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Fitness Cost of Imazamox Resistance in Wild Poinsettia (*Euphorbia heterophylla* L.)

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Abstract: Wild poinsettia (*Euphorbia heterophylla* L.) is a difficult-to-control weed in soybean production in Brazil that has developed resistance to herbicides, including acetolactate synthase inhibitors. We investigated the potential fitness cost associated to the Ser-653-Asn mutation that confers imazamox resistance in this weed. Plant height, leaf and stem dry weight, leaf area and seed production per plant as well as the growth indices of specific leaf area, leaf area ratio, relative growth rate and net assimilation in F_2 homozygous resistant (R) and susceptible (S) wild poinsettia progenies were pairwise compared. S plants were superior in most of the traits studied. Plant heights for S and R biotypes, recorded at 95 days after planting (DAP), were 137 and 120 cm, respectively. Leaf areas were 742 and 1048 cm^2 in the R and S biotypes, respectively. The dry weights of leaves and stems in the S plants were 30 and 35%, respectively, higher than in the R plants. In both biotypes, the leaves had a greater share in dry weight at early development stages, but from 50 DAP, the stem became the main contributor to the dry weight of the shoots. The R biotype produced 110 ± 4 seed plant^{-1}, i.e., $12 \pm 3\%$ less seeds per plant than that of the S one (125 ± 7 seed plant^{-1}). The growth indices leaf area ratio and specific leaf area were generally higher in the S biotype or similar between both biotypes; while the relative growth rate and net assimilation rate were punctually superior in the R biotype. These results demonstrate that the Ser-653-Asn mutation imposed a fitness cost in imazamox R wild poinsettia.

Keywords: fitness penalty; herbicide resistance; leaf area ratio; net assimilation rate; relative growth rate; seed production; Ser-653-Asn mutation; specific leaf area

1. Introduction

The evolution of herbicide-resistant weeds due to the consecutive application of herbicides has become a great challenge for sustainable agriculture [1] and currently, 514 unique cases of resistance have been identified across the world [2]. Herbicide-resistant weeds may have a competitive advantage under the selective pressure of herbicide compared to susceptible populations, however, in the absence of that herbicide, resistance may come at a fitness cost [3]. The fitness cost is defined as the adverse pleiotropic effect of an allele associated with herbicide resistance on fecundity or the survival of a herbicide-resistant weed [4], and can be considered as the final outcome of changes in the physiology, biochemistry and genetic of a weed due to a mutation responsible for herbicide resistance [5]. The mutation may lead to negative, neutral or positive effects [6,7] and the cost may vary depending on the mutation [5].

The fitness cost associated with herbicide-resistant alleles may be studied via various plant attributes such as seed germination, vegetative characteristics, phenology, yield and fecundity [8]. Du et al. [9] investigated several mutations in the Acetyl-coenzyme A carboxylase (ACCase) encoding gene on the growth characteristics of *Beckmannia syzigachne* (Steud.) Fernald and reported that the plants carrying the Ile-1781-Leu mutation had higher relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio compared to the susceptible plants. However, these indices were lower in plants carrying the Trp-2027-Cys, Ile-2041-Asn, Asp-2078-Gly, and Gly-2096-Ala mutations, indicating a fitness cost compared to the susceptible plants. The seed production of *Amaranthus tuberculatus* (Moq.) J. D. Sauer populations with multiple resistance was similar to susceptible populations [10]. The fitness cost of herbicide-resistance has also been studied in numerous weeds such as *Lolium multiflorum* Lam. [11], *Lolium rigidum* (Gaudin) Weiss ex Nyman [12], *Amaranthus palmeri* S. Wats. [13], *Avena ludoviciana* (Durieu) Nyman [6,14], etc.

Wild poinsettia (*Euphorbia heterophylla* L.), a weed species native to South America [15], causes losses of crop yields in crops such as cotton, soybean, peanut [16], maize and tomato in Africa, Asia, the Mediterranean region and North America [17]. Furthermore, this weed is reported to reduce the quality of the crop at the harvest [18].

Wild poinsettia has become a serious problem in soybean production in Brazil for its ability to select for herbicide resistance. There are numerous reports regarding the occurrence of resistance to various herbicides in wild poinsettia, including acetolactate synthase (ALS) inhibitors [19], ALS and protoporphyrinogen oxidase (PPO) inhibitors [20], and glyphosate [18,21]. Additionally, a new case of imazamox resistance in wild poinsettia was reported recently by Rojano-Delgado et al. [22]. A better understanding about the fitness cost of herbicide resistance in the weeds will immensely help to develop integrated weed management methods to mitigate the adverse effects of herbicide-resistant weeds [3,5]. Thus, the present study was conducted to investigate the potential fitness cost associated to the Ser-653-Asn mutation that confers imazamox resistance in a wild poinsettia.

2. Materials and Methods

2.1. Plant Material

The experiment was conducted using the imazamox-resistant biotype described by Rojano-Delgado et al. [22], which had the Ser-653-Asn mutation and ^{14}C-imazamox root exudation governing its herbicide resistance. Two segregating genotype progenies, one susceptible (S) and one resistant (R), were derived from heterozygous R plants of this biotype, i.e., both progenies shared a common genetic background except for the Ser-653-Asn mutation. Homozygous S or R progenies from a segregating F_2 population with similar genetic backgrounds were identified, propagated and used as the source of the homozygous R or S plants for this study.

2.2. Growth Conditions and Measurement of Fitness Traits

Five seeds from each of R and S wild poinsettia biotypes were sown in 40×40 cm plastic pots filled with a soil which was a mixture of sandy loam soil (50%) and peat (50%). The pots were arranged in a completely randomized design with three replicates in a greenhouse per evaluation time, and each pot served as one replicate. The greenhouse temperature was 22/16 °C (day/night) with 12/12 h period of light/darkness and a relative humidity of ~70%. The pots were regularly irrigated and fertilized throughout the growth season to maintain the vigorous growth of the plants. Spontaneous weeds different from the wild poinsettia initially sown were removed by hand during the growth season.

At 30, 40, 50, 60, 75 and 95 days after planting (DAP), the height was measured in all plants of three pots (15 plants per biotype) per evaluation time. Then, the plants were cut at the ground level and transferred to the lab. The leaves were separated from the shoots and the leaf area was determined using a leaf area meter apparatus (Delta-T, Burwell, England). Shoots and leaves were dried separately at 75 °C for 72 h, and finally, the dry weight of these plant sections was determined.

Growth indices of specific leaf area (SLA), leaf area ratio (LAR), relative growth rate (RGR), and the net assimilation rate (NAR) were calculated with the following equations:

$$SLA = \frac{LA}{LW} \tag{1}$$

$$LAR = \frac{LA}{W} \tag{2}$$

$$RGR = \frac{lnW2 - lnW1}{t2 - t1} \tag{3}$$

$$RGR = \frac{(W2 - W1)(lnLA2 - lnLA1)}{(t2 - t1)(LA1 - LA2)} \tag{4}$$

where LA is the leaf area per plant, LW is the leaf dry weight per plant, W is the dry weight per plant and *t* is time. Seed production per plant was measured at the end of the experiment period (95 DAP).

2.3. Statistical Analysis

The experiments were conducted twice. Since no interaction was observed between the treatments and experiments, the data from the two experimental runs were pooled, i.e., data from 30 plants composed the averages presented. The comparison of means within and between the R and S biotypes were done using the least significant difference (LSD) method and *t*-test at $p < 0.05$, respectively. Statistical analysis and plots were done using the software's SAS v.9 (SAS Institute, Cary, NC, USA) and Sigma Plot v.10 (Systat Software Inc., San Jose, CA, USA), respectively.

3. Results

3.1. Plant Height

Both wild poinsettia biotypes had similar heights at earlier stages, but from the 60 DAP the S plants were taller than the R. The maximum plant heights for S and R biotypes, recorded at 95 DAP, were 137 and 120 cm, respectively (Figure 1).
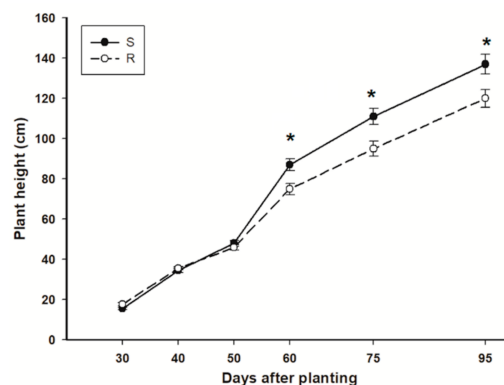

Figure 1. Plant height of imazamox-resistant (R) and susceptible (S) wild poinsettia plants. * indicates significant differences at $p < 0.05$. Vertical bars ± standard error ($n = 30$).

3.2. Leaf Area

The leaf area per plant increased at a similar rate and without differences between wild poinsettia biotypes up to 50 DAP. Between 50 and 60 DAP, the leaf area more than doubled and in S plants this increase was higher than that of the R plants. From 60 DAP, the foliar area remained constant or decreased slightly and at the end of the experiment, the R and S biotypes recorded 742 and 1048 cm^2,

respectively, of leaf area (Figure 2). Both biotypes have lobed and ovate leaves; however, the R plants mainly had lobed leaves.
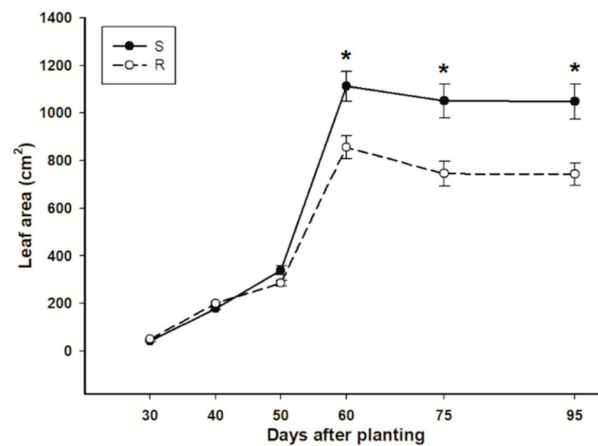

Figure 2. Leaf area per plant of imazamox-resistant (R) and susceptible (S) wild poinsettia plants. * indicates significant differences at $p < 0.05$. Vertical bars ± standard error ($n = 30$).

3.3. Dry Weights

Leaf and stem dry weights were similar in S and R wild poinsettia biotypes up to 60 DAP, but the S biotype accumulated more dry biomass thereafter and was higher compared to the R biotype. The highest values of leaf and stem dry weights for the R biotype were 1.9 and 2.6 g, respectively, whereas for the S biotype these values were 2.5 and 3.5 g (Figure 3).
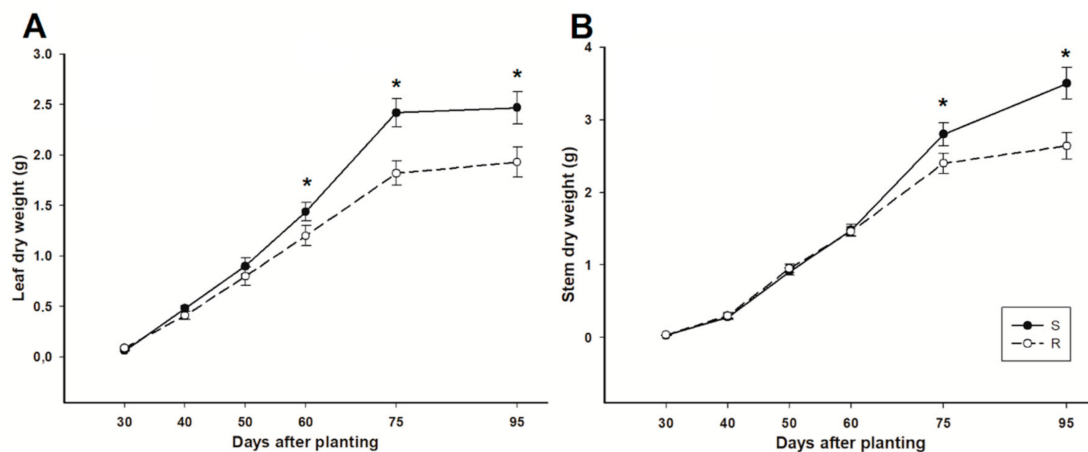

Figure 3. Leaf (**A**) and stem (**B**) dry weight of imazamox-resistant (R) and susceptible (S) wild poinsettia plants. * indicates significant differences at $p < 0.05$. Vertical bars ± standard error ($n = 30$).

3.4. Share of Weight and Seed Production

In both wild poinsettia biotypes, the leaves had a greater share in the dry weight of the shoots in the earlier development stages. This relationship changed clearly from 50 DAP in the R biotype and between 50 to 60 DAP for the S, when the stem became the main contributor to the dry weight of the shoots (Figure 4). In addition, biotype R produced 110 ± 5 seeds plant^{-1} while biotype S produced 125 ± 7 seeds plant^{-1}, i.e., biotype R produced 12.3% fewer seeds than biotype S (Figure S1).
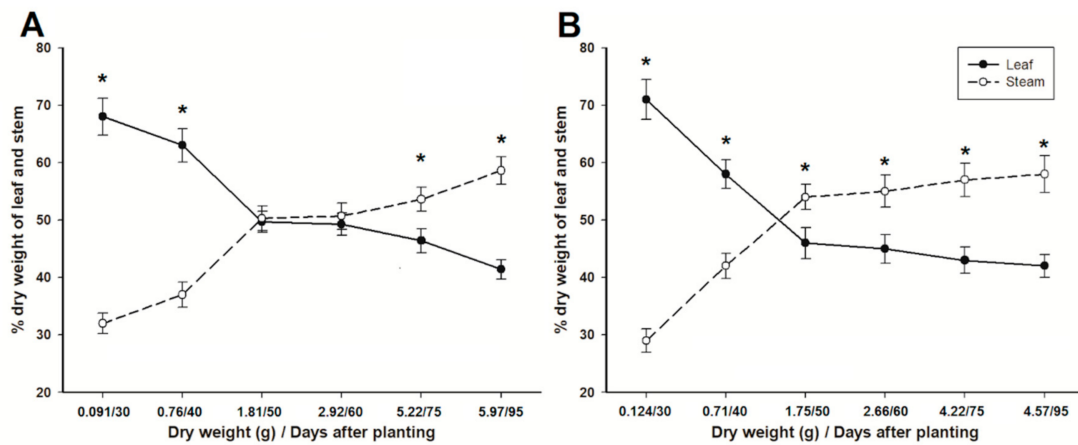
Figure 4. Dry weight ratio of the leaves and stems in imazamox-susceptible (**A**) and -resistant (**B**) wild poinsettia plants. * indicates significant differences at $p < 0.05$. Vertical bars ± standard error ($n = 30$).

3.5. Leaf Area Ratio and Specific Leaf Area

Both wild poinsettia biotypes showed an initial decrease in LAR and SLA followed by a strong increase between 50 and 60 DAP. These traits decreased again thereafter and remained constant from 75 DAP. Exceptionally at 40 DAP, the R biotype had higher LAR and SLA compared to the S one; however, the rest of the time these traits were higher in the S biotype or similar between both biotypes (Figure 5).
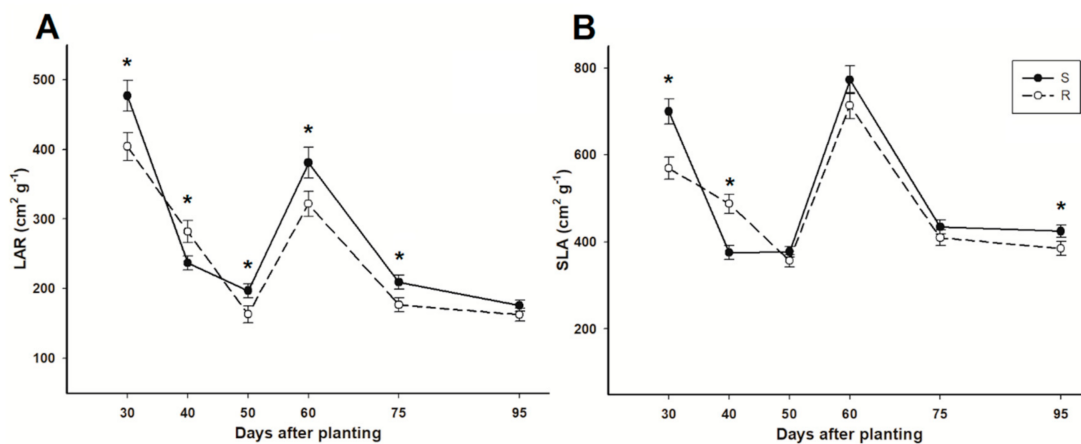

Figure 5. LAR—leaf area ratio (**A**) and SLA—specific leaf area (**B**) of imazamox-resistant (R) and susceptible (S) wild poinsettia plants. * indicates significant differences at $p < 0.05$. Vertical bars ± standard error ($n = 30$).

3.6. Relative Growth Rate and Net Assimilation Rate

The RGR and NAR decreased over time in both wild poinsettia biotypes. These parameters were generally similar between biotypes, and only on two specific occasions RGR and NAR were higher in the S biotype (RGR at 40 DAP and NAR at 50 DAP) (Figure 6).
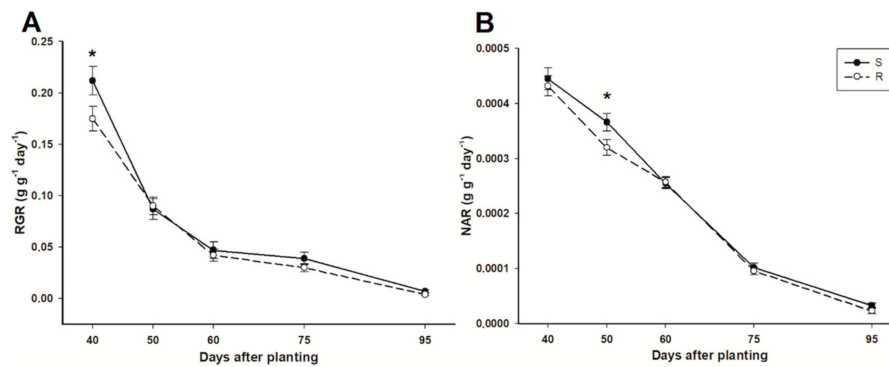
Figure 6. RGR—relative growth rate (**A**) and NAR—net assimilation rate (**B**) of imazamox-resistant (R) and susceptible (S) wild poinsettia plants. * indicates significant differences at $p < 0.05$. Vertical bars ± standard error ($n = 30$).

4. Discussion

The lower height of the wild poinsettia R plants in stages of advanced vegetative development can obstruct pollination, since in this weed, pollination is mainly done by insects [23]; thus, it is possible that R plants may produce fewer seeds per plant than S plants. Additionally, taller plants better intercept solar radiation [24], and at the same time shade smaller plants [25]. Reduction in plant height as a pleiotropic effect of herbicide resistance was also reported in glyphosate-resistant *Lolium perenne* L. [26] and atrazine-resistant *Brachypodium hybridum* Catalan, Joch. Muell., L. A. J. Mur & T. Langdon [27].

That the R biotype had mainly lobed leaves may explain the lower leaf area. However, it cannot state that this leaf shape is a trait associated with the fitness cost or with resistance to imazamox, since the wild poinsettia presents foliar polymorphism, regardless of the growing conditions, being the characteristic linked to its scientific name (*E. heterophylla*). In the absence of herbicide selection pressure, decreasing the amount of a photosynthetically active area per unit of plant mass may decrease carbon assimilation [24]. In contrast, species with a higher leaf area (up to an optimum leaf area) tend to be more successful in competition and interception for light and carbon assimilation [28]. In this context, imazamox-R wild poinsettia plants have a competitive disadvantage in relation to the S plants that can be reflected in the production of total biomass. Lower leaf area production was observed in several herbicide R plants compared to their S counterparts, such as ALS-inhibitor resistant *Bromus tectorum* Huds. [29], *L. multiflorum* with multiple resistance to glyphosate, iodosulfuron-methyl and pyroxsulam [11]. In contrast, the leaf area in *A. ludoviciana* [6] and *L. rigidum* [30] resistant to the of ACCase-inhibitors carrying the Ile-2041-Asn and Ile-1781-Leu mutations, respectively, was similar to their respective S biotypes.

The lower biomass production in imazamox-R wild poinsettia plants could be due to the lower height and leaf area recorded in this biotype. Usually, smaller plants are less competitive than taller ones. In addition, having a lower biomass limits the competitiveness of a plant on its neighboring plants [31]. The shoot biomass of glyphosate-R *L. perenne* plants was ~50% lower than S plants [26]. Atrazine-R and S *B. hybridum* plants had a similar shoot dry weight under natural environmental conditions, whereas the shoot dry weight of R plants was lower than that of the S ones under low light and nitrogen deficiency conditions [27]. There are also reports on the similarity of shoot dry weight between herbicide-R and -S plants [7,13,32].

Biotype R presented an inversion in the percentage of biomass allocated to the stem earlier than biotype S. This anticipated inversion in biomass allocation may be associated with the lower height observed in the R biotype. The increase in the allocation of biomass to the stem in smaller plants is an adaptative response to avoid shading from taller plants [33]. Smaller plants often capture less radiation and consequently fix less carbon. This competitive adaptation also implies a lower

requirement of nutrients and water and is known as functional balance [34]. This suggests that although R plants have a competitive disadvantage for light, they optimized the use of resources anticipating their vegetative development. On the other hand, the number of produced seeds is a crucial fecundity trait which affects the absolute fitness of a population [26]. Thus, low seed production in a R biotype will lead to a reduced frequency of the resistance alleles in the absence of herbicide selection pressure [14]. Lower seed production has been also reported in ACCase-R *B. syzigachne* [9], ACCase-R *Hordeum murinum* L. [35], glyphosate-R *Kochia scoparia* (L.) Schrad., ALS-R *Echinochloa crus-galli* (L.) P. Beauv. [36], among other weeds.

Plants with higher LARs often occupy better space and have better capacity to intercept light and consequently, have a competitive advantage [11]. The lower LAR in the R wild poinsettia biotype confirms a fitness cost of the Ser-653-Asn mutation, but at the same time, shows that this biotype allocated photoassimilates to stems and reproductive structures earlier than the S biotype as a competitive adaptation mechanism, as observed in the *L. multiflorum* [11]. The comparison of the LAR between herbicide-R and S plants have also been done in *B. syzigachne* [9], *L. rigidum* [30], among other weeds. SLA plays a crucial role in plant growth [37]. The S plants of *Sinapis arvensis* had a higher SLA than the plants resistant to ALS inhibitors [38], which is in accordance with the results of the present study. In contrast, *Chenopodium album* with multiple resistance to atrazine and dicamba had a higher SLA compared to S plants [37].

Plants with high RGR are able to rapidly occupy large spaces which gives them advantage in competition. The higher RGR in the S wild poinsettia biotype at 40 DAP shows that this biotype is more competitive at early stages of growth. In addition, high RGR will allow the plant to complete its life cycle more rapidly, which is an essential attribute for weeds [39]. *Eleusine indica* and *L. rigidum* plants, resistant to glyphosate and ACCase-inhibitors, respectively, had a lower RGR compared to their S counterparts [30]. In contrast, there are reports regarding the similarity of RGR between the R and S plants [32,40]. NAR is determined by different factors such as photosynthesis and respiration rates, biomass allocation, canopy architecture, light interception, among others [41]. The similar NAR patterns in both wild poinsettia biotypes suggest that these factors have not yet been altered in the R biotype. In several studies, reductions in NAR rates in weed biotypes have been observed as a fitness cost trait of herbicide resistance [6,9,30,37].

5. Conclusions

The evolution of resistance to imazamox in wild poinsettia, governed by the Ser-653-Asn mutation, is associated with a fitness cost. Hence, S plants may be able to make use of this superiority to gain the edge in competition with the R ones in the absence of imazamox herbicide selection pressure. This is despite the fact that characteristics such as the relative growth rate, net assimilation rate, leaf area ratio, specific leaf area, and dry weight ratio of leaves and stems of R and S plants were similar in some stages.

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