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Article

Fitness costs of susceptible and resistant radish biotypes to ALS-inhibitor herbicides

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Abstract

Radish is an important weed that causes yield reduction in winter crops in southern Brazil. The control chemical of radish is an important and essential step to prevent damage on crops. However the intensive use of ALS-inhibitors herbicides favors the selection resistant biotypes. The selection can influence the adaptive traits of biotypes with physiological changes and growth variables and plant reproduction. The objective of the study was compare the fitness costs of susceptible and resistant radish biotypes to ALS-inhibitors herbicides. The experiment was conducted in greenhouse using completely randomized design with five replications. The treatments were arranged in factorial arrangement corresponding to susceptible and resistant biotypes (B_1 and B_4) and nine sampling times (14, 28, 42, 56, 70, 84, 98, 112 and 126 days after emergency), respectively. The variables evaluated were plant height, shoot dry matter, root dry matter, total dry matter, leaf area, growth rate, relative growth rate, leaf area ratio, number of siliques and seeds produced per plant. The results showed that the resistant biotype (B_4) had no fitness costs when compared to the susceptible biotype (B_1).

Keywords: adaptability, competition, resistance evolution, weed

Introduction

Raphanus sativus L. (radish) is important dicotyledonous weed cross-fertilization that cause high yield losses in wheat, barley and canola crops (Vargas & Roman, 2005). The ALS-inhibitors herbicides is the main tool used for radish control in winter crops and the intensive use of this herbicide class favors the selection of resistant biotypes (Pandolfo et al., 2013). The weed control is a practice that increases the selection pressure and can influence the evolution of adaptive traits (Délye, 2013).

In agricultural areas, crops and weeds compete and utilize the limited resources of environment for defense, growth and reproduction (Vila-Aiub et al., 2005). The survival of resistant biotypes when submitted to spray of herbicide may be considered an adaptive advantage when compared to susceptible biotypes (Délye, 2013). However, resistant biotypes could have different fitness costs when compared to susceptible biotypes in herbicide-free environments (Délye et al., 2013; Vila-Aiub et al., 2015).

Changes in physiological characteristics and growth rate of resistant biotypes may change the competitiveness and dynamics of a population, affecting the management and the prevention of resistance (Christoffoleti, 2001; Li et al., 2013). However, fitness costs of resistant

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biotypes depend on resistance mechanism (target-site or non-target-site of the enzyme) and species involved (Tardif et al., 2006; Yu et al., 2010), which may occur less vegetative growth and lower allocation of resources in reproductive phase (Vila-Aiub et al., 2009; Li et al., 2013).

Studies about fitness costs of biotypes allow evaluating the capacity of survival and the persistence of the species as a function of available resources in ecological niche, helping to establish the management strategies and prevention of resistance.

The objective of the study was to compare the fitness costs of susceptible and resistant radish biotypes to ALS- inhibitors herbicides.

Material and Methods

The experiment was conducted in greenhouse using a completely randomized design with five replicates. The experimental units were composed of plastic pots with eight liters volumetric capacity containing loamy red-yellow soil and GerminaPlant® substrate at 2:1 proportion, which were previously corrected according to soil analysis. The susceptible (B_1) and resistant (B_4) biotypes from Três de Maio-RS city had their susceptibility and resistance proven to ALS-inhibitors herbicides (Cechin et al., 2016).

The treatments were arranged in factorial design where the factor A was the susceptible and resistant biotypes (B_1 and B_4) and the factor B was composed by nine sampling times (at 14, 28, 42, 56, 70, 84, 98, 112 and 126 days after seedling emergency).

The variables evaluated in each time were plant height, shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM), leaf area, growth rate (GR), relative growth rate (RGR), leaf area ratio (LAR), number of siliques and seeds per plant, according to the methodology proposed by Benincasa (Benincasa, 2004). The plant height was determined with a millimeter ruler, measuring the distance from the ground to the last extended leaf. The SDM (leaves and stem) and the RDM were determined by drying the material in an oven with forced air circulation at 60°C for 72 hours. The TDM was obtained by addition of SDM and RDM at each sampling time. The leaf area was determined in leaf area meter (model LI 3100C) using the leaves without petiole.

The growth rate was obtained by equation: $GR_{n-1} = (W_2-W_1) / (t_2-t_1) + GR_1$, where: W_2 and W_1 are SDM of two successive samplings, t_2 and t_1 are days elapsed between two observations and GR_1 is the growth rate observed in the previous sample. The relative growth rate was obtained by equation: $RGR= (\ln W_2-\ln W_1) / (t_2-t_1)$, where: $\ln W_2$ and $\ln W_1$ are neperian logarithm the SDM of two successive samplings and, t_2 and t_1 the days elapsed between two observations. The leaf area ratio was determined by equation: LAR= Leaf area/SDM. The number of siliques and seeds per plant was determined at 126 days after emergency.

The results were analyzed for normality (Shapiro-Wilk test) and submitted to analysis of variance ($p \leq 0.05$). Thereafter, it was performed regression analysis using SigmaPlot 12.0 software (Sigmaplot, 2012). For the variables number of siliques and seeds per plant, the means were compared by *t* Test ($p \leq 0.05$).

Results and Discussion

The Shapiro Wilk test indicated not to be necessary the data transformation data. The analysis of variance demonstrated interaction between the factors biotypes and sampling times for all evaluated variables, except for root dry matter that presented simple effect for the sampling times factor (Figures 1 and 2). For the variables number of siliques and seeds produced per plant it was not evidenced statistical differences between susceptible and resistant biotypes (Table 1).

For plant height variable, the results demonstrated similar development between biotypes until 112 DAE where the height of resistant biotype (B_4) was 24% higher than the susceptible biotype (B_1) at 126 DAE (Figure 1A). Plants with higher height become more competitive, especially in solar radiation absorption and they can express greater productive potential (Rigoli et al., 2009). Yet, in *Brassica rapa* L. resistant biotypes to atrazine herbicide, the plants had lower height without reduction the yield when compared to susceptible biotype (Newell, 2006).

The results of SDM indicate increase and similar accumulation between the biotypes until

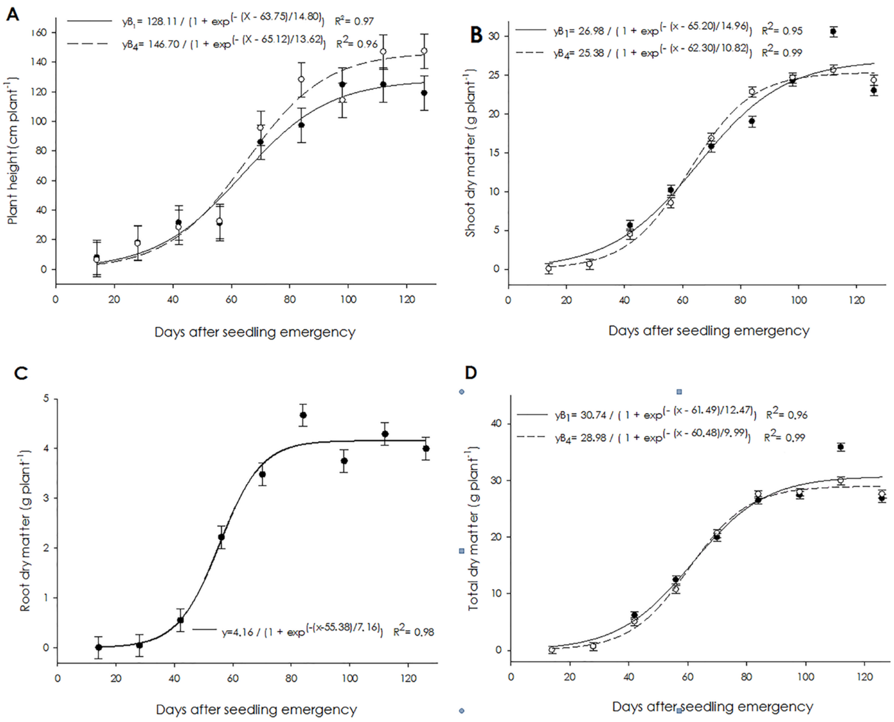

Figure 1. Plant height (A), shoot dry matter (B), root dry matter (C) and total dry matter (D) of radish susceptible (B_1) and resistant (B_4) biotypes to ALS-inhibitors herbicides, and evaluated at 14 until 126 days after seedling emergency. FAEM/UFPel, Capão do Leão/RS.

112 DAE, and the higher value was 30.62 and 25.67 g plant^{-1} to B_1 and B_4 biotypes, respectively (Figure 1B). Similar results were obtained in *Raphanus raphanistrum* L. resistant biotypes to ALS-inhibitors herbicides, but there was no significant change of SDM (Li et al., 2013). The SDM accumulation is dependent of the photosynthetic activity and plants that produce higher quantities of SDM in a shorter time are more competitive and can suppress the growth of other plants in environments with limited resources (Fleck et al., 2006).

For RDM, there was no significant difference between B_1 and B_4 biotypes where the accumulation was above 4 g plant^{-1} after 84 DAE (Figure 1C). Similar results were obtained in *Euphorbia heterophylla* L. biotypes, with no difference for RDM accumulation between susceptible and resistant biotypes to ALS-inhibitors herbicides (Brighenti et al., 2001). However, plants with higher root system development may be more efficient at absorption of soil resources and more competitive (Li et al., 2013; Darmency et al., 2013).

The results obtained for TDM demonstrated that the accumulation was similar between the biotypes in the sampling times with accumulation of 35.8 and 29.9 g plant^{-1} to B_1 and B_4, respectively, at 112 DAE (Figure 1D). In *Amaranthus retroflexus* L. it was not detected differences of TDM accumulation between susceptible and resistant biotypes to triazine and sulfometuron herbicides indicating that there were no fitness costs (Sibony & Rubin, 2003).

For leaf area variable, it was found that there were no differences between susceptible and resistant biotypes during the sampling times (Figure 2A). The results demonstrated that leaf area at 70 DAE was 1536 and 1445 cm^2 plant^{-1} to B_1 and B_4, respectively (Figure 2A). Similar results were found in *Amaranthus retroflexus* L. resistant biotypes to sulfometuron and triazine herbicides, registering higher leaf area at 69 DAE and no difference to susceptible biotype (Sinoby & Rubin, 2003).

The results indicate that the GR of radish biotypes was similar during the sampling times (Figure 2B). Similar results were found in *Cyperus difformis* L. resistant biotypes to pirazasulfuron-ethyl where the GR not differed from susceptible biotype (Dal Magro et al., 2011). The higher increase was evidenced between 28 and 98

DAE where the GR was 0.34 g plant^{-1}day^{-1} for both biotypes (B_1 and B_4) proving their relationship with SDM accumulation and leaf area (Figure 2). In *Fimbristylis miliacea* (L.) Vahl, the results reported that the resistant biotype GR to ALS-inhibitors herbicides was 15% higher when compared to susceptible biotype (Schaedler et al., 2013). Changes of GR can express higher competitive capacity and improve plant development (Vila-Aiub et al., 2005). For RGR variable, the results show that there was a similar reduction between B_1 and B_4 biotypes during the sampling times (Figure 1C). The absence of differences in RGR is an indicative that the biotypes may have similar competitive capacity and yield (Brighenti et al., 2001). The RGR decrease with time elapsing occurs due to the increasing dry matter and assimilate demand to maintenance of the plant structures, decreasing its availability to grow (Benincasa, 2004).

The LAR decreased about 3.36 cm^2 g^{-1}day^{-1} and it was similar to B_1 and B_4 biotypes during the sampling times (Figure 2D). The decrease can have occurred by the formation of plant vegetative and reproductive structures, which are competitive drains that reduce assimilates for the leaves (Benincasa, 2004). Plants that have higher LAR may be more competitive for essential resources for their survival (Ferreira et al., 2008).

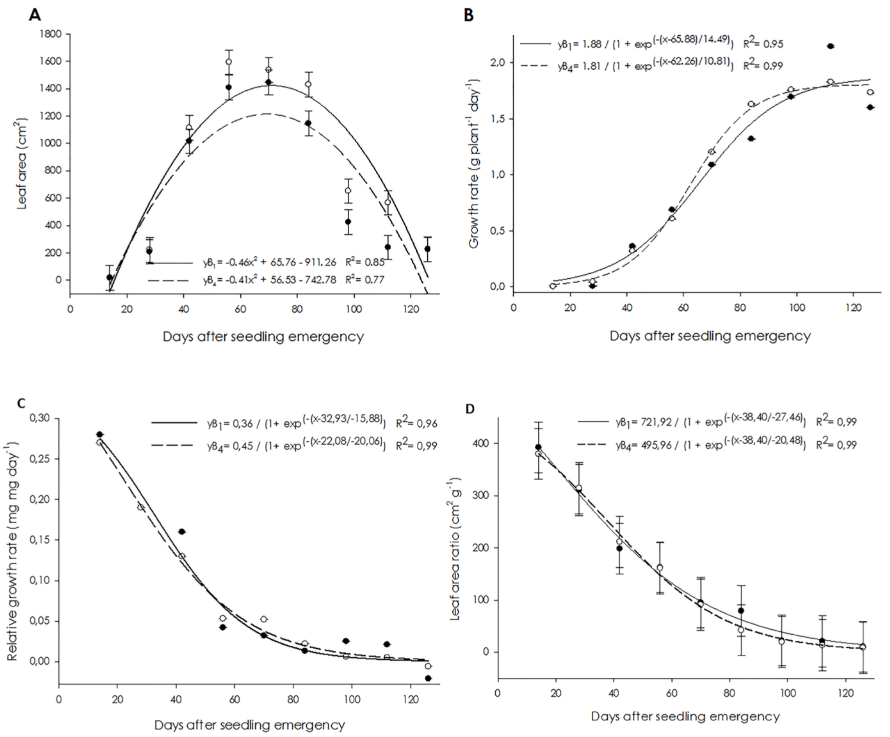

Figure 2. Leaf area (A), growth rate (B), relative growth rate (C) and leaf area ratio (D) of radish susceptible (B_1) and resistant (B_4) biotypes to ALS-inhibitors herbicides evaluated at 14 until 126 days after seedling emergency. FAEM/UFPel, Capão do Leão/RS. Points represent the means values and the bars represent least significant difference ($p<0.05$).

For the number of siliques and seeds produced per plant, the results demonstrated that there was no difference between susceptible (B_1) and resistant (B_4) biotypes at 126 DAE (Table 1). Similar results were found in *Raphanus raphanistrum* L. resistant biotypes to ALS-inhibitors herbicides who reported no differences for number of seeds per plant (Roles & Conner, 2008). However, the seeds production of susceptible biotype was 38% higher when compared to the resistant one (Table 1).

Results demonstrated that the seed production can be 30% less in plants that presented ALS gene mutation when compared with plants without mutation (Darmency et al., 2013). Seed production is an important factor that impact the life cycle of biotypes and that may be used as a management tool of resistant weeds (Vila-Aiub et al., 2015).

The results obtained in this study are similar to other researches with weeds resistant to ALS-inhibitors herbicides, with no reported fitness costs for resistant biotypes (Yu et al., 2010; Dal Magro et al., 2011; Li et al., 2013). The absence of fitness costs is a factor that may contribute to a rapid evolution and dispersion of resistance in agricultural areas and, thus, implicating agricultural sustainability in different crops.

Table 1. Number of siliques and seeds produced per plant in radish susceptible (B_1) and resistant (B_4) biotypes to ALS-inhibitors herbicides at 126 days after emergency. FAEM/UFPel, Capão do Leão, RS.

Biotypes	Number of siliques (no. plant^{-1})	Number of seeds (no plant^{-1})
B_1 (susceptible)	209 A	778 A
B_4 (Resistant)	199 A	482 A
V.C. (%)	42,3	30,2

*means followed by the uppercase letter (column) do not differ by *t* Test (p≤0,05).

Conclusions

The radish resistant biotype (B_4) to ALS-inhibitors herbicides does not show fitness costs when compared to susceptible biotype (B_1).

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