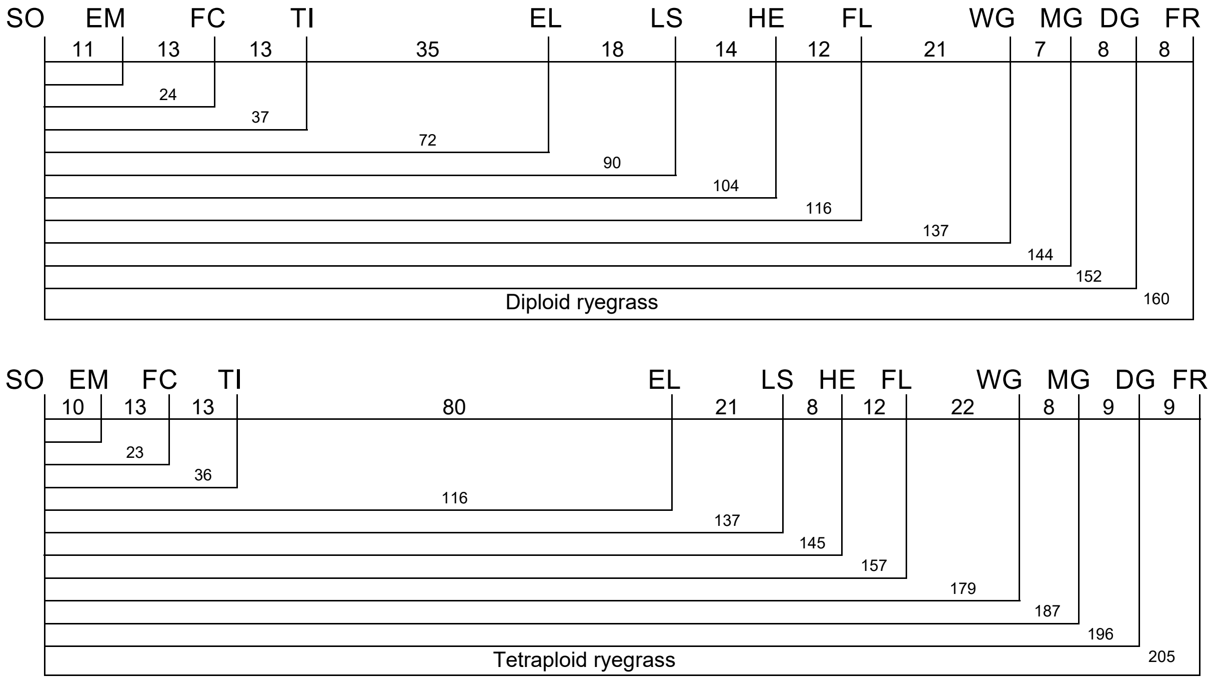
RESULTS AND DISCUSSION

This topic is presented following the sequence of activities detailed in the Material and Methods section.

Phenological development of genotypes

The length in days of ryegrass phenological stages, i.e., the period from emergence to physiological maturity, differed between genotypes. Diploid ryegrass completed its phenological development in 160 days, while the tetraploid required 205 days (Figure 1). Tillering was the main phenological stage accountable for this difference between genotypes, in which tetraploid ryegrass needed 35 days longer than the diploid to complete this stage. The longest vegetative period of the tetraploid genotype is the result of genetic improvement, which aimed to lengthen the grazing period when the bromatological forage quality is higher (Tonetto et al., 2011).

In the interaction of plants in an environment with limited resources, the cycle is one of the factors that interfere with the competition for resources and with the efficiency of their use for growth. In this sense, late-cycle cultivars tend to be more efficient in resource use when in



Sowing (SO); emergence (EM); first leaf through coleoptile (FC); tillering (TI); elongation (EL); leaf sheath flag begins to stretch (LS); heading (HE); flowering (FL); watery grain (WG); milky grain (MG); dough grain (DG); fully ripe (FR).

Figure 1 **-** Length, in days, of phenological stages of diploid (wild type) and tetraploid (cv. INIA TITAN®) ryegrass based on the phenological scale adapted from Bleiholder et al. (1991).

competition when compared to early-cycle cultivars, resulting in lower yield losses (Balbinot Jr. et al., 2003).

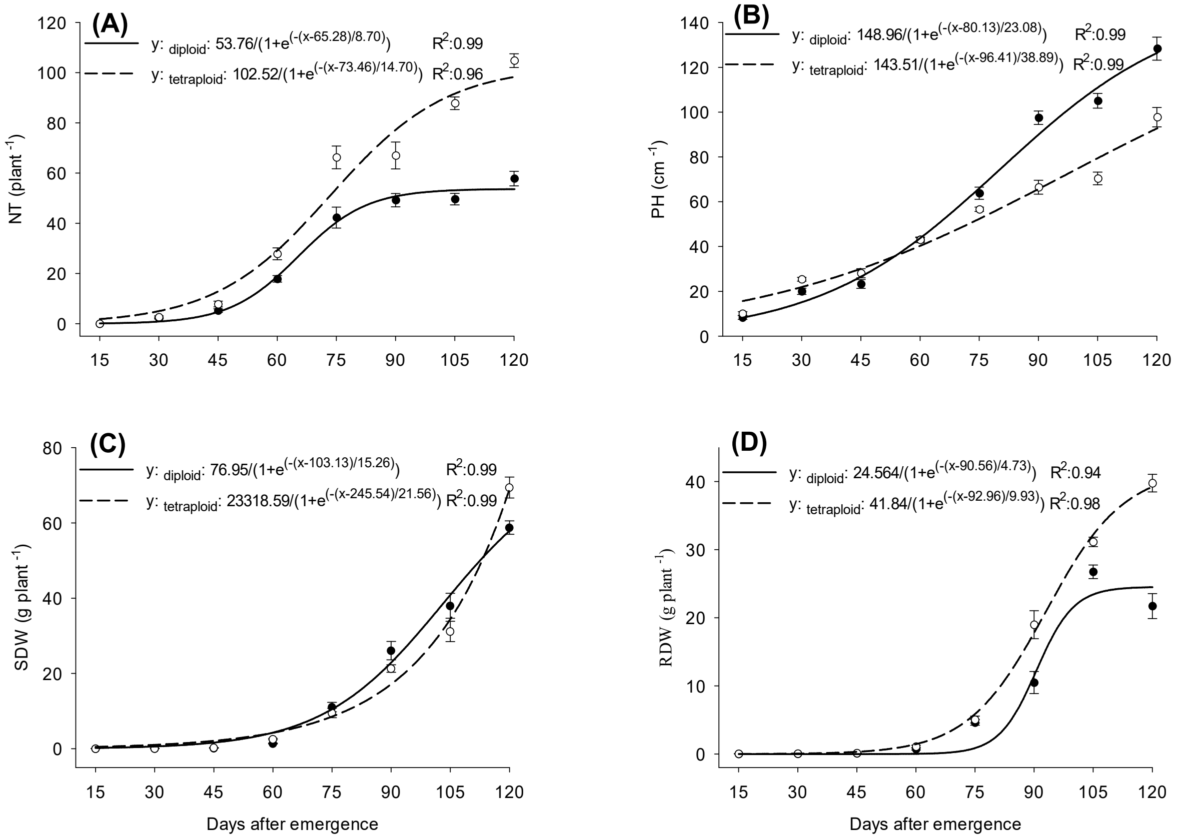
Also, planning should be paramount when seed production of tetraploid genotype is aimed either for harvesting or for supplying the seed bank in the soil, without compromising the proper time of establishment of summer crops adopted in succession. According to the results, tetraploid ryegrass has to be sown until the half of May to allow the plants to complete the phenological stage leading to the soil seed bank replenishment as well as the soybean sowing at an appropriate time.

Fitness cost

The analysis of results obtained in the experiment showed that data transformation was not necessary based on the Shapiro-Wilk test. The analysis of variance indicated an interaction between the factors genotype and assessment times for all variables. The sigmoidal model presented a satisfactory adjustment, with coefficients of determination (R^2) varying from 0.88 to 0.99 (Figures 2 and 3).

The variable NT showed, through the confidence interval, a significant difference between genotypes from 60 DAE; in the last assessment (120 DAE), NT was 47% higher for tetraploid ryegrass (Figure 2A). The parameter $x0$ of the equation showed that the time required to produce 50% of NT was 65 and 73 DAE for diploid and tetraploid ryegrass, respectively. The parameter a, which represents the difference between maximum and minimum points of the equation, showed that tetraploid plants were 47% higher when compared to diploid plants. Plants with a high NT have advantages in space occupation and, consequently, can suppress the growth of neighboring plants (Tironi et al., 2014).

The tetraploid genotype showed a 20% higher PH when compared to the diploid up to 45 DAE (Figure 2B). It may be due to a high initial vigor, which allows a rapid plant establishment a desirable trait when we want to increase competitiveness with weeds. At 60 DAE, PH of both



Points represent the mean values of replications of each genotype at each time, while bars indicate the respective 95% probability confidence intervals showed by each treatment.

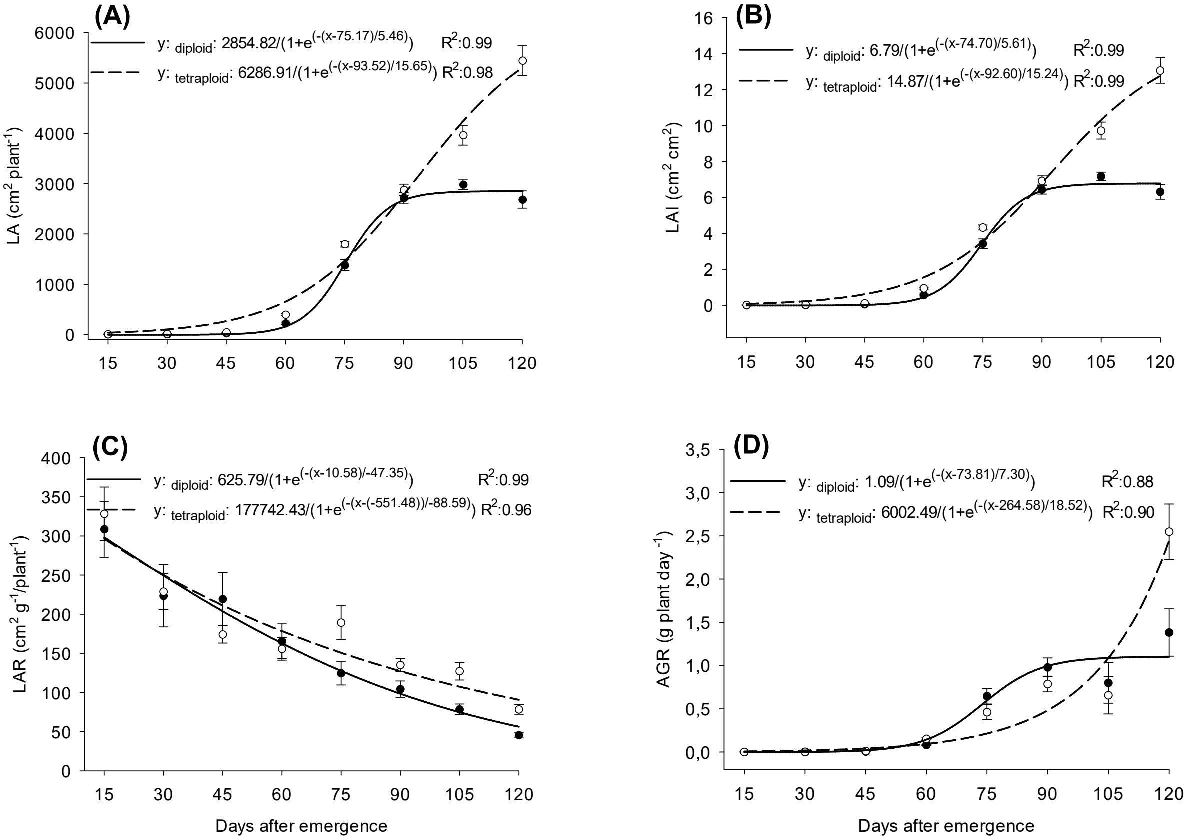
Figure 2 - Number of tillers (NT) (A), plant height (PH) (B), shoot dry weight (SDW) (C), and root dry weight (RDW) (D) of diploid (wild type) and tetraploid (cv. INIA TITAN®) ryegrass assessed from 15 to 120 DAE.

genotypes was similar, but in the assessment times, the other diploid ryegrass presented a higher height when compared to the tetraploid, with values 24% higher than that observed at 120 DAE. It is an essential morphological trait related to light capture, especially in the initial stage of establishment, in which plants with higher height and leaf area have advantages to intercept solar radiation, favoring carbon assimilation rate (Pontes et al., 2003).

The behavior of SDW accumulation between genotypes was similar up to 75 DAE (Figure 2C). The confidence interval showed that diploid ryegrass presented accumulation 18% higher when compared to the tetraploid at 90 DAE and 15% lower at 120 DAE. SDW accumulation is dependent on the stem to leaf ratio, in which water content in these organs influences this trait (Tonetto et al., 2011). Thus, the highest SDW accumulation of diploid ryegrass from 90 DAE and tetraploid ryegrass at 120 DAE coincides with plant elongation and the highest stem to leaf ratio (Figures 1 and 2C). The highest final SDW accumulation by the tetraploid ryegrass cv. INIA TITAN® was already expected because the plants had more time to accumulate photoassimilates than diploid genotypes (Oliveira et al., 2014).

For RDW, genotypes showed similar behavior up to 60 DAE, while in the other evaluation times, tetraploid ryegrass showed higher accumulation; at 120 DAE, the value was 45% higher than that of diploid ryegrass, with 39.78 g per plant (Figure 2D). Tetraploid ryegrass plants have a more developed root system, contributing to efficient use of water and assimilation of limited nutrients in the environment, reducing soil erosion and improving soil biological characteristics due to the increased carbon and other nutrients source (Deru et al., 2014).

A differentiated behavior of LA was observed for the genotypes. LA accumulation reached the maximum at 90 DAE for diploid ryegrass, with no maximum LA point for the tetraploid during the experimental period (Figure 3A). These results can be observed by the curve slope (parameter *b*



Points represent the mean values of replications of each genotype at each time, while bars indicate the respective 95% probability confidence intervals showed by each treatment.

Figure 3 **-** Leaf area (LA) (A), leaf area index (LAI) (B), leaf area ratio (LAR) (C), and absolute growth rate (AGR) (D) of diploid (wild type) and tetraploid (cv. INIA TITAN®) ryegrass assessed from 15 to 120 DAE.

of the equation), which was about three times higher for tetraploid ryegrass. LAI presented a behavior similar to that of LA since the growth of both genotypes occurred in a limited area free of competition and under the same availability of resources (Figure 3B). LA and LAI values are directly related to light interception and photosynthetic capacity, once plants with higher LAI and LA are more efficient in fixing CO_2, with advantages when submitted to the competition (Fraga et al., 2012). Thus, tetraploid ryegrass may have less impaired growth when in competition.

The highest required period of time (DAE) for tetraploid ryegrass to reach a 50% response (parameter *x0* of the sigmoidal equation) for the variables NT, PH, SDW, and LA occurred because plants presented a longer phenological development, especially the vegetative period, when compared to diploid genotypes (Figures 1, 2A, B, C, and 3A). Although tetraploid plants need a longer time to express maximum productive potential for NT, PH, SDW, and LA, a higher production during the entire assessment period for NT, initial for PH and AF, and final for SDW may favor competitive capacity of tetraploid genotypes in comparison to the diploid since when environmental resources are limiting, plants more efficient in using environmental resources can suppress the growth of less efficient plants when they occupy the same ecological niche (Fleck et al. 2006).

In both genotypes, a decrease was observed in LAR (Figure 3C). This behavior was due to the investment of photoassimilates for the development of shoots, roots, and reproductive structures, making the plant less efficient in energy conversion due to the reduction in the photosynthetic surface (Urchei et al., 2000). Due to the more extended vegetative period, tetraploid ryegrass plants showed an increase in leaf area for a longer period, while diploid plants allocated photoassimilates to stems and reproductive structures earlier. Therefore, the decrease in LAR was more accentuated for diploid genotype, being these results observed from 75 DAE, when the diploid ryegrass started elongation.

Regarding the genotypes, an increasing trend was observed for AGR as plants developed, with its stabilization at 90 DAE for diploid ryegrass (Figure 3D). On the other hand, the tetraploid ryegrass did not present a maximum AGR during the assessed period. AGR behavior was similar to that observed for LA and LAI, being a reflection of it since the optimal LAI is reached when crop AGR is also maximal (Alvarez et al., 2012).

For yield components, tetraploid ryegrass presented NSP and NS 44% higher in comparison to the diploid (Table 1). The highest seed yield of the tetraploid genotype was due to the higher amount of fertile tillers, resulting in an increase in the number of seeds and contributing to a higher yield of the cultivar (Mandic et al., 2014).

Table 1 **-** Yield components of diploid (wild type) and tetraploid (cv. INIA TITAN®) ryegrass after physiological maturation

Yield component	Diploid ryegrass	Tetraploid ryegrass	CV (%)
Number of spikes per plant (NSP)	40.60*	75.75	13.64
Number of seeds per plant (NS)	10161*	18085	10.90
Number of spikelets per spike (SS)	24.00*	29.80	5.70
Number of filled seeds per spikelet (NFS)	11.00*	8.00	8.86
Number of seeds per spike (NSS)	252.00^{ns}	228.14	8.30
Seed weight per spike (SWS)	0.78^{ns}	0.88	8.64
One thousand-seed weight (TSW)	3.08*	3.87	4.87

^{ns} Not significant and * significant when comparing ryegrass genotypes for each response variable by the t-test ($p \leq 0.05$).

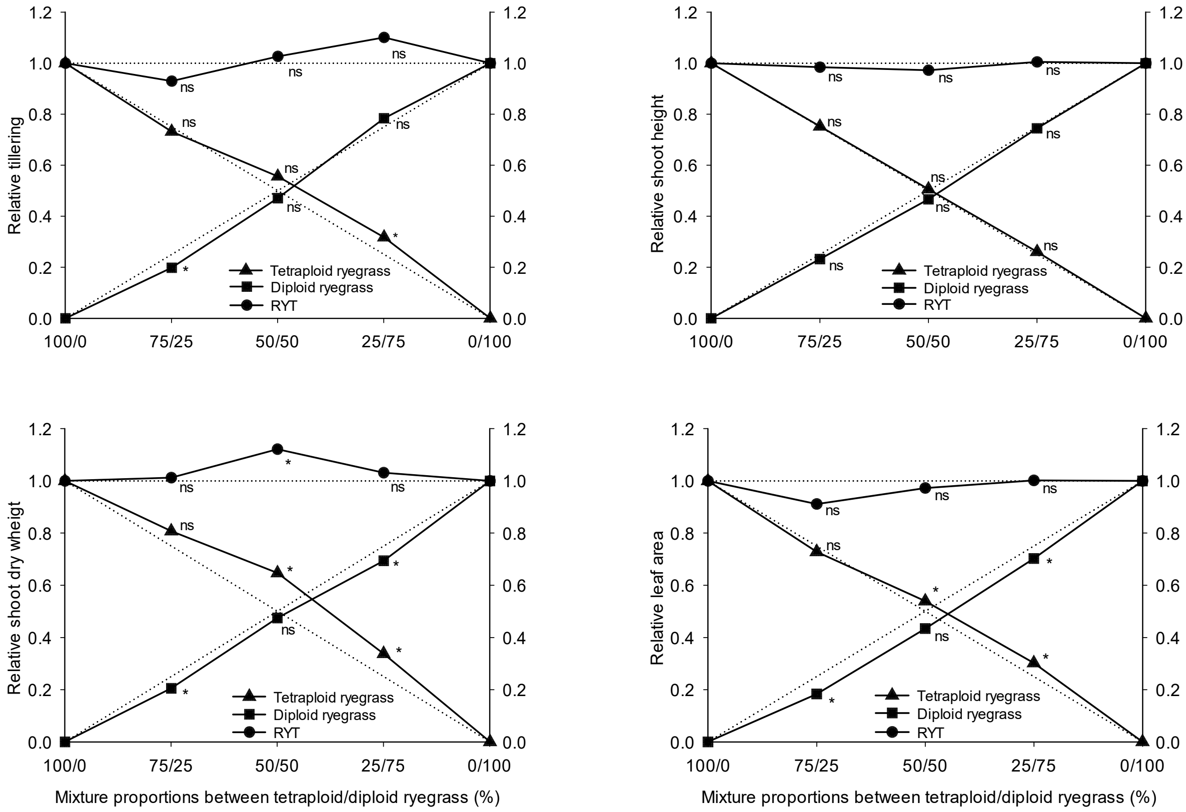
The tetraploid genotype also presented a higher SS, which was 19% higher when compared to the diploid, but with a lower NFS, resulting in similar values of NSS among genotypes. NFS was 27% higher for the diploid genotype, while TSW was 20% higher for the tetraploid ryegrass (Table 1). These opposite results contributed to SWS presented no difference between genotypes. The highest TSW favors the initial vigor in seedling development (Mandic et al., 2014) and the highest TSW of the tetraploid genotype is due to doubling the number of chromosomes, benefiting the increase in seed size (Bustamante et al. 2015).

Opposite results were found in a study carried out under field conditions, in which the tetraploid cultivar INIA TITAN® was submitted to cut levels and presented lower seed yield when compared to wild diploid ryegrass, regardless of the cutting frequency (Tonetto et al., 2011). However, cutting regime can promote drastic and negative changes in morphogenic traits and ryegrass seed yield (Cunha et al., 2016).

Competitive ability

The graphical analyses of the competitive ability experiment for RY of ryegrass genotypes in the variables NT and PH were, in general, equivalent, as the obtained results were similar to the hypothetical straight lines (Figure 4). Exceptions occurred when the tetraploid genotype occupied 25% of the niche proportion, allowing the tillering of this genotype to increase, but a reduction in tillering was reached when the genotype was present in 75% of the proportion. This result indicates that the tetraploid genotype was able to carry out tillering and occupy a larger area of soil when at a lower proportion. However, when this genotype held a more substantial proportion, tillering remained constant, only reducing that of its competitor, i.e., the diploid genotype.

Tetraploid ryegrass increased the production of the variables LA and SDW when present at an equal or lower proportion of plants (Figure 4). On the contrary, the diploid genotype showed a reduction concerning the hypothetical straight line when at a lower or higher mixture proportion of plants. Thus, there was a loss to one genotype and benefit to the other, showing that the competition occurred by the same environmental resources and that the tetraploid genotype was more efficient in using them for growth. Similar results were observed in tetraploid cultivars of *L. perenne*, which were more competitive than diploid cultivars, increasing their proportion in the pasture established above the seeded ratio (Gilliland et al., 2011, 2016).



Triangles (▲) represent RY (relative yield) of the tetraploid ryegrass genotype, squares (■) represent RY of the diploid ryegrass genotype, and circles (●) indicate RYT (relative yield total). Dotted lines refer to the hypothetical relative yields when there is no interference of one species over another. ^{ns} Not significant and *significant when comparing ryegrass genotypes for each response variable by the t-test ($p \leq 0.05$).

Figure 4 **-** Relative Yield (RY) and relative yield total (RYT) computed for different mixture proportions of tillering, shoot height, shoot dry weight, and leaf area of diploid (wild type) vs. tetraploid (cv. INIA TITAN®) ryegrass.

In combinations of diploid: tetraploid *L. perenne*, when the tetraploid genotype represented 10 and 30% at sowing, the proportion in the pasture increased to 32.2 and 56.8% of the tetraploid, respectively, and when the genotypes were sown at proportions equal to the composition of tetraploid plants, they occupied 65% of space after two years of sowing (Gilliland et al., 2011). These results indicate that the tetraploid ryegrass was more efficient in the use of resources, occupying a higher proportion of the niche. Canopy structure was the likely factor that affected the competitive ability of genotypes (Griffith et al., 2016).

Regarding RYT, differences were observed only for SDW at a mixture proportion of 50:50, in which the estimated values were higher than expected (Figure 4). RYT values higher than 1 and above the hypothetical straight line for both tested genotypes indicate no competition for resources. However, for SDW, tetraploid genotypes showed a higher RY, and diploid genotypes had values similar to RY in relation to the hypothetical straight lines, providing an observed value higher than the expected for RYT. This behavior shows that the tetraploid genotype was more competitive than the diploid and contributed more than expected for RYT.

The morphological variables NT, LA, and SDW of the diploid genotype were reduced as the proportion of tetraploid plants increased in the associations (Table 2). For these same variables, an inverse response was observed for the tetraploid genotype, which increased the production as a function of the presence of diploid plants in the association. Thus, the higher the proportion of tetraploid genotypes in the association with diploid is, the higher the damage to the variables of diploid ryegrass. Also, the competition between tetraploid genotypes was less harmful when compared to that intragenotypic in the monoculture. For PH, the effect was similar to the other variables, but with no difference between plant associations.

Table 2 **-** Responses to the number of tillers, plant height, leaf area, and shoot dry weight of diploid ryegrass (wild type) competing with tetraploid ryegrass (cv. INIA TITAN®) under different proportions of plants

	Mixture proportion of plants (tetraploid: diploid)					CV (%)
	100:0	75:25	50:50	25:75	0:100	
Genotype	Number of tillers (No. per plant)					
Tetraploid	5.62	5.49^{ns}	6.25^{ns}	7.14*	-	7.50
Diploid	-	4.35*	5.18^{ns}	5.74^{ns}	5.50	8.12
	Plant height (cm per plant)					
Tetraploid	37.70	37.80^{ns}	38.16^{ns}	39.27^{ns}	-	5.59
Diploid	-	37.44^{ns}	37.58^{ns}	40.02^{ns}	40.31	6.19
	Leaf area (cm^2 per plant)					
Tetraploid	75.61	73.38^{ns}	81.51^{ns}	90.48*	-	7.82
Diploid	-	51.15*	60.58*	65.37^{ns}	69.80	7.26
	Shoot dry weight (g per plant)					
Tetraploid	0.35	0.38^{ns}	0.46*	0.48*	-	7.15
Diploid	-	0.30*	0.35^{ns}	0.34^{ns}	0.37	7.05

^{ns} Not significant and * significant to the respective monoculture (100%) by the Dunnett test (p≤0.05). CV – coefficient of variation.

By means of the CR, K, and A indices, considering that tetraploid ryegrass is more competitive than the diploid, when CR > 1, Ka > Kb, and C > 0 (Hoffman and Buhler, 2002), proved that tetraploid ryegrass presented for all variables a higher competitiveness than the diploid genotype (Table 3). The competitive ability of ryegrass genotypes can be affected by several factors, including the environment, edaphic parameters, sowing time, intrinsic characteristics of the cultivar (ploidy), and different managements, which may or may not include animals for grazing. Therefore, field experiments under different conditions and with different tetraploid cultivars are important tools to verify the efficiency of tetraploid genotypes in the suppression of herbicide-resistant diploid populations.

Table 3 **-** Competitive indices of tetraploid ryegrass (cv. INIA TITAN®) competing with diploid ryegrass (wild type) expressed by the competitive ratio (CR), relative crowding coefficients (K), and aggressivity (A) indices

	CR	Ka(5)	Kb(6)	A
LA(1)	1.25 (±0.07)*	1.17 (±0.04)*	0.77 (±0.07)	0.11 (±0.02)*
SDW(2)	1.38 (±0.09)*	1.84 (±0.10)*	0.92 (±0.09)	0.17 (±0.03)*
PH(3)	1.09 (±0.01)*	1.03 (±0.05)^{ns}	0.88 (±0.05)	0.04 (±0.01)*
NT(4)	1.18 (±0.05)*	1.26 (±0.10)*	0.89 (±0.03)	0.09 (±0.02)*

(1) Leaf area; (2) shoot dry weight; (3) plant height; (4) number of tillers; (5) tetraploid ryegrass; (6) diploid ryegrass; ^{ns} Not significant and * significant by the t-test (p≤0.05). Values in parentheses represent the standard error of the mean.

Considering that ryegrass has crossed fertilization and nuclear resistance, which is transferred via pollen to susceptible plants (Vargas et al., 2007), and that differences in phenological development provided a flowering delay in tetraploid genotypes in relation to diploid ryegrass, the use of tetraploid genotypes in areas with herbicide-resistant ryegrass aiming the depletion of the frequency of herbicide-resistant ryegrass is a good management tool, because crossing between genotypes tends to be reduced due to a difference between flowering time. As reported by Bustamante et al. (2015), if diploid and tetraploid crosses occur, the probability of selecting viable triploids is very low (1/166) since the presence of unpaired chromosomes in meiosis makes it difficult to maintain alive the genotype, leading to germination problems or even death of the seedling. Therefore, the probability of the transfer of genes that confer herbicide-resistance to the offspring in tetraploid x diploid-resistant crosses tends to be negligible.

In summary, tillering is the main stage responsible for differences in phenological development between genotypes. The tetraploid genotype is superior when compared to the diploid for NT, LA, RDW, and seed production when plants were free of competition. These characteristics of growth allowed confirming in the competitive ability test that the tetraploid ryegrass is more competitive when compared to the diploid when the species occur at different proportions and occupy the same ecological niche.

From these results, is possible to conclude that because of the tetraploid higher competitive potential, the sowing of tetraploid genotype can be useful as an integrated weed management tool aiming at reducing the frequency of herbicide-resistant diploid ryegrass in troublesome areas.

ACKNOWLEDGMENTS

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for granting the scholarship.

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