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Article

Population Interference of Glyphosate Resistant and Susceptible Ryegrass on Eucalyptus Initial Development

Interferência Populacional de Azevém Resistente e Suscetível ao Glyphosate no Desenvolvimento Inicial do Eucalipto

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ABSTRACT - The repetitive use of herbicides with the same mechanism of action causes the selection of resistant weeds, such as ryegrass. Considering the occurrence of ryegrass (*Lolium multiflorum*) in eucalyptus, a crop on which glyphosate is used, it is necessary to study its interference. The objective of this study was to evaluate the effect of densities (0, 10, 20, 30 and 50 plants m^{-2}) of two ryegrass biotypes (resistant and susceptible to glyphosate) on seedlings of two eucalyptus clones (I-144 and 1407). The used experimental design was in randomized blocks with four replications, following a 2 x 5 factorial arrangement for each clone. The height and diameter of the clones were evaluated at 0, 14, 28, 42, 56 and 70 days after transplantation (DAT), and at 70 DAT, the leaf area and dry biomass of eucalyptus and ryegrass were also evaluated. There was no interaction between the biotype and densities factors for the clones, but all characteristics were affected by the factors separately. The increase in ryegrass densities affected all the characteristics evaluated in the clones, being the leaf area of the eucalyptus clones the most affected one, with reductions of up to 72%. The resistant biotype was less competitive, causing reductions in clones of up to 39% in leaf area, 5% in diameter and 1% in height, while the susceptible biotype resulted in reductions of 51%, 13% and 6%, respectively. Thus, the tolerable density by the culture to the resistant biotypes may be greater than that of the susceptible biotypes.

Keywords: *Lolium multiflorum, Eucalyptus urograndis*, competition.

*RESUMO - O uso repetitivo de herbicidas com o mesmo mecanismo de ação ocasiona a seleção de plantas daninhas resistentes, como o azevém. Tendo em vista a ocorrência de azevém (**Lolium multiflorum**) em eucaliptais, cultura na qual se usa o glyphosate, torna-se necessário estudar sua interferência. Neste trabalho, objetivou-se avaliar o efeito de densidades (0, 10, 20, 30 e 50 plantas m^{-2}) de dois biótipos de azevém (resistente e suscetível ao glyphosate) em mudas de dois clones de Eucalyptus urograndis (I-144 e 1407). O delineamento experimental adotado foi em blocos casualizados com quatro repetições, seguindo esquema fatorial 2x5 para cada clone. Aos 0, 14, 28, 42, 56 e 70 dias após o transplante (DAT), foram avaliados a altura e o diâmetro dos clones, e aos 70 DAT, a área foliar e a biomassa seca de parte aérea do eucalipto e do azevém. Não houve interação entre os fatores biótipos e densidades para os clones, mas todas as características foram afetadas pelos fatores isoladamente. O aumento das densidades do azevém alterou todas as características avaliadas nos clones, sendo a área foliar dos clones de eucaliptos a mais afetada, com reduções de até 72%. O biótipo resistente foi menos competitivo, ocasionou reduções nos clones de até*

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39% em área foliar, 5% em diâmetro e 1% em altura, ao passo que o biótipo suscetível ocasionou reduções de 51%, 13% e 6%, respectivamente. Desse modo, a densidade tolerável pela cultura dos biótipos resistentes pode ser maior que a dos biótipos suscetíveis.

Palavras-chave: *Lolium multiflorum, Eucalyptus urograndis*, competição.

INTRODUCTION

Eucalyptus crops have expanded in Brazil over the last decades and are prominent in the agricultural business, mainly due to the production of paper and cellulose (Sperotto, 2014). Exports of forest products reached US$ 2,565 million, with cellulose and paper accounting for 75% of this market (Brazil, 2016). Increased exports demand from the market greater productivity per planted area, and the success of the crop is associated with good agricultural practices. Weed management is a fundamental practice, since, when neglected, it causes damages that can lead to losses in volumetric productivity by more than 50%, depending on the weed community (Zen, 1987). Controlling these plants is important especially in the early years of the crop, and several studies report the damage generated by weeds in that period (Costa et al., 2004; Cruz et al., 2010). However, there are no reports comparing the initial interference generated by ryegrass biotypes in the crop, considering the growing importance of this weed in eucalyptus.

Ryegrass (*Lolium multiflorum*) is a weed with high seed production characteristics; their management is important mainly in winter cereal crops and in forest areas where the chemical control is limited due to the few products registered at MAPA – Ministério da Agricultura, Pecuária e Abastecimento (Rodrigues and Almeida, 2011). Another aggravating factor of this weed is that the continuous use of glyphosate caused the selection of resistant biotypes, making its control even more difficult (Roman et al., 2004).

The degree of weed interference in a crop is related to the environment, the crop (spacing, density and cultivar), the weed community (specific composition, density and distribution) and the time and period in which the coexistence occurs (Pitelli, 1985). As for the specific composition, studies show that there are competitiveness differences between ryegrass biotypes, and this difference can often be related to the cost that resistance generates for the biotype (Ferreira et al., 2008; Vila-Aiub et al., 2011). On the other hand, weed density is one of the most important factors; the greater the density, the greater the dispute of the individuals for the same resources of the environment and the more intense the competition with the culture (Barroso et al., 2012).

As for the eucalyptus crop, breeding programs try to develop superior hybrids in wood volume and quality, focusing only on economic characteristics. However, according to Pitelli (1985), the increase in the economic productivity of the cultivated species is often accompanied by a decrease in its competitive potential, and Medeiro et al. (2016) emphasize the need for studies that help identifying less sensitive genotypes to weed interference, in order to reduce the losses and costs for their management.

Based on the hypothesis that ryegrass biotypes with resistance to glyphosate may present changes in their competitive capacity against eucalyptus and that this competitiveness depends on their population density, the objective of this study was to evaluate the competitive capacity of two ryegrass biotypes, susceptible and resistant to glyphosate, and their interference in increasing population densities on the growth of the eucalyptus clones (*Eucalyptus urograndis*) I-144 and 1407.

MATERIAL AND METHODS

Dose-response curve

Before the competition experiment, glyphosate dose-response curves were performed with the ryegrass biotypes, in order to verify the resistance to the herbicide. Seeds were collected in the State of Rio Grande do Sul, Brazil, in Ijuí (28°23’18,72" S; 53°55’13,75" W) and Três de Maio (27°47’02,77" S; 54°14’05,06" W), which are sites of supposed susceptibility and resistance, respectively. They were sown in trays filled with horticultural substrate and, when seedlings were at the two-leaf stage, they were transplanted to 0.5 L plastic pots. With the expansion of the fourth leaf, plants were submitted to glyphosate application through a CO_2 pressurized backpack sprayer, equipped with four fan-jet type spraying nozzles (8002), calibrated to distribute 200 L ha^{-1} at a constant pressure of 200 kPa.

In this test, a completely randomized design (CRD) with four replications was used, where there were two ryegrass biotypes (R, resistant; and S, susceptible to glyphosate) under five glyphosate doses (180, 360, 720, 1,440, and 2,880 g a.e. ha^{-1}), and the control treatment without herbicide application. Twenty-one days after herbicide application, visual control scores were given, estimated between 0 and 100%, where 0% represented the absence of control and 100% the death of plants (SBCPD, 1995). The obtained results were submitted to log-logistic non-linear regression analysis, after the means of the treatments presented significant statistical differences in the analysis of variance ($p \leq 0.05$). Statistical analyses were performed using the Agroestat software, and regressions using the Origin software. In order to compare resistance, the resistance factor (RF) was calculated among the populations, and was expressed by the ratio between the doses required to control 50% of the population of resistant (R) and susceptible (S) plants, RF = D50 (R)/D50 (S) (Barroso et al., 2014).

Competition experiment

In the competition study, two experiments were carried out in the same period in 2015, under semi-controlled conditions, in plastic pots (21 L) filled with dystrophic Dark Red Latosol (Embrapa, 1999) mixed with sand (3:1 v/v). Physical and chemical analyses of the soil showed the following characteristics: clay texture; $CaCl_2$ 58; 15 g dm^{-3} of organic matter; 29 mg dm^{-3} of P in resin; and K, Ca, Mg, and H + Al contents of 2.3, 33, 13 and 22 $mmol_c$ dm^{-3}, respectively.

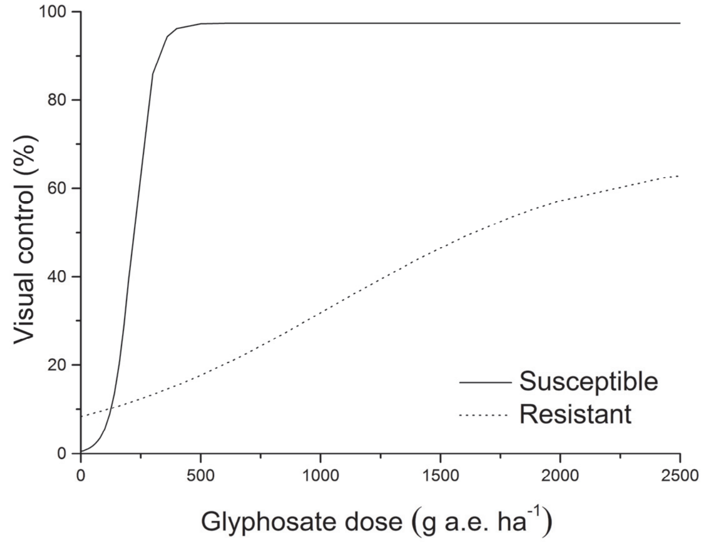
In each experiment, a eucalyptus plant was used in coexistence with ryegrass biotypes at increasing densities. The eucalyptus clones were I-144 and 1407, which are hybrids of the crossings of *Eucalyptus grandis* with *Eucalyptus urophylla*, known as *Eucalyptus urograndis*. The coexistence densities with ryegrass were 0 (no coexistence); 10, 20, 30 and 50 plants m^{-2}. Eucalyptus seedlings were transplanted in the center of the pots, and ryegrass seedlings were transplanted radially at a mean distance of 5 cm from the eucalyptus seedlings. Planting fertilization was done by depositing in each pot the 4-14-8 formulated fertilizer in an amount equivalent to 500 kg ha^{-1}. Twenty-one and 35 days after planting, top-dressing was carried out with urea diluted in water at a concentration of 0.5% (p/v).

The adopted experimental design was a randomized complete block (RCB) with four replications, with treatments arranged in a 2 x 5 factorial arrangement for each clone, in which the two main ryegrass biotypes in five increasing densities were the main factors. Plant height and stem diameter (Zaas, digital 6") were evaluated on eucalyptus plants 0, 14, 28, 42, 56 and 70 days after transplanting (DAT), and at 70 DAT, in addition to these measurements, the leaf area of the eucalyptus (LiCor, LI3100A) and the dry biomass of the shoot and stem of eucalyptus and ryegrass were also evaluated, after drying in a forced air circulation oven at 60 °C, until constant weight. The obtained data were submitted to analysis of variance by F test and, when significant, regression analyses were performed within each evaluation period. In the analyses, the Agroestat software was used, and, for the regressions, the Origin software.

RESULTS AND DISCUSSION

Dose-response curve

The percentage of biotype control according to increasing doses of glyphosate was different between the two biotypes (Figure 1). The biotype from Três de Maio (hereinafter referred to as R) presented a Resistance Factor (RF) of 7.50 in relation to the Ijuí (S) biotype, demonstrating that the resistant population needs 7.50 times more herbicide to control 50% of its population. Another study with *L. multiflorum* seeds from the municipality of Vacaria, also in the State of Rio Grande do Sul, showed that ryegrass biotypes presented a resistance factor of 16.8 (Vargas et al., 2005).



R: Y=67.41/(1+exp(-0.0018(x-1,062.42))), R^2=0.99; S: Y=88.17/(1+exp(-0.021(x-339.74))), R^2=0.79.

Figure 1 **-** Visual control (%) of resistant and susceptible genotypes, 21 days after the application of increasing doses of glyphosate.

Roman et al. (2004) observed that the 360 g a.e. ha^{-1} dose of glyphosate was enough to fully control the susceptible ryegrass biotype, and in another biotype, doses of up to 1,440 g a.e. ha^{-1} caused toxicity below 15%, and doses up to 5,760 g a.e. ha^{-1}, below 50%, thus confirming its resistance. In literature there are some differences in relation to the resistance factor of ryegrass biotypes, but these differences may be related to environmental conditions, herbicide formulas and conditions of application and growth of the species (Vargas et al., 2005).

Competition experiment

In the competition study, there was no significant interaction between biotypes and density factors for the characteristics evaluated on the two eucalyptus clones; however, the plant height, stem diameter, leaf area and dry biomass of the two clones were affected by the factors separately.

For the eucalyptus plant height (Figure 2A and B), evaluations prior to 56 DAT were not significant. However, from this evaluation, ryegrass densities caused reductions in both clones. At 70 DAT, the density of 10 plants m^{-2} of ryegrass reduced the height of the two eucalyptus clones by 17%, compared to the control treatment. With 50 m^{-2} of ryegrass plants, eucalyptus plants were reduced by 25% and 26% in clones I-144 and 1407, respectively.

Medeiro et al. (2016), evaluating only the presence and absence of weeds in the eucalyptus crop, reported height reductions. Eucalyptus clones in coexistence with *Panicum maximum* had their height reduced by about 26%, and in coexistence with *Ipomoea nil*, the reduction reached 40% at 60 DAT. For Londero et al. (2012), at day 378, weeds reduced eucalyptus height by 25.9%, while Costa et al. (2004) observed a reduction of 21% in only 40 days of coexistence. The height decrease of the clones with the increase of ryegrass densities, in relation to the other evaluated characteristics, was the one presenting smaller losses. These data corroborate those reported by Cruz et al. (2010), who also found that, among the analyzed characteristics, the smallest decreases were in height, when evaluating eucalyptus in the presence of Guinea grass (*Panicum maximum*).

High plants have an advantage over some weeds, because they are able to capture more solar radiation and, therefore, they demonstrate greater competition capacity. However, for eucalyptus plants, this characteristic does not determine competitiveness. Height increments alone are not interesting, because with them, the plant diameter is reduced, which is a part of economic interest. In addition, studies report that height does not influence the competitive abilities of tree species (Kunstler et al., 2016).

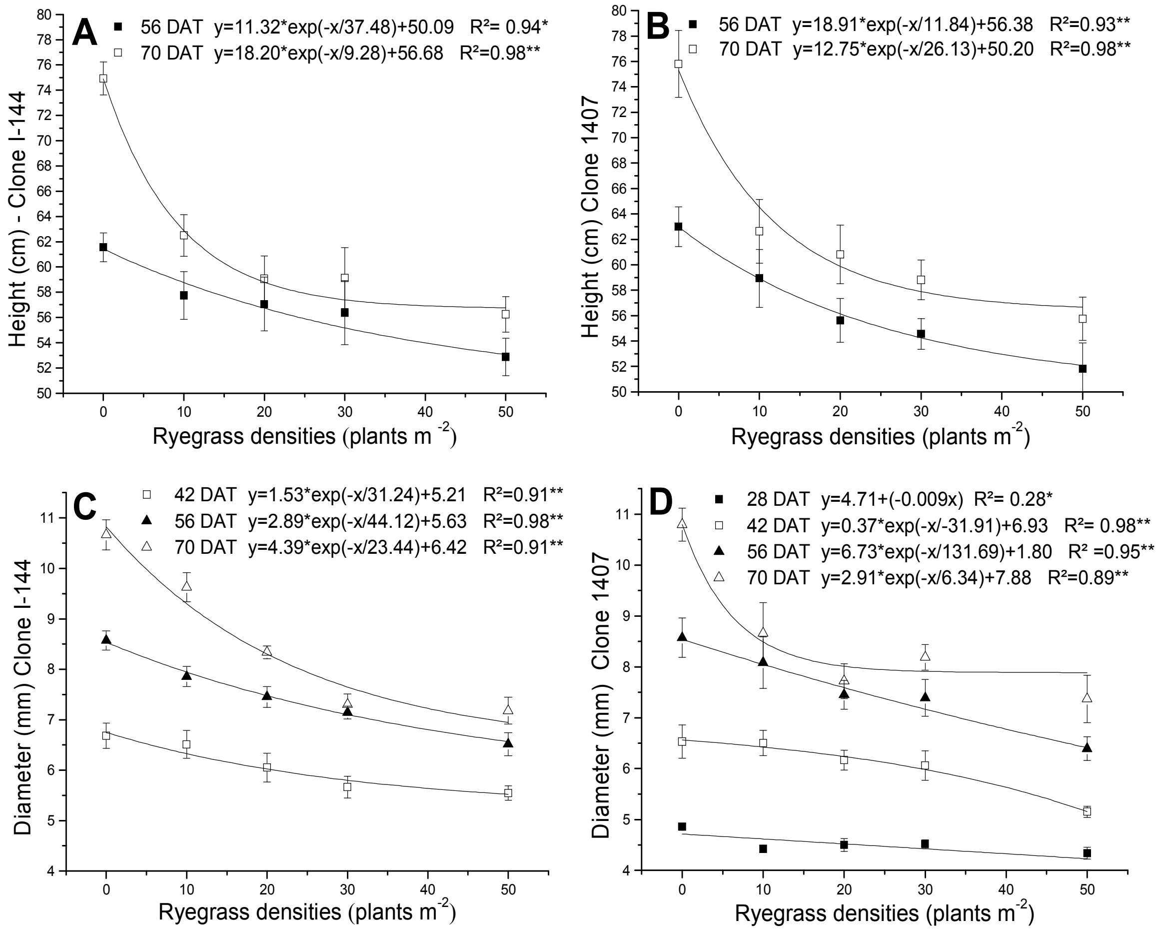


Figure 2 - Mean values ± standard deviation of height (A and B) and diameter (C and D) of clones I-144 and 1407 in coexistence with increasing ryegrass densities.

On the other hand, the diameter or thickening of the stem is directly related to the production of wood/cellulose. According to Wink et al. (2012), it is only possible to measure the diameter of eucalyptus plants to make inferences about characteristics that are more difficult to measure and to estimate forest productivity. Thus, the diameter is one of the most important characteristics in growth studies. Only the presence of ryegrass plants at low densities (10 m^{-2} plants) did not cause remarkable losses. The stem diameter of clone I-144 (Figure 2C) was affected by ryegrass densities from 42 DAT only at a density above 10 m^{-2} plants. At 56 and 70 DAT, the 50 plants m^{-2} density was the most damaging, reducing by 24% (56 DAT), and from the 30 plants m^{-2} density, losses were equal (70 DAT). In clone 1407 (Figure 2D), at 42 DAT, only the 50 plants m^{-2} density differed from the others, and the diameter decreased by about 21%; the other densities had no differences in relation to the control treatment. At 56 DAT, there was greater reduction from the density of 20 plants m^{-2}. It is possible to observe that, with the increase of the coexistence time, clone 1407 appeared to be more sensitive to the interference of ryegrass with the increase of the population densities; at 70 DAT, the 10 plants m^{-2} density caused a 20% decrease in the diameter, and the 50 plants m^{-2} density caused a 32% decrease in relation to the control treatment.

Diameter reductions, in a greater coexistence period with *P. maximum*, reached 36% at 90 DAT (Cruz et al., 2010). Bacha et al. (2016) observed similar results at 75 DAT, when the reduction in the stem diameter at the density of 10.4 plants m^{-2} of *Urochloa decumbens* was 30%. Aparício et al. (2010) verified that the diameter was the most sensitive variable in clones of *Eucalyptus* x *urograndis* when coexisting with weeds.

According to Fleck et al. (2006), plants that produce more dry biomass cause greater reduction in the resources of the environment, causing suppression in the growth of neighboring plants.

Studies that evaluate dry biomass make it possible to infer the competitive capacity of one species over the other when they grow together. The densities of 30 and 50 plants m^{-2} reduced the dry biomass of clone I-144 leaves (Figure 3A) by 64% and 66%, followed by the density of 20 plants m^{-2} (50% reduction). The least damaging was 10 plants m^{-2}, which caused a 37% reduction in relation to the control treatment.

The stem dry biomass (Figure 3A) was also lower at the density of 50 plants m^{-2}, which decreased by 66% compared to the control treatment, followed by densities of 20 and 30 (48% and 55% reduction); the least damaging was the lowest density, with a 24% reduction. Clone 1407 was also sensitive to ryegrass interference as for leaf dry biomass (Figure 3B) at the density of 50 plants m^{-2} (65% reduction), followed by densities of 20 and 30 (53 and 54% reduction), and 10 m^{-2} plants (41% reduction). The dry biomass of the stem (Figure 3B) maintained a similar behavior: the density of 50 plants m^{-2} was the most damaging (68% reduction), followed by densities of 20 and 30 plants m^{-2} (52% and 55%), and the lowest density caused a 36% reduction in relation to the control treatment.

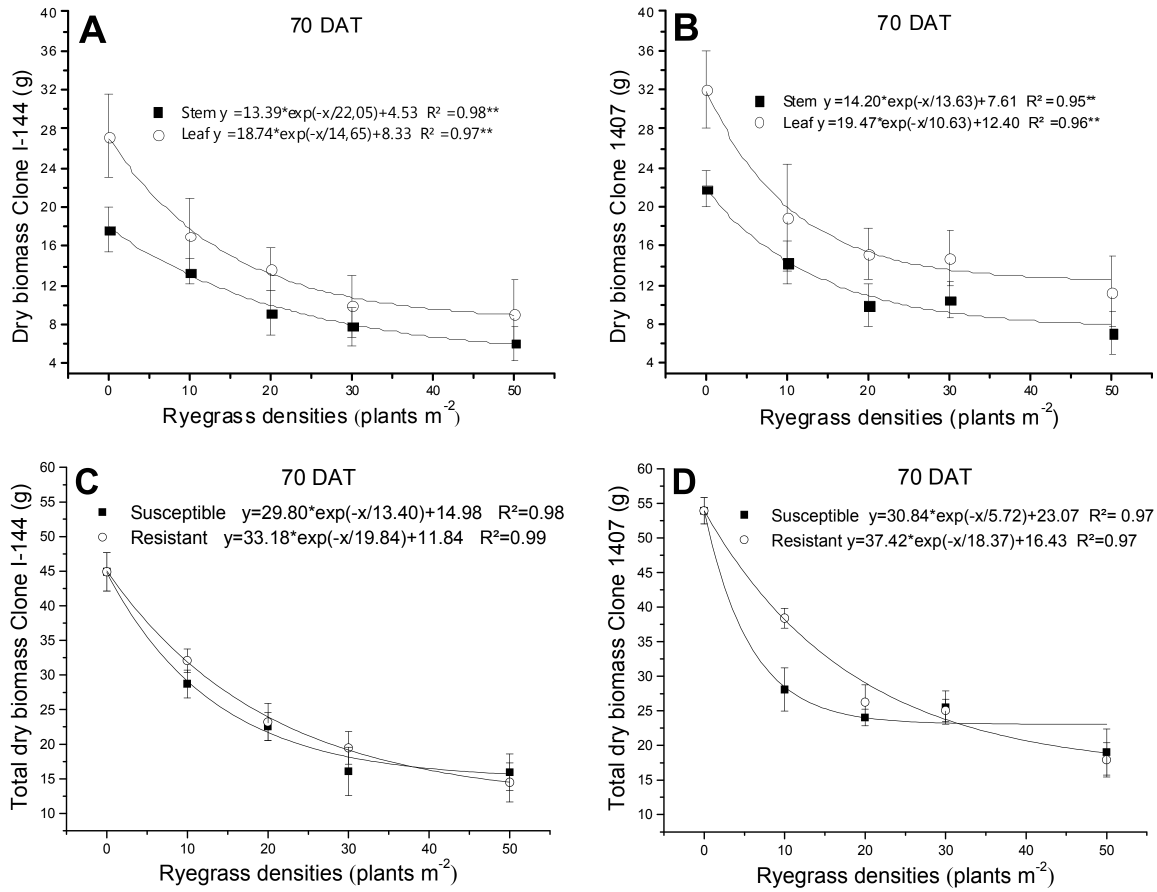


Figure 3 - Average values ± standard deviation of dry biomass of stem and leaves of clones I-144 (A) and 1407 (B); and total dry biomass (C and D) in coexistence with increasing densities of ryegrass biotypes.

When analyzing the total dry biomass values of the clones in competition with the two ryegrass biotypes, it was observed that the eucalyptus clone I-144 (Figure 3C) showed no difference as for total dry biomass in relation to the presence of the two biotypes; whereas 1407 (Figure 3D), at the density of 10 plants m^{-2}, produced less biomass in competition with the susceptible biotype. At other densities, the behavior was similar. According to Fleck et al. (2006), a higher biomass production would lead to a greater competitive ability, but when the dry biomass production of the susceptible biotype was compared with that of the resistant biotype in the presence of clone 1407 (Figure 4B), data were very similar, differing only at the density of 10 plants m^{-2}, where the resistant biotype produced a higher amount of shoot dry biomass. Thus, the suppression difference of the biotypes observed in Figure 3D was not a result of the higher shoot dry biomass production of the susceptible biotype.

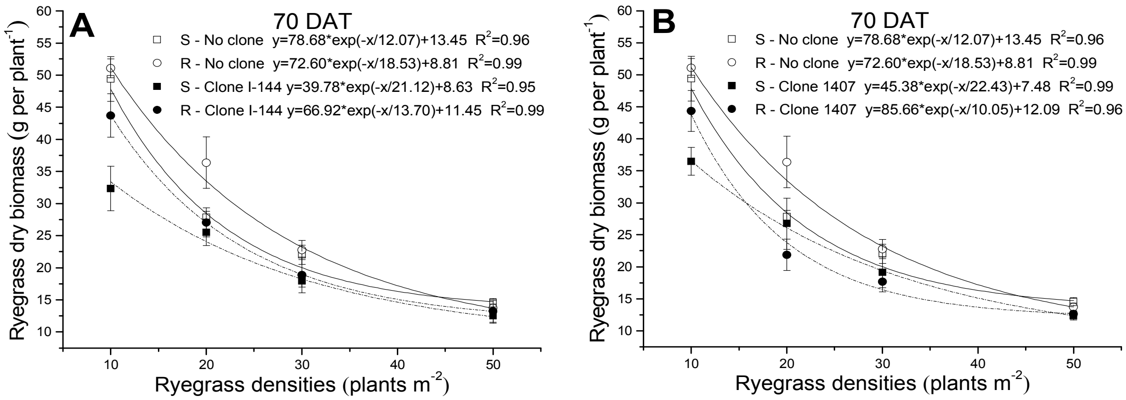


Figure 4 - Mean values ± standard deviation of shoot dry biomass of S (susceptible) and R (resistant) ryegrass biotypes at increasing densities, in the presence and absence of eucalyptus clones I-144 (A) and 1407 (B).

The presence of clones I-144 (Figure 4A) and 1407 (Figure 4B) also reduced the dry biomass of the two ryegrass biotypes, and this reduction was proportional to the increase of the densities, that is, with the increase of ryegrass densities, competition ceases to be interspecies and becomes intraspecies. The reduction of dry biomass resulting from increased plant densities may be influenced by some factors, such as light, nutrients and water, in which increased plant densities enhance the competition for these resources (Swanton et al., 2015).

According to Cruz et al. (2010), leaf area and dry biomass are good characteristics to be evaluated in eucalyptus plants to diagnose the effects of weed interference, and in their work, these were the most sensitive characteristics. In this study, these characteristics were also the most impaired in the experimental period; the greater the population density of ryegrass plants, the greater the reductions. Among all the analyzed characteristics in both clones, the leaf area was the most sensitive to ryegrass interference.

The leaf area of clone I-144 (Figure 5A) showed the greatest decrease between the control treatment and the density of 10 plants m^{-2}, that is, this density caused a marked reduction for this characteristic; the other densities were detrimental, but showed a tendency to stabilize losses. This decrease was 46% at the density of 10 plants m^{-2} and 69% with 50 plants m^{-2}. For clone 1407 (Figure 5A), the reduction was 53% at the lowest density and 72% at the greatest. A greater reduction was observed in clone 1407 as for leaf area when comparing it with the control treatment, mainly with increasing densities, but it maintained its averages higher than clone I-144. The decrease in the leaf area is detrimental to the development of clones, since it is related to a lower investment in branches and leaves, due to the stress imposed by the competition, which can compromise the survival of the seedlings on the field or generate substantial losses in productivity, mainly because it reduces the photosynthetic apparatus of plants (Medeiro et al., 2016).

Clone I-144 (Figure 5B) in coexistence with the resistant biotype had a leaf area 18% higher than the clone that coexisted with the susceptible biotype. There was a significant difference between the susceptible and the resistant biotypes, also for the diameter of clone I-144 (Figure 5C) and for the height of clone 1407 (Figure 5D). At 42 DAT, the resistant biotype was less competitive than the susceptible one, because the diameter was about 8% larger in clone I-144 than in the susceptible one. Clone 1407 was also less affected by the interference of the resistant biotype at 28 and 42 DAT, where height was about 8% and 5% more, respectively. Ferreira et al. (2008) observed that the susceptible ryegrass biotype reduced the values of dry biomass, leaf area and height of wheat plants, but when they competed with the resistant biotype, the decrease in these characteristics tended to be lower.

The resistant ryegrass biotype, regardless of the population density, was less competitive than the susceptible biotype. Several studies comparing the competitive ability between resistant and susceptible weed biotypes demonstrated greater damage to the resistant than the susceptible biotype (Ferreira et al., 2008; Galon et al., 2013). These competitiveness differences in the biotypes can often be related to the different sites of collection and occurrence; however, for the

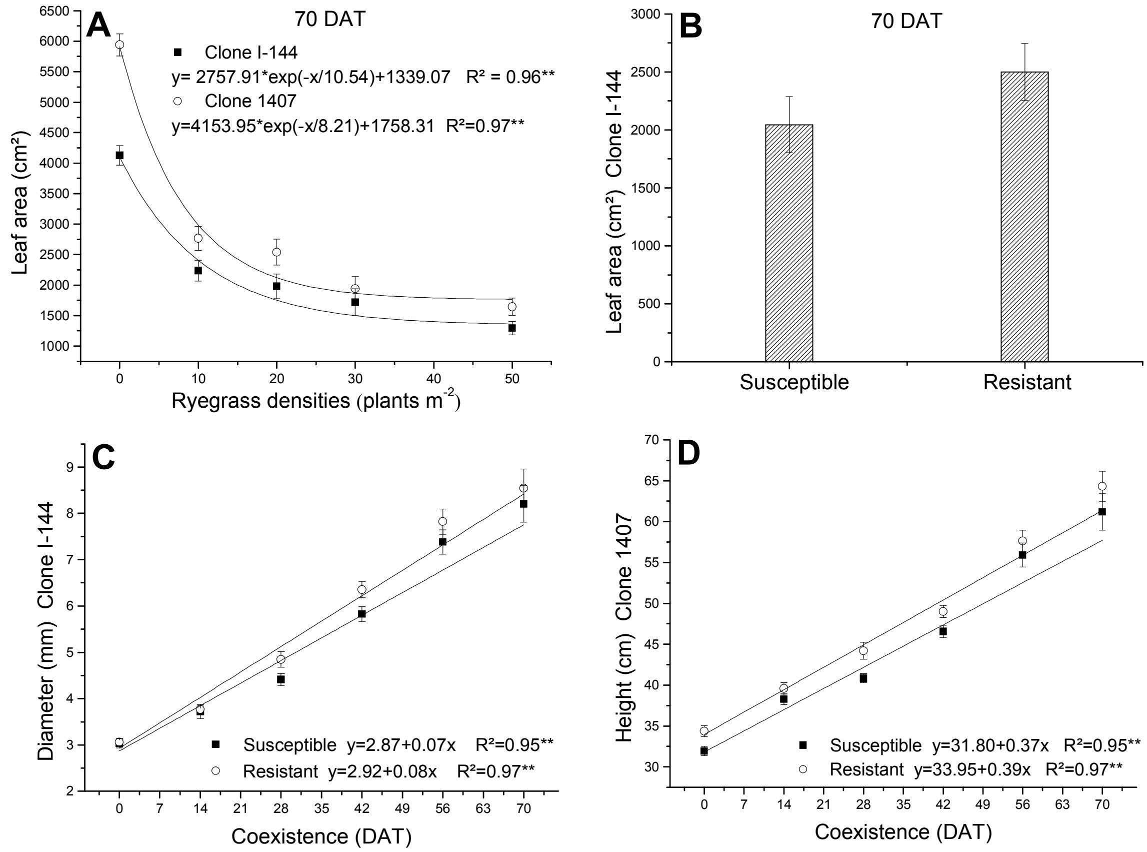


Figure 5 **-** Mean values ± standard deviation of the leaf area of clones I-144 and 1407 in increasing ryegrass densities (A) and of clone I-144 in coexistence with ryegrass biotypes (B), 70 days after transplanting (DAT), diameter of clone I-144 (C) and height of clone 1407 (D) in different evaluation periods, in coexistence with ryegrass biotypes.

studied biotypes, the collection sites are close, since the occurrence of the species is restricted to Rio Grande do Sul and to regions of milder climates, and thus, they probably present a common evolution origin.

The resistant biotype tends to predominate in crops with a repetitive use of glyphosate - sorting agent, but in natural environments, this would probably not happen (Galon et al., 2013). This is because susceptible biotypes have demonstrated to be more adapted, and the more adapted, the greater their competitive capacity in the absence of the herbicide (Christoffoleti et al., 1997). However, it is possible to infer that the occurrence of physiological and adaptive changes in weeds resulting from resistance can be varied depending on each species and on the used herbicide (Dal Magro et al., 2011).

Vila-Aiub et al. (2015) explain that when resistant biotypes show an adaptability reduction, without the application of herbicides, it is due to a "cost" that resistance has for plants. This resistance cost is generated because weeds would be using resources for their defense against herbicides, rather than using them in their growth and reproduction (Vila-Aiub et al., 2011). Yanniccari et al. (2016), comparing ryegrass biotypes, observed height, leaf area, biomass and seed number reductions in the resistant ones in relation to the susceptible ones, showing that, without the presence of a selective agent, the susceptible biotypes are more vigorous and productive. Thus, the competitiveness difference observed in this study between ryegrass biotypes and eucalyptus clones may be related to the cost that the resistance generated for this biotype. From a practical point of view, the resistant biotype, being less competitive, would require more plants per area to cause the same losses generated by the susceptible biotype.

Considering the competitiveness difference between biotypes, the number of plants per area (m^{-2}) of each ryegrass biotype required to cause 5% to 20% losses in the total dry biomass characteristic of the eucalyptus clones was estimated (Table 1). This estimate was done based on the equations obtained from the regression analyses presented in Figure 3C and 3D.

Table 1 - Estimated values of susceptible and resistant ryegrass densities (plants m^{-2}) required to cause losses from 5% to 20% in the total dry biomass of eucalyptus clones I-144 and 1407 at 70 DAT

Biotype	Clone I-144				Clone 1407			
	5%	10%	15%	20%	5%	10%	15%	20%
Susceptible	1	2	3	5	1	1	2	3
Resistant	1	3	5	6	1	3	3	8

Clone 1407, when admitting losses of 20%, would require approximately 8 plants m^{-2} of the resistant biotype and only about 3 plants m^{-2} of the susceptible one. This difference in behavior may be due to possible requirement differences of each biotype, such as the cost that resistance generated for them (Vila-Aiub et al., 2011), and caused them to decrease their competitive capacity.

In conclusion, it is known that glyphosate is the mostly used herbicide for weed management on eucalyptus crops, and in fields with resistant biotypes, after controlling susceptible biotypes, it is possible to admit a greater population of resistant biotypes than susceptible biotypes. However, for further elucidation, it would be necessary to conduct other experiments on the field, in order to study the periods of interference with each biotype and their coexistence densities, in addition to the molecular and physiological characteristics.

Increased population density of ryegrass, whether or not resistant to glyphosate, caused gradual reductions in the growth of clones I-144 and 1407. The resistant biotype was less competitive for certain growth traits; thus, its tolerable density for the culture may be greater than the one of susceptible biotypes.

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