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Article

Fitness Cost and Competitive Ability of Ryegrass Susceptible and with Multiple Resistance to Glyphosate, Iodosulfuron-Methyl, and Pyroxsulam

Custo Adaptativo e Habilidade Competitiva de Azevém Suscetível e com Resistência Múltipla a Glyphosate, Iodosulfuron-Methyl e Pyroxsulam

HENCKES, J.R.[1]*
CECHIN, J.[2]
SCHMITZ, M.F.[2]
PIASECKI, C.[2]
VARGAS, L.[3]
AGOSTINETTO, D.[2]

ABSTRACT - Ryegrass is an important weed in wheat cultivations due to the evolution of resistance to different mechanisms of action. This study aimed to compare the phenological development, fitness cost, and competitive ability between ryegrass biotypes susceptible and with multiple resistance to 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) and acetolactate synthase (ALS) inhibiting herbicides. Fitness cost and phenological development were evaluated from biotypes grown in pots, and morphological growth variables were determined up to 140 days after emergence. Phenological development was evaluated with a fitness cost. Competitive ability was determined in a replacement-series experiment with proportions of resistant and susceptible ryegrass. The tested proportions were 100:0 (pure resistant ryegrass stand), 75:25, 50:50, 25:75, and 0:100% (pure susceptible ryegrass stand), where the number of tillers, height, leaf area, and shoot dry matter were evaluated at 50 days after emergence. The longer tillering period of resistant ryegrass was the main factor involved in the difference in phenological development between biotypes. The resistant biotype was superior to the susceptible regarding height, shoot dry matter, and absolute growth rate, while the susceptible biotype had a higher number of tillers and leaf area ratio. Thus, the resistant biotype had no fitness cost. For competitive ability, no difference was observed between biotypes in the different tested proportions when they occupied the same ecological niche.

Keywords: *Lolium multiflorum*, EPSPs, ALS, competition.

RESUMO - O azevém é planta daninha importante nos cultivos de trigo devido à evolução da resistência a diferentes mecanismos de ação. O objetivo deste estudo foi comparar o desenvolvimento fenológico, custo adaptativo e habilidade competitiva entre biótipos de azevém suscetível e com resistência múltipla aos herbicidas inibidores da 5-enolpiruvilchiquimato-3-fosfato sintase (EPSPs) e acetolactato sintase (ALS). Para avaliar o custo adaptativo e o desenvolvimento fenológico, os biótipos foram cultivados em vasos, e as variáveis morfológicas de crescimento foram avaliadas até os 140 dias após a emergência. O desenvolvimento fenológico foi avaliado juntamente com o custo adaptativo. A habilidade competitiva foi determinada em experimento de série de substituição com proporções de azevém resistente e suscetível. As proporções testadas foram: 100:0 (estande puro de azevém resistente), 75:25, 50:50, 25:75 e 0:100% (estande puro de suscetível), onde o número de afilhos, a estatura, área foliar e massa seca da parte aérea foram avaliados aos 50 dias após a emergência. O maior período de afilhamento do azevém resistente é o principal fator envolvido na diferença do desenvolvimento fenológico entre os biótipos. O biótipo resistente é superior ao

*** Corresponding author:**
<jhenckes94@gmail.com>

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[1] Universidade Estadual de Maringá, Maringá-PR, Brasil; [2] Universidade Federal de Pelotas, Pelotas-RS, Brasil; [3] Embrapa Trigo, Passo Fundo-RS, Brasil.

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suscetível quanto a estatura (EP), massa seca da parte aérea (MSPA) e taxa de crescimento absoluto (TCA), enquanto o suscetível possui maior número de afilhos (NA) e razão de área foliar (RAF). Assim, o biótipo resistente não apresenta custo adaptativo. Para a habilidade competitiva, verificou-se que não há diferença entre os biótipos nas diferentes proporções testadas quando ocupam o mesmo nicho ecológico.

Palavras-chave: *Lolium multiflorum*, EPSPs, ALS, competição.

INTRODUCTION

Ryegrass (*Lolium multiflorum* L.) is an allogamous, annual monocotyledonous species adapted to different soil types and highly productive in fertile soils, where it supports intense grazing and presents resprout with a large tillers number (Carámbula, 2007). These characteristics make ryegrass an important winter forage for temperate regions of southern Brazil. In addition, the natural reseeding of ryegrass allows its permanence in the soil seed bank and heterogeneous emergence flows, making the management of this species onerous when present in fields cultivated with winter cereals (Tironi et al., 2014).

The reduction in the negative impact of ryegrass on yield and quality of harvested grains is indispensable, and the chemical control with 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) and acetolactate synthase (ALS) inhibiting herbicides are considered the main management tool. Chemical control combines practicality, efficiency, and low cost compared to other control methods (Correa et al., 2014). However, herbicide-resistant of weed biotypes have evolved in response to the intensive use of the same active principle or mechanism of action, which makes control more costly and difficult, especially in wheat crop in which the herbicide options are limited (Vargas et al., 2006).

In a scenario with the evolution of resistance in different weed populations and types of involved resistance mechanism, the survival of biotypes is considered an adaptive advantage (Délye, 2013). However, in environments without herbicide application, resistant biotypes may present adaptation costs because of physiological changes due to resistance, which may have effects on the competitive ability and dynamics of a population in a given environment (Li et al., 2013; Vila-Aiub et al., 2015).

Competitive ability studies have been widely used to verify the response of the interaction of a particular species or biotype in the presence of its competitor to evaluate the ability of one plant to suppress the development of another with or without prejudice to its growth (Goldberg and Landa, 1991). Also, these studies, together with the fitness value, are important for developing management strategies and to help prevents the evolution of weed resistance. Thus, it is essential to evaluate different responses of ryegrass biotypes in relation to their fitness value and competitive ability in environments where resources are limited. In this context, this study aimed to compare the phenological development, fitness cost, and competitive ability between ryegrass biotypes susceptible and with multiple resistance to EPSPs (glyphosate) and ALS (iodosulfuron-methyl and pyroxsulam) inhibiting herbicides.

MATERIAL AND METHODS

The experiments were carried out in a greenhouse belonging to the Faculdade Eliseu Maciel, Federal University of Pelotas, from April to October 2017. Treatments were arranged in a completely randomized experimental design with four replications. Each experimental unit consisted of plastic pots filled with sandy loam textured Red-Yellow Argisol belonging to the mapping unit of Pelotas (Embrapa, 2013), with pH and fertility previously corrected, according to soil analysis for the cultivation of cold season grass forages (Tedesco et al., 2004). Ryegrass biotypes were susceptible (Susc) and resistant (110) to glyphosate, iodosulfuron-methyl, and pyroxsulam from Roncador - PR (24°31'08.59" S - 52°15'38.8" W and 24°35'23.93" S - 52°15'02.12" W).

Phenological development of biotypes

The Bleiholder et al. (1991) scale was used to determine the required time interval for ryegrass genotypes to reach the following phenological stages: sowing (SO), emergence (EM), first leaf through coleoptile (FC), tillering (TI), elongation (EL), flag leaf sheath extending (FS), heading (HE), flowering (FL), watery grain (WG), milky grain (MG), dough grain (DG), and fully ripe (FR). Eight plants of each biotype were evaluated. The plants were considered to have reached the predetermined phenological stages when 50% or more of them had the same phenotypic characteristics.

Fitness cost

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 susceptible (Susc) and resistant (110) ryegrass biotypes, and factor B was composed of eight collection seasons: 15, 30, 45, 60, 80, 100, 120, and 140 days after emergence (DAE).

The growth variables analyzed at each time were the number of tillers (NT), plant height (PH), shoot dry weight (SDW), leaf area index (LAI), leaf area ratio (LAR), and absolute growth rate (AGR). Leaf area 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 (g $plant^{-1}$) was obtained by collecting shoot samples and drying them in a forced air circulation oven at 60 °C for 72 h, with subsequent weighing on an analytical balance. LAI expresses the ratio between the total leaf area per unit soil area, indicating the available surface for interception and absorption of light. LAR (cm^2 g^{-1}) represents the ratio of LA and SDW, demonstrating the leaf area available for photosynthesis, and was obtained by the equation $LAR = (LA_1 + LA_2)/(SDW_1 + SDW_2)$. AGR (g day^{-1}) measures the mean growth rate in a given period, being 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, 1985).

The data were analyzed for normality (Shapiro-Wilk test) and then submitted to analysis of variance ($p \leq 0.05$). In case of statistical significance, regression analysis was performed for the factor evaluation times for all variables, using the non-linear sigmoidal model $y = a/(1 + x/x_0)^b$, where y is the response variable, x is the days after emergence, and a, x_0, and b are the equation parameters, in which a is the difference between the maximum and minimum points of the curve, x_0 represents the days that provide 50% of response of the variable, and b is the curve slope.

Competitive ability

Before the competitive ability study, an additive series experiment was carried in pots with a diameter of 19 cm and a volume of 4 dm^3. Monoculture (additive series) of ryegrass biotypes (susceptible and resistant) 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, 1,120, 2,240, and 4,480 plants m^{-2}, respectively). SDW was collected at 50 DAE, and the data were analyzed by the reciprocal production method. The average results of genotypes showed that SDW was constant and independent of the population for 1693 plants m^{-2}, equivalent to 48 plants pot^{-1} (data not shown).

A replacement-series experiment was carried out based on the obtained population, in which genotypes were maintained under monoculture or associated in mixture using different proportions, as follows: 100:0 (resistant ryegrass monoculture), 75:25, 50:50, 25:75, and 0:100 (susceptible ryegrass monoculture). Resistant biotype plants were identified with colored narrow ribbon wires wrapped in the stem at the transplanting time. Biotypes were previously sown in trays and equidistantly transplanted at 4 DAE, with the evaluation 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 response variable. RY of each genotype was calculated by dividing the mean of association by the mean of the monoculture, while 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 points 100% for RYT. The occurrence of a concave line for RY has a negative effect on the growth of one or both biotypes, while a convex line shows a growth benefit. For RYT, a convex line shows no competition for environmental resources, while a concave line indicates losses to the growth of both biotypes (Cousens, 1991).

The competitive ratio (CR), relative crowding coefficient (K), and aggressivity (A) were calculated for 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 the resistant biotype (Ka) over the susceptible biotype (Kb), and A shows the most competitive biotype (Cousens, 1991), with the following reference values for a higher competition: CR > 1, Ka > Kb, and C > 0.

The statistical analysis of RY was performed by 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. The differences in the RYD, RYT, CR, K, and A indices were compared by the t-test ($p<0.05$) (Roush et al., 1989; Hoffman and Buhler, 2002). For this, they were considered as null hypothesis (Ho = 0) to test differences of RYD and C; for RYT and CR, they should be considered equal to 1 (Ho = 1); for K, the means of differences between Ka and Kb should be considered null [Ho = (Ka - Kb) = 0]. The difference between RY and RYT curves of theoretical straight lines and CR, K, and A values was considered when at least two proportions were statistically different by the t-test (Bianchi et al., 2006).

The mean results obtained for LA, DW, PH, and NT were evaluated for normality (Shapiro-Wilk test) and then submitted to analysis of variance ($p\leq0.05$). When a statistical significance was found for these variables, the means of treatments were compared by the Dunnett test ($p\leq0.05$), being the standard treatment the respective monocrop.

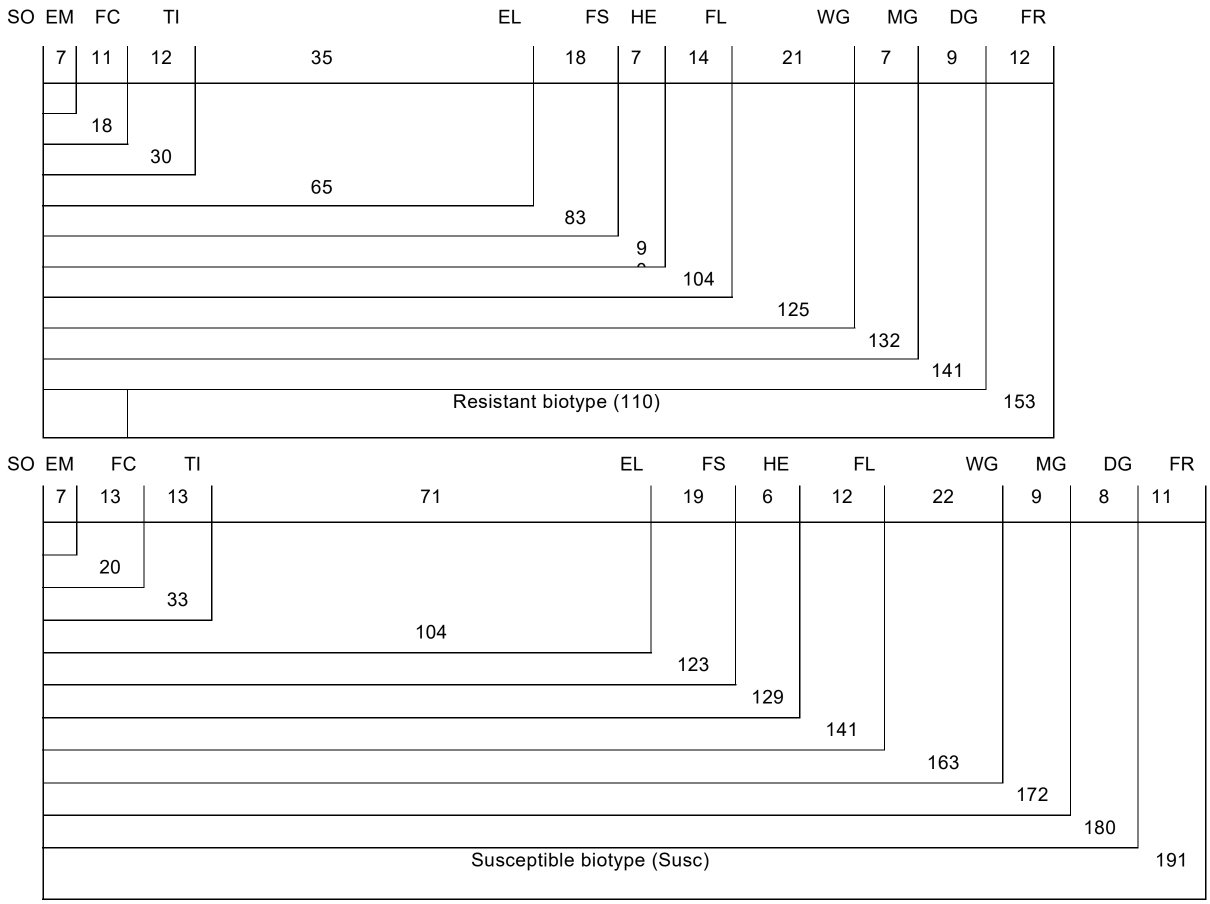
RESULTS AND DISCUSSION

Phenological development of biotypes

The results showed differences in the length of phenological stages of ryegrass susceptible (Susc) and with multiple resistance (110) for periods between emergence and physiological maturity (Figure 1). The life cycle of resistant and susceptible biotypes was 153 and 191 days, respectively. This difference occurred mainly at the tillering stage. The shorter cycle for resistant ryegrass can be considered an aggravating factor since this species shows cross-fertilization and a nuclear resistance to EPSPs and ALS inhibiting herbicides, being transferred via pollen to susceptible plants (Vargas et al., 2007). Thus, these plants will cross to each other before flowering of susceptible plants, increasing the homozygosis of genes that confer resistance, in addition to occupy the seed bank earlier, reducing the probability that the plant be eliminated before completing the cycle and contributing to a fast dispersion of the resistant population within infested areas and other adjacent areas.

Another important characteristic is related to the emergence rate and the rapid initial growth of seedlings, making them more competitive due to a better space occupation and use of environmental resources (Gustafson et al., 2004). Faster plant development provided by increased light interception capacity, rapid leaf area expansion, and better canopy colonization at the top layer through sheath, petiole, and stem internode elongation result in increased competitive ability (Mckenzie-Gopsill et al., 2016).

The shorter cycle of resistant weed populations requires the adoption of different management practices to minimize and/or avoid seed dispersal, and thus, contribute to the reduction of seed banks. Complementary and joint management practices that include crop rotation and the use of herbicides with different mechanisms of action in the pre- and post-emergence may contribute to reducing germination and establishment of resistant plants. Another alternative, as in the



Sowing (SO); emergence (EM); first leaf through coleoptile (FC); tillering (TI); elongation (EL); flag leaf sheath extending (FS); 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 resistant (110) and susceptible (Susc) ryegrass (*Lolium multiflorum*) biotypes based on the phenological scale adapted from Bleiholder et al. (1991).

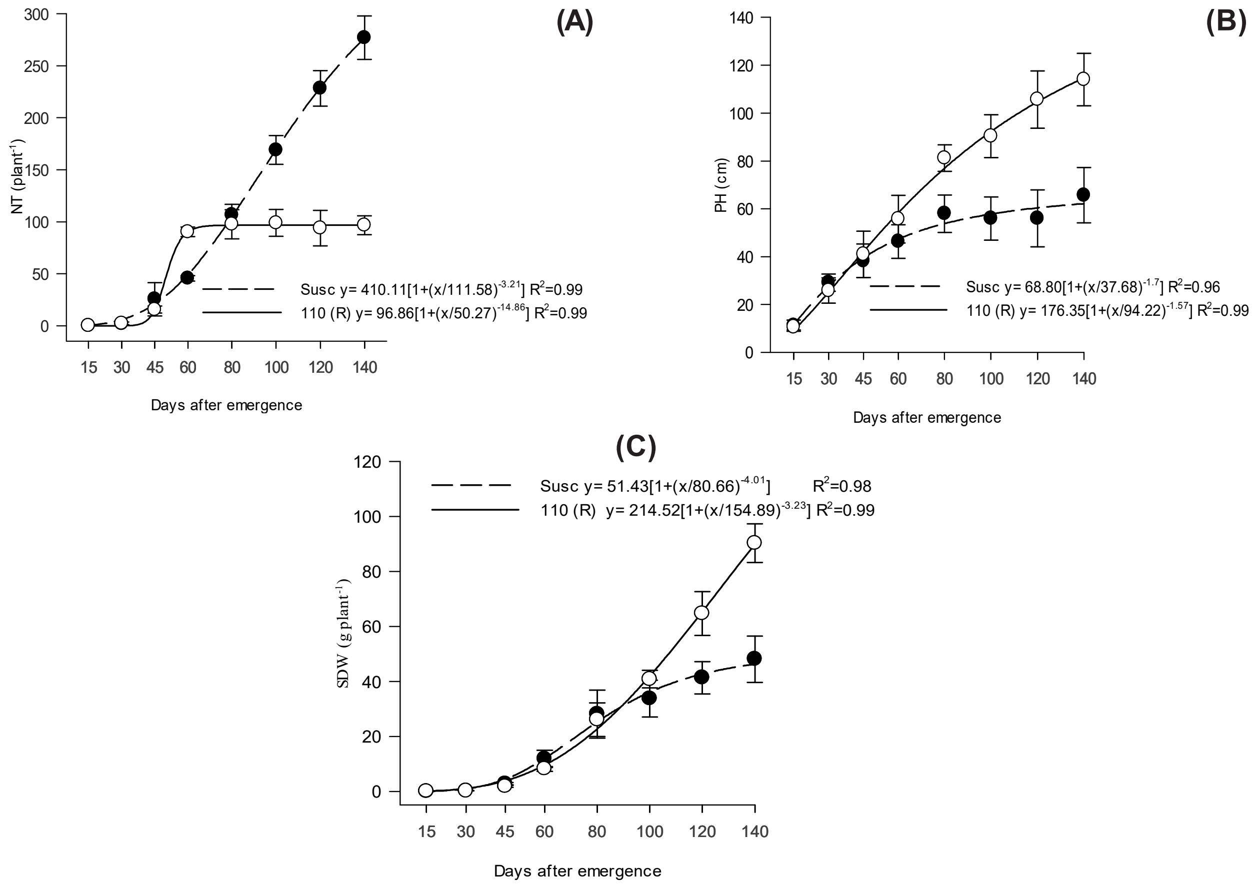
case of biotype 110, which has a shorter cycle, is the adoption of integrated crop-livestock, where it is possible to use a more intensive grazing with higher animal stocking in the first months of ryegrass establishment aiming at reducing seed production of the resistant biotype, resulting in a gradual seed reduction of this biotype in the soil seed bank.

Fitness cost

No data transformation was required according to the Shapiro-Wilk test. The analysis of variance showed an interaction between the factors biotypes and evaluation times for all variables, with an adjustment to the sigmoidal regression model, in which the coefficients of determination (R^2) ranged from 0.95 to 0.99 (Figures 2 and 3).

The confidence interval showed a difference between biotypes for the variable NT from 80 DAE, with a value 31% higher for the susceptible biotype when compared to the resistant in the evaluation carried out at 140 DAE (Figure 2A). Higher NT in plants is considered a competitive advantage in space occupation and, consequently, in suppressing the growth of neighboring plants (Tironi et al., 2014). Also, the occurrence of a longer cycle for the susceptible biotype corroborates the longer tillering period, evidencing a higher capacity in the allocation of limited resources in a given environment (Figure 1).

Regarding the variable PH, results showed that the resistant biotype was 43% higher compared to the susceptible at 140 DAE, with similar values between biotypes in evaluations carried out up to 60 DAE (Figure 2B). PH is an important morphological characteristic that interferes with light competition (Fleck et al., 2008) due to the ability to prevent the shade, allowing plants a



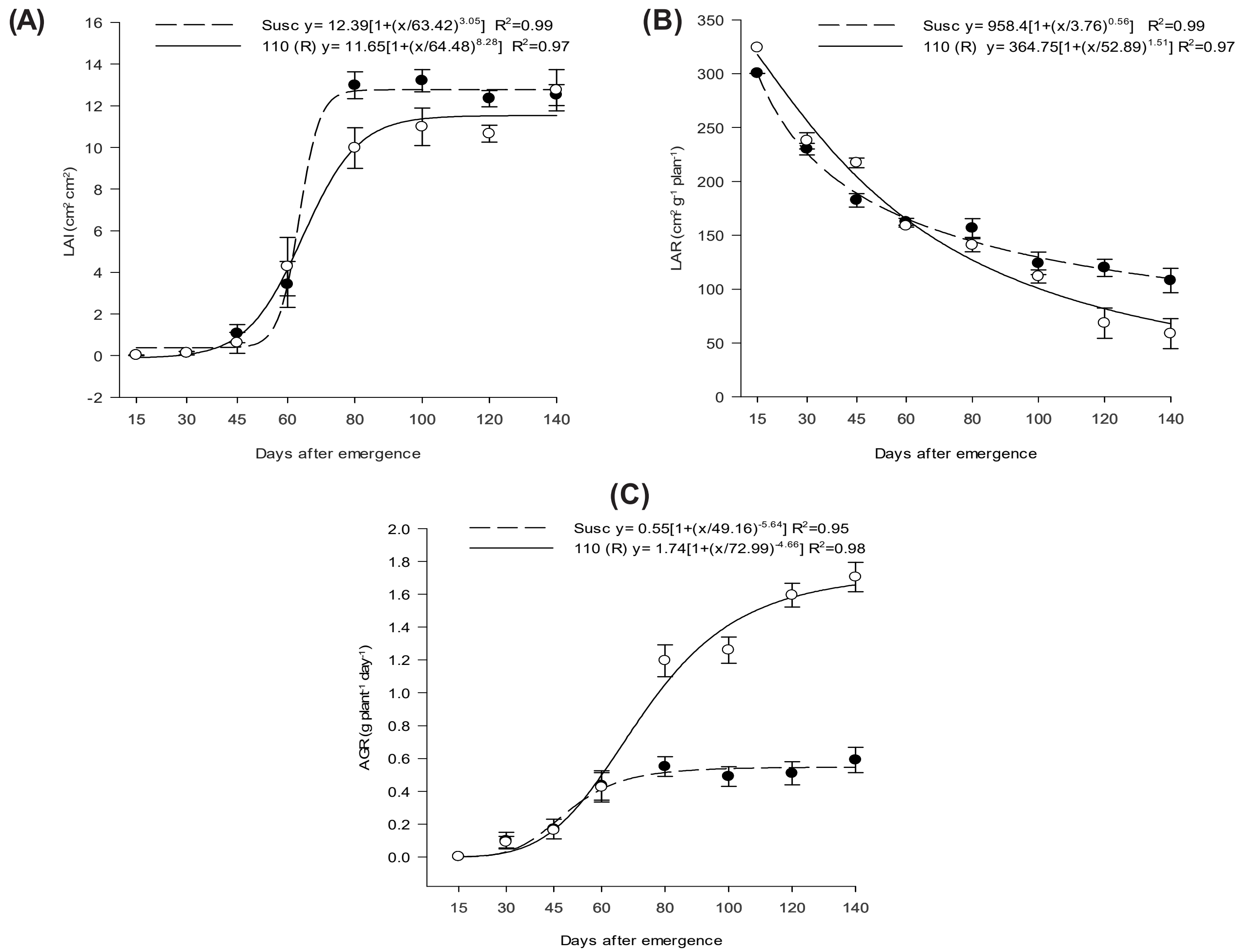
Dots represent the mean values of replications of each biotype at each time, while bars indicate the respective 95% probability confidence intervals showed by each treatment.

Figure 2 **-** Number of tillers (A), plant height (B), and shoot dry weight (C) of susceptible (Susc) and resistant (110) ryegrass biotypes evaluated from 15 to 140 DAE.

higher competitive ability with surrounding plants (Fleck et al., 2006). Similar results were observed in ryegrass biotypes resistant to the herbicides iodosulfuron-methyl and fluazifop, with a higher PH observed in evaluations from 60 DAE (Fraga et al., 2013; Mariani et al., 2016). Moreover, evaluating different population levels in pure forage Poaceae species, an inverse relationship was found between PH and the number of tillers due to the differential partition of assimilates for tillers (Martínez Calsina et al., 2012). The increased density leads to a reduction in NT and tends to stimulate plant etiolation, favoring light capture because it occurs in response to the light quality detected by phytochrome photoreceptors, causing physiological changes in plants (Fleck et al., 2006; Mckenzie-Gopsill et al., 2016).

Biotype SDW accumulation was similar up to 80 DAE, with differences based on the confidence interval, with values 47% higher in the resistant biotype at 140 DAE (Figure 2C). SDW accumulation depends on the stem: leaf ratio, as in the PH and NT values of the resistant biotype. The ability of a species to accumulate biomass is one of the characteristics that determine its competitiveness (Carvalho et al., 2005), so that the higher the SDW production is, the higher the reduction in environmental resources, which can lead to the suppression in the growth of surrounding plants (Fleck et al., 2006).

For LAI, adjustments of the data to the model showed a similar behavior between biotypes, with a difference higher than 20% only at 80 and 100 DAE (Figure 3A). Upon reaching a maximum LA during flowering, plants direct their reserves to the reproductive stage, reducing LAI and SDW accumulation (Peixoto and Peixoto, 2004). Plants with a higher LAI are more efficient in fixing CO_2 due to the higher light interception, which makes them better adapted to the exploitation of a given environment (Fraga et al., 2013).



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

Figure 3 **-** Leaf area index (A), leaf area ratio (B), and absolute growth rate (C) of susceptible (Susc) and resistant (110) ryegrass biotypes evaluated from 15 to 140 DAE.

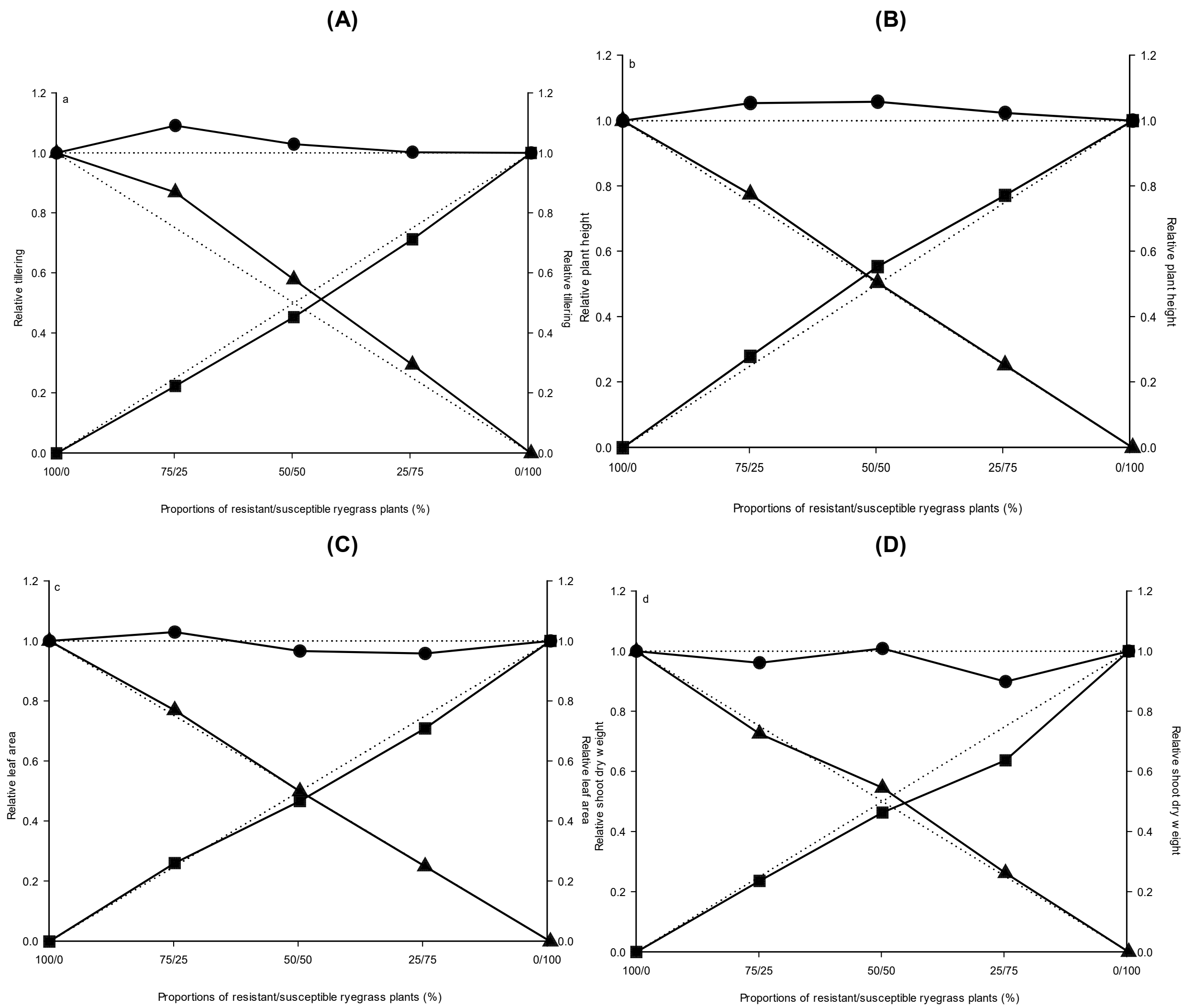
A decrease was observed in the LAR values of biotypes throughout the evaluated period (Figure 3B). LAR shows the plant response during growth and development as a function of photoassimilate allocation to tissues and assimilatory structures, such as stems, roots, and reproductive structures. Thus, the plant becomes less efficient in energy conversion due to a reduction in the photosynthetic surface, self-shading, as well as senescence of leaves throughout the plant cycle (Urchei et al., 2000). For the resistant biotype, photoassimilate allocation to stems and reproductive structures was earlier than that in the susceptible biotype, resulting in a rapid decrease of LAR from 80 DAE (Figure 3B). Higher LAR values indicate higher plant capacity to intercept light energy and occupy space, making it more competitive (Ferreira et al., 2008).

The AGR values of both biotypes increased throughout the cycle, with stabilization at 80 DAE for the susceptible biotype and 120 DAE for the resistant biotype (Figure 3C). However, resistant biotype had a higher growth rate compared to the susceptible, being considered a competitive advantage for photoassimilate accumulation.

The absence of fitness costs in resistant weed populations is an important factor involved for the resistance evolution rate and serves to estimate the ecological adaptability of biotypes resistant and susceptible to herbicides (Gray et al., 1995; Christoffoleti et al., 1997). In this sense, the adoption of management practices and/or associated with other control methods that favor susceptible biotypes should be considered an essential strategy to manage areas with resistant weeds (Vargas et al., 2005).

Competitive ability

According to the statistical criteria used to consider the occurrence of differences between RY and RYT curves in at least two proportions, no differences were observed between the evaluated biotypes in any of the analyzed variables (Figure 4 and Table 1). Although not significant by the adopted criteria, a difference was observed in RY for the variable NT. An increased NT was observed when the biotype 110 occupied the greater proportion of niche, leading to a reduction of tillering in the biotype Susc, i.e., a loss was observed only the biotype Susc as its proportion in the niche was reduced (Figure 4A and Table 1).



Triangles (▲) represent RY of the resistant ryegrass biotype, squares (■) represent RY of the susceptible ryegrass biotype, and circles (●) indicate RYT. Dotted lines refer to hypothetical relative yields when there is no interference of one species over another.

Figure 4 - Relative Yield (RY) and relative yield total (RYT) for tillering (A), plant height (B), leaf area (C), and shoot dry weight (D) of susceptible (Susc) and resistant (110) ryegrass biotypes.

Regarding PH, an increase in RY and RYT was observed for the susceptible biotype when both biotypes occupied the niche at equal proportions (50:50) (Figure 4B and Table 1). The formation of longer stems due to etiolation is a physiological process that affects the gene expression involved in light response and avoid shading. Higher competitive ability and plant capacity to intercept of light, the higher its ability to assimilate CO_2 and produce photoassimilates. Also, the increased height allows the shading of plants that are under competition, decreasing the net photosynthesis and, consequently, suppressing its development.

Table 1 **-** Relative yield differences (RY) and relative yield total (RYT) for the variables number of tillers, plant height, leaf area, and shoot dry weight in the proportions of susceptible (Susc) and resistant ryegrass plants (110)

Biotype	Mixture proportion of plants (%) (resistant: susceptible)		
	75:25	50:50	25:75
	Number of tillers (no. per plant)		
110 (R)	0.12 (±0.05)^{ns}	0.08 (±0.04)^{ns}	0.04 (±0.02)^{ns}
Susc	-0.03 (±0.01)*	-0.05 (±0.03)^{ns}	0.05 (±0.03)^{ns}
Total	1.09 (±0.05)^{ns}	1.03 (±0.06)^{ns}	1 (±0.02)^{ns}
	Plant size (cm per plant)		
110 (R)	0.02 (±0.02)^{ns}	0.00 (±0.02)^{ns}	0.00(±0.02)^{ns}
Susc	0.03 (±0.01)^{ns}	0.05 (±0.01)*	0.02 (±0.02)^{ns}
Total	1.05 (±0.04)^{ns}	1.06 (±0.03)*	1.02 (±0.02)^{ns}
	Leaf area (cm^2 per plant)		
110 (R)	0.02 (±0.03)^{ns}	0.00 (±0.03)^{ns}	0.00 (±0.02)^{ns}
Susc	0.01 (±0.03)^{ns}	-0.03 (±0.04)^{ns}	-0.04 (±0.05)^{ns}
Total	1.03 (±0.03)^{ns}	0.97 (±0.05)^{ns}	0.96 (±0.06)^{ns}
	Shoot dry weight (g per plant)		
110 (R)	-0.02 (±0.02)^{ns}	0.05 (±0.04)^{ns}	0.01 (±0.02)^{ns}
Susc	-0.01 (±0.02)^{ns}	-0.05 (±0.05)^{ns}	-0.11 (±0.05)^{ns}
Total	0.96 (±0.03)^{ns}	1.01 (±0.09)^{ns}	0.90 (±0.05)^{ns}

^{ns} Not significant; * significant by the t-test ($p \leq 0.05$). Values in parentheses represent the standard errors of the means.

No statistical difference was found for LA between biotypes at the different mixture proportions. A tendency to reduce RYT was observed for SDW in the proportion of 25:75. Only Susc reduced RY more than expected for this variable, indicating that lower proportions of the resistant biotype may lead to an impairment of the susceptible population growth (Figure 4d and Table 1). In other words, there was competition for resources and a greater period of competition associated with the selection pressure imposed by the herbicide, in which the biotype 110 is resistant, favoring the predominance of the resistant population over the generations.

No difference was observed between biotypes for the morphological variables NT, PH, LA, and SDW, showing that they are not affected by intraspecific competition for the analyzed variables (Table 2). In studies evaluating the intraspecific competition of biotypes resistant to ALS inhibiting herbicides, no differences of competitive ability have been found for several species (Sibony and Rubin, 2003; Ashigh and Tardif, 2007; Lamego et al., 2011; Légère et al., 2013). Ryegrass biotypes with a low level of resistance to fluazifop also did not present differences in competitive ability (Fraga et al., 2013).

The indices CR, K, and A showed a significant difference for the variable PH, where CR was higher than 1 and C below zero for the susceptible biotype, being considered more competitive than the resistant biotype. The observed results were already expected because the biotypes belong to the same species and geographical region (Tables 2 and 3).

The lack of negative effects on resistant biotypes favors evolution, propagation, and competitiveness of these species in agricultural systems (Légère et al., 2013). Special attention should be given to resistant biotypes that do not have a fitness cost, as they may compromise cultivation systems, making them unfeasible in areas with these biotypes, especially in crops such as wheat, in which ryegrass is the main weed.

Considering this scenario, it is essential to think of the productive system as a whole. The adoption of integrated weed management should be sought, i.e., in addition to chemical methods, other methods, such as physical and cultural, should be used to minimize reproduction and replenishment of banks of weed seeds in the soil, as well as their dispersion to other areas. Proactive attitudes are essential to contribute to the sustainability of agricultural activity from the standpoint of weed resistance to herbicides, ensuring maximum crop yield and profitability to farmers.

Table 2 **-** Responses to the number of tillers, plant height, leaf area, and shoot dry weight of susceptible ryegrass (Susc) competing with resistant ryegrass (110) under different plant proportions

Biotype	Mixture proportion of plants (resistant: susceptible)					CV (%)
	100:0	75:25	50:50	25:75	0:100	
	Number of tillers (no. per plant)					
110 (R)	5.49	5.83^{ns}	6.23^{ns}	6.344^{ns}	-	12.75
Susc	-	6.50^{ns}	6.57^{ns}	6.88^{ns}	7.29	9.19
	Plant size (cm per plant)					
110 (R)	40.85	42.20^{ns}	41.21^{ns}	41.24^{ns}	-	5.34
Susc	-	40.57^{ns}	40.34^{ns}	37.37^{ns}	36.34	6.38
	Leaf area (cm^2 per plant)					
110 (R)	93.85	96.24^{ns}	93.68^{ns}	93.77^{ns}	-	10.05
Susc	-	94.31^{ns}	84.60^{ns}	85.51^{ns}	90.52	13.08
	Shoot dry weight (g per plant)					
110 (R)	0.42	0.41^{ns}	0.46^{ns}	0.44^{ns}	-	12.08
Susc	-	0.45^{ns}	0.45^{ns}	0.48^{ns}	0.41	16.64

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

Table 3 **-** Competitive indices of resistant (110) and susceptible (Susc) ryegrass (*Lolium multiflorum*) biotypes expressed by the competitive ratio (CR), relative crowding coefficients (K), and aggressivity (A)

	CR	Ka$^{(5)}$	Kb$^{(6)}$	A
LA$^{(1)}$	1.07 (±0.03)^{ns}	1.02 (±0.12)^{ns}	0.88 (±0.07)	0.03 (±0.02)^{ns}
SDW$^{(2)}$	1.20 (±0.09)^{ns}	1.26 (±0.20)^{ns}	0.91 (±0.17)	0.08 (±0.04)^{ns}
PH$^{(3)}$	0.91 (±0.03)*	1.03 (±0.09)^{ns}	1.24 (±0.05)	-0.05 (±0.01)*
NT$^{(4)}$	1.30 (±0.05)^{ns}	1.45 (±0.23)^{ns}	0.84 (±0.12)	0.13 (±0.05)^{ns}

$^{(1)}$ Leaf area; $^{(2)}$ shoot dry weight; $^{(3)}$ plant height; $^{(4)}$ number of tillers; $^{(5)}$ biotype 110; $^{(6)}$ biotype Susc; ^{ns} Not significant and * significant by the t-test (p≤0.05). Values in parentheses represent the standard error of the mean.

In summary, the higher tillering period of resistant ryegrass is the main factor involved in the difference of phenological development between biotypes. No fitness cost was observed for resistant biotype, which was superior to the susceptible regarding PH, SDW, and AGR, while the susceptible biotype had a higher NT and LAR. Thus, resistant biotype had no fitness cost. For competitive ability, no difference was observed between biotypes in the different tested proportions when they occupied the same ecological niche.

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