DOI: 10.1017/wet.2025.26

Junglerice Resistance Dynamics

Intra- and interspecific competition between glyphosate-resistant and susceptible junglerice (*Echinochloa colona*) populations and soybean

Gabriel Picapietra[1,2*] and Horacio A. Acciaresi[1,3]

[1]Weed Researcher, EEA INTA Pergamino, Ruta N° 32 Km 4.5 (2700), Pergamino, Buenos Aires, Argentina; [2]Assistant professor (ORCID 0000-0002-0129-603X), Weeds, Escuela de Ciencias Agrarias, Naturales y Ambientales, Universidad Nacional del Noroeste de la provincia de Buenos Aires, Ruta N° 32 Km 4 (2700), Pergamino, Buenos Aires, Argentina; and [3]Research associate, Comisión de Investigaciones Científicas, Calle 256 e/10 y 11 (1900), La Plata, Buenos Aires, Argentina

***Author for correspondence:** Gabriel Picapietra; Email: picapietra.gabriel@inta.gob.ar

Abstract

Herbicide-resistant weeds demand particular attention in the selection of management strategies, considering the characteristics and fitness differences between susceptible and resistant plants. This study aimed to analyze two heterogeneous junglerice populations, glyphosate-susceptible (EC-S) and glyphosate-resistant (EC-R), derived from field-collected seeds, to quantify the productivity of EC-S and EC-R, to analyze the impact of soybean plants, and to evaluate density dependence. Experiments were conducted in a growth chamber using plastic pots, combining three factors: junglerice density (2, 4, and 8 plants pot^{-1}), the proportion of EC-S and EC-R (100% EC-S, 50% EC-S:50% EC-R, 100% EC-R), and soybean density (0 and 1 plant pot^{-1}). One-leaf junglerice seedling and soybean seeds were planted in plastic pots. After 65 days, plant height (PLH), total number of tillers (TIL), seed number (SEN), and seed weight (SEW) per plant, weight of 100 seeds (HSW), and aboveground dry matter (ADM) were measured. The variables measured were primarily affected by junglerice density and the presence of soybean. Vegetative and reproductive structures of EC-S and EC-R exhibited strongly density-dependent patterns. One soybean plant increased ($p<0.05$) junglerice productivity at low densities but had no effect at high densities (8 plants pot^{-1}). Mostly EC-S and EC-R showed the same competitive ability. However, EC-S presented a higher index of competitive ability than EC-R in SEN and SEW at lower densities with a soybean plant. A resource complementarity (RYT~1) between EC-S and EC-R was observed ($p<0.05$), regardless of the presence of the crop. These results demonstrate that, even in the absence of glyphosate, fitness differences favor heterogeneous resistant populations. This highlights the inherent adaptive advantage of resistant populations and, considering the extensive reliance on glyphosate in current production systems underscores the urgent need for integrated weed management strategies to mitigate the evolution and spread of resistant populations.

Nomenclature: Glyphosate; junglerice, *Echinochloa colona* L. ECHCO; soybean, *Glycine max* (L.) Merr.

Keywords: fitness, herbicide resistance, weeds.

Introduction

The weed community in Argentina's agricultural systems has undergone remarkable transformations, primarily driven by changes in tillage practices (Oreja et al. 2024) and the near-exclusive reliance on herbicides as the sole weed control method (Shaner 2014). These shifts have favored a select group of species, particularly those capable of adapting to these new conditions (Satorre et al. 2020). Among the weeds that have thrived under such circumstances, the genus *Echinochloa* is notable for its rapid growth, high seed production with varying levels of dormancy, and adaptability to diverse environments (Shabbir et al. 2019).

Within this genus, junglerice stands out due to its fast growth rate and significant dry matter accumulation during early stages, enhancing its competitive ability and their adaptation to agricultural systems (Vulchi et al. 2024). Intensive herbicide use facilitates the selection of resistant individuals, exerting pressure on heterogeneous populations where susceptible and resistant individuals coexist (Asaduzzaman et al. 2021). The proportion of susceptible and resistant individuals within these populations is dynamic and can shift depending on the ability of each biotype to produce propagules and replenish the soil seedbank. Resistance mechanisms may also impose a fitness cost, potentially resulting in lower fitness for resistant individuals in the absence of herbicide selection pressure (Damalas and Koutroubas 2024).

Analyzing the fitness of these biotypes is crucial for understanding the evolution of resistance within a population (Chauhan et al. 2022). Assessing fitness under herbicide-free conditions allows researchers to evaluate the potential costs of resistance (Damalas and Koutroubas 2024). Some studies have reported that glyphosate-susceptible biotypes of junglerice exhibit higher fitness, while others have found no significant differences in seed production between susceptible and resistant plants (Mutti et al. 2019; Mollaee et al. 2020). In certain cases, however, glyphosate-resistant populations have been shown to exhibit higher fitness compared to susceptible ones, both under non-restrictive conditions and in response to saline stress (Shrestha et al. 2018).

Interactions among individuals, in addition to determining population fitness differences, provide insights into the competitive dynamics of plant-plant relationships (Radosevich et al. 2007). These interactions are generally negative, meaning one species may experience greater suppression than the other. Understanding these dynamics is essential for elucidating patterns of species composition and dominance within agroecosystems (Lau and Funk 2023). Competition studies enable the assessment of competitive ability through indices such as aggressiveness (AGR) and resource complementarity using the total relative yield (RYT), which ultimately shapes the success of a species within a community (Roush and Radosevich 1985; Swanton et al. 2015).

Given the growing importance of junglerice in the Pampas region, particularly due to the selection of glyphosate-resistant populations, understanding the competitive dynamics between susceptible and resistant individuals is essential. Such insights will not only clarify potential fitness costs associated with resistance but also inform the development of improved management programs and more effective control strategies (Mutti et al. 2019). Furthermore, studies involving both weed-weed and weed-crop interactions under a density-dependent gradient are critical for enhancing management strategies (Baucom 2019).

The objective of this study was to quantify the individual and areal productivity of two junglerice populations, glyphosate-resistant (EC-R) and glyphosate-susceptible (EC-S), at different densities and under coexistence with a soybean plant. It was hypothesized that glyphosate-susceptible individuals have greater biological fitness (as determined by aboveground dry matter, seed number, and seed weight) and superior competitive ability (AGR > 0). Additionally, it was hypothesized that intraspecific interactions between susceptible and resistant individuals would result in full competition (RYT = 1), while interspecific interactions with soybean would reduce the productivity of both susceptible and resistant individuals.

Materials and Methods

Two experiments were conducted at the National Institute of Agricultural Technology (INTA) in Pergamino, Argentina (33.947° S, 60.564° W), under controlled conditions in 2018 and 2019. A growth chamber was used with a 12-hour photoperiod and a constant temperature of 26 C. Light was provided by 1000-Watt full-spectrum LED panels. These conditions replicate the ideal environment for the development of junglerice.

Sample Collection and Seedling Obtention

Two junglerice seed samples were used, following the procedure described by Sherstha et al. (2018), ensuring that the progeny originated from field-collected seeds rather than clonal propagation. Seeds were collected in March 2018 and stored in a dry chamber at 21 C. According to Goh et al. (2016), the collected seeds were sown and treated with glyphosate at the labeled dose (0.86 kg ae ha^{-1}) to corroborate resistance. The glyphosate-susceptible population (EC-S) exhibited 100% mortality, while the resistant population (EC-R) had a survival rate of 93.3%, exceeding the 85% threshold proposed by Goh et al. (2016).

Although species in the *Echinochloa* genus exhibit a high degree of self-pollination, genetic differences between the seed samples and the mother plants may exist (Liu et al. 2022). Due to the possibility that field-collected seed samples contain individuals with varying levels of susceptibility or resistance to glyphosate, it is appropriate to consider these as heterogeneous populations –glyphosate-susceptible and -resistant– rather than biotypes (Asaduzzaman et al., 2021).

After five months of storage (Picapietra and Acciaresi, 2022), seeds were placed in plastic boxes with a moistened paper substrate inside the same growth chamber. Once the seedlings fully developed their first leaf, they were transplanted into containers. The germination and transplant process were repeated in August 2019 using the same seeds stored in 2018 for a second experimental cycle.

Pots and Experimental Design

Plastic pots of 6.2 L were used as experimental units. They were filled with soil substrate of a silty loam texture (22.7% clay, 64.8% silt, and 12.5% sand) and a nitrogen ($N\text{-}NO_3$) content of 11.8 mg kg^{-1}. Containers were watered twice a week with 100 mL of water per irrigation to ensure non-restrictive water conditions.

The experiment was conducted under a completely randomized design with a factorial arrangement involving three factors (α, β, γ). Factor α was the density of junglerice with three levels (i=3) defined by the number of plants per pot (2, 4, and 6 plants pot^{-1}). Factor β was the proportion of EC-S and EC-R individuals, with four levels (j=4): pure EC-S (100% EC-S), pure EC-R (100% EC-R), and mixed populations where EC-S and EC-R individuals grew at 50:50 ratio, but where evaluated separately as EC-S in mixture and EC-R in mixture. Factor γ was the inclusion of one soybean plant, with two levels (k=2), defined as "with" and "without" soybean (0 and 1 plant pot^{-1}).

Junglerice seedlings were transplanted equidistantly between the center and the margin of each pot (Figure 1a/c). Then, two seeds of the soybean cultivar DM 4612 (treated and inoculated) were sown at the center of the designated pots (Figure 1b). Five days after sowing, soybean plants emerged and were subsequently thinned to leave only one per pot. Each pot with an α-β-γ

treatment combination had three replicates, which were labeled and randomly arranged inside the growth chamber.

Plant Measurements

At plant maturity (65 days after transplanting), plant height (PLH, cm) was measured from the base to the most distant panicle, and the number of tillers per plant (TIL, no. plant^{-1}) was counted. Seeds were extracted from the panicles, cleaned, and placed in glasses. Seed number (SEN, no. plant^{-1}), seed weight (SEW, g), and the hundred-seed weight (HSW, g) per plant were measured using a digital balance (d=0.01 g). Finally, the aerial parts of each plant were placed in paper bags, oven-dried at 52 C for 72 hours, and weighed to determine aboveground dry matter (ADM, g) per plant.

Determination of Competitiveness and Aggressiveness

Relative yield (RY) for TIL, ADM, SEN, and SEW per pot was calculated (Equation 1). Subsequently, the relative yield total (RYT, Equation 2) and aggressiveness (AGR, Equation 3) were determined, following the methods described by Cheng et al. (2022) and Cao et al. (2024):

(Eq. 1) $RY_a = Y_{ab}/Y_{aa}$ and $RY_b = Y_{ba}/Y_{bb}$

(Eq. 2) $RYT = RY_a + RY_b$

(Eq. 3) $AGR = RY_a - RY_b$

Where Y_{ab} (or Y_{ba}) is the yield per pot of susceptible (or resistant) individuals grown in mixtures with resistant (or susceptible) individuals, and Y_{aa} (or Y_{bb}) is the yield per pot of susceptible (or resistant) individuals grown in monoculture.

An RYT of 1.0 indicates full competition between EC-R and EC-S, whereas RYT > 1.0 suggests resource complementarity, and RYT < 1.0 indicates antagonism or mutual negative effects. Similarly, aggressiveness (AGR) identifies whether one population has greater competitive ability (AGR > 0) or if both populations share equal ability (AGR = 0).

Data Analysis

Individual productivity data were analyzed using a linear mixed model (LMM) that included the main effects of density (α), population proportion (β), and soybean presence (γ), as well as their respective two-way and three-way interactions. Given the experimental design, all factors were treated as fixed effects. Since no significant differences were detected between the two experimental years, data from both cycles were combined for analysis.

To assess fitness differences, an LMM was applied based on the type of junglerice individuals per pot. The analysis considered three groups: pure EC-S pots, pure EC-R pots, and EC-S:EC-R mixture pots, with this new three-level variable defined as 'combination'. Data were further analyzed by partitioning according to α and γ effects, allowing comparisons among individual types without accounting for variations from intra- or interspecific interactions.

The RYT and AGR index were analyzed using ANOVA with a factorial arranged that included the effects of density (α) and soybean presence (γ), as well as their interaction (α×γ). Additionally, two bivariate comparisons (Snaydon and Satorre, 1989) were conducted to contrast the productivity of EC-S and EC-R (TIL, ADM, SEN, and SEW). In the first comparison, RY was evaluated to illustrate the RYT and AGR indices. In the second, double-logarithmic scales of productivity from pure EC-S and EC-R pots were constructed to visualize intra- and inter-specific relationships projections.

Results and Discussion

Individual Productivity of Junglerice

The density of junglerice (α) and the inclusion of soybean (γ) were the only treatments that significantly affected all analyzed variables ($p<0.05$) (Table 1). On one hand, the intraspecific density effect demonstrated that the species exhibited a density-dependent behavior, showing extensive plasticity in its vegetative structures (number of tillers, aboveground biomass) and reproductive variables (seed weight and seed number). This finding aligns with previous studies (Weiner 2004; Romillac et al. 2023), which emphasize that these traits are highly plastic and responsive to neighbor presence. Additionally, no significant differences related to glyphosate susceptibility or resistance were observed in these variables, suggesting that resistance does not directly impact individual vegetative productivity.

On the other hand, when soybean was included, the productivity of ADM, SEN, and SEW of junglerice significantly increased ($p<0.05$), particularly at densities of 2 and 4 plants pot^{-1}. However, at 8 plants pot^{-1}, no significant differences were observed ($p>0.05$). In contrast to previous reports on competition between junglerice and crops such as beans (Mutti et al. 2019), soybean (Matloob and Chauhan 2021), or rice (Hu et al. 2023), this experiment showed that the presence of one soybean plant increased individual productivity at a density of 2 plants pot^{-1}. This increase could be attributed to greater resource acquisition by junglerice and the soybean's ability to supply nitrogen through biological fixation, given that water and light were not limiting factors and soybean seeds were inoculated in soil with an initial availability of 11.8 ppm of N-NO_3.

The proportion of EC-S and EC-R individuals (β) did not significantly affect PLH, ADM, or TIL. Adjustments in aboveground biomass, tiller number, and plant height were strongly conditioned by population density. However, variations in SEN, SEW, and HSW were significantly more favorable for EC-R individuals (Table 2). These findings are consistent with Shrestha et al. (2018), who reported that resistant individuals produce more seeds under intraspecific competition, although these differences tend to disappear with greater resource limitations.

The interaction α×β was significant for SEN and SEW, whereas for HSW, only the β effect was significant ($p<0.05$). The lowest SEN per plant was observed at a density of 8 plants pot^{-1}, with no significant differences between EC-S and EC-R individuals ($p>0.05$), yielding values of 225 and 250 seeds plant^{-1}, respectively. The highest seed number per plant was recorded for EC-R individuals at a density of 2 plants pot^{-1}, averaging 505–519 seeds plant^{-1}.

Similarly to SEN, the lowest SEW plant^{-1} was observed at a density of 8 plants pot^{-1}, regardless of the junglerice individual type. However, at densities of 2 and 4 plants pot^{-1}, EC-R individuals showed the highest SEW (Table 2). Accordingly, HSW values were also higher for EC-R individuals ($p<0.05$). At the pot level, adjustments in junglerice productivity were strongly influenced by plant density. However, at 8 plants pot^{-1}, no significant differences were found between EC-S and EC-R individuals, whether alone or mixed, with or without soybean. Among the variables analyzed, the total number of tillers per pot varied the least in relative terms. This is consistent with Vulchi et al. (2024), who observed that tiller number tends to stabilize, but does not agree with Matloob and Chauhan (2021) who observed that the presence of cover crop plants in the vicinity suppressed the tillering of junglerice. At lower densities, EC-R individuals exhibited higher SEN and SEW, partially supporting the findings of Mahajan et al. (2020), who reported that glyphosate resistance does not significantly influence junglerice productivity in mixed populations.

Fitness Differences Between Glyphosate-Susceptible and -Resistant Junglerice Individuals

The results cannot be strictly interpreted as the absence of a fitness cost associated with resistance, as the EC-S and EC-R populations analyzed originate from distinct environments and may differ in genetic and adaptive backgrounds. The observed results indicated that SEN and SEW per plant, was higher in EC-R individuals compared to EC-S (Figure 2). This difference was particularly evident at a density of 2 plants pot^{-1}, where soybean significantly increased the productivity of EC-R individuals, leading to an increase in SEN by 46±14 seeds plant^{-1} and in SEW by 0.14±0.02 g plant^{-1}. However, at a density of 8 plants pot^{-1}, no productivity differences were observed between EC-R and EC-S, nor were significant differences found in junglerice plants growing with or without soybean ($p>0.05$).

No differences were observed at 8 plants pot-1 in vegetative and reproductive productivity, and considering that resource availability was not measured in this study, this behavior could be associated with allometric relationships, where increasing density leads to an asymptotic decrease in plant size (Weiner 2004). Nevertheless, this pattern will be further analyzed in the following section, considering relative yield and aggressivity indices.

Regarding areal productivity, junglerice density had a statistically significant effect on all studied variables ($p<0.05$), similar to individual productivity. However, soybean significantly affected ADM, SEN, and SEW ($p<0.05$), but not TIL ($p>0.05$). When evaluating productivity per pot, the total number of tillers was only related to population density, with no differences between EC-S and EC-R individuals. These results suggest that under the evaluated conditions, glyphosate resistance does not appear to be associated with a significant fitness cost. The findings presented here align with those of Damalas and Koutroubas (2024), who stated that the biological cost associated with herbicide resistance may not express or may only occur under specific conditions. Additionally, they are consistent with the findings of Tillería et al. (2024), who observed no differences in fitness components between susceptible and resistant plants, even in the absence of the herbicide.

The variation in ADM pot^{-1} was explained by junglerice density and soybean presence, independently of the EC-S and EC-R (Figure 3). In terms of reproductive productivity, at low densities (2 and 4 plants pot^{-1}), soybean significantly increased SEN and SEW in EC-R individuals, showing greater population fitness compared to EC-S individuals. At high density (8 plants pot^{-1}), no significant differences were observed between populations. The SEN and SEW increase by soybean in EC-R individuals, contrasting with the results of Barroso et al. (2022), who observed that soybean competence significantly reduced the relative fitness of resistant individuals due to decreased catalytic activity of the EPSPS enzyme. These results reinforce Li et al. (2023) hypothesis that not all herbicide-resistant weeds have fitness costs and that herbicide resistance could lead to improved fitness even in the absence of herbicides.

This behavior is striking given that, despite the difference in resistance traits, both EC-R and EC-S belong to the same species. However, EC-R individuals appear to have a greater advantage in resource acquisition, particularly at low densities, in the presence of soybean, and in the absence of glyphosate. Nevertheless, the lack of productivity differences at 8 plants pot^{-1} suggests a reduction in EC-R performance at higher densities. This could be attributed to resource limitations and, more importantly, a lower resource-use efficiency, which might be linked to differences in fitness associated with the genetic background of these individuals.

Additionally, although no significant differences in ADM were observed between populations, while EC-R exhibited a higher SEN and SEW, this pattern suggests that resistant individuals may be reallocating resources toward reproduction, thereby gaining a differential advantage in biomass allocation. Such a compensatory mechanism could confer a net reproductive benefit. This phenomenon may be driven by pleiotropic effects or inherent differences in the genetic background of the resistant populations. This association with genetic background could be linked to phenotypic plasticity or genetic diversity, as suggested by Romillac et al. (2023), supporting the need for further studies to better understand these mechanisms.

Relative Yield and Aggressiveness of Junglerice

The analysis of the RYT index adapted to TIL and ADM variables showed no significant differences ($p>0.05$). This indicates that the index was statistically similar to 1.0 across all density levels (2, 4, and 8 plants pot^{-1}), both with (C) and without (O) soybean. These results suggest that EC-S and EC-R individuals grew under full competition (Figure 2). These findings align with their classification as the same species, where symmetric competition relationships are typically described under intraspecific competition between plants of similar size (Tomiolo et al. 2022).

However, for SEN and SEW variables, significant differences ($p<0.05$) were observed only at a density of 2 plants pot^{-1} with soybean, where RYT indices were 1.25 and 1.36, respectively. This suggests resource complementarity under these conditions. In the remaining treatments, the indices were not significantly different from 1.0. This situation, suggesting resource complementarity, could be attributed to the fact that at 2 plants per pot in association with soybean, resources were not limiting for junglerice seed formation and filling.

Regarding the AGR index, no significant differences were found in the total number of tillers or aboveground biomass of junglerice between EC-S and EC-R individuals (AGR~0). However, differences emerged in reproductive variables. For SEN, at a density of 2 plants pot^{-1}, AGR was 0.19, while for SEW, in the same density, AGR reached values of 0.29 and 0.26 when plants grew C and O, respectively (Figure 2). These results indicate that under these conditions, EC-S individuals showed higher competitive ability than EC-R individuals ($p<0.05$). This is reflected by the greater reduction in relative yield when EC-S and EC-R grew in mixture. These differences were evidenced in the bivariate diagram, as described by Snaydon and Satorre (1989).

The double-logarithmic bivariate representations for EC-S and EC-R per pot (Figure 4) indicated no differences in ADM and SEN between EC-S and EC-R individuals (Figures 4b–c). However, slight differences were observed in TIL at a density of 4 plants pot^{-1} without soybean (Figure 4a), and in SEW at densities of 2 and 4 plants pot^{-1} with soybean and 8 plants pot^{-1} without soybean (Figure 4d). In these cases, EC-R individuals outperformed EC-S, as evidenced by the differences in confidence intervals ($\alpha=0.05$).

These results indicate that the differences between EC-S and EC-R are primarily observed at low densities in the determination of seed number and weight. Under the experimental conditions evaluated, no differences were observed in the production of tillers, aboveground dry matter, seed number, and seed weight that would indicate a fitness difference favoring susceptible individuals over resistant ones, leading to the rejection of the first hypothesis.

The higher productivity observed in EC-R at low densities can be interpreted as an intrinsic characteristic of that population rather than being solely an attribute associated with resistance biology. While this trait has implications for resistance ecology—by conferring an intraspecific competitive advantage that could potentially favor the evolution of a higher frequency of resistant individuals—at a density of 8 plants pot^{-1}, these individuals exhibited a greater relative loss in yield. This resulted in a higher competitive ability for EC-S, particularly under this condition, supporting the partial acceptance of the hypothesis regarding the aggressiveness index.

This suggests that the productive advantage of resistant individuals is highly dependent on the competitive context (i.e., density-dependent). This dynamic underscores the importance of evaluating competition between populations of different origins, as might occur in a scenario where resistant individuals invade or are introduced into a predominantly susceptible population.

Regardless, our findings generally indicate that the populations were engaged in full competition in relative terms, which is partly attributable to their intraspecific nature. The only exception was observed at a density of 2 plants pot^{-1} in the presence of soybean, where resource complementarity was evident for both seed weight and seed number. Consequently, this supports the partial acceptance of the third hypothesis.

Finally, the competitive advantage of junglerice was not only attributed to its growth traits but also to its earlier emergence relative to soybean. At the time of soybean emergence, junglerice seedlings had already developed one to two fully expanded leaves, likely providing an initial establishment advantage and greater resource capture by the weed. Instead of exerting negative effects, the presence of soybean significantly improved junglerice productivity at the lowest density and maintained or slightly increased weed productivity at the remaining densities. Consequently, the fourth hypothesis is completely rejected, as soybean did not decrease the productivity of susceptible or resistant populations.

This finding underscores the importance of carefully timing junglerice management strategies, particularly considering that soybean is the predominant crop in Argentina and cultural management decisions are made early in the season. Therefore, factors such as planting date, crop density, and row spacing should be carefully evaluated due to their potential influence on the competitive advantage of junglerice. In this context, the widespread adoption of glyphosate-resistant soybean plays a key role in the coevolution of herbicide resistance, further emphasizing the relevance of herbicide resistance ecology.

Practical Implications

The findings of this study provide valuable insights into the competitive dynamics between glyphosate-resistant and -susceptible junglerice populations in the presence and absence of soybean. These results demonstrate that glyphosate-resistant individuals can produce a higher seed number and weight, particularly at lower densities, which may enhance their persistence and proliferation in agroecosystems where glyphosate use is frequent. However, susceptible individuals exhibit a greater competitive ability when grown alongside resistant ones, especially under conditions of resource limitation or soybean competition.

For field practitioners, this information underscores the importance of considering population heterogeneity in weed management strategies. The coexistence of resistant and susceptible populations could influence the effectiveness of cultural and chemical control methods. For example, integrated management practices that reduce seed production at low densities, such as targeted herbicide applications or increased crop competition through adjustments in crop stand density, row spacing, planting date, and the selection of a competitive cultivar, may help to suppress resistant populations. Furthermore, these findings highlight the potential risks of relying solely on glyphosate-based management and the need to implement diverse approaches, such as crop rotation and the use of pre-emergence herbicides, to mitigate the spread of resistance.

Overall, this study emphasizes the ecological dynamics driving herbicide resistance and provides actionable knowledge for developing sustainable weed management strategies in glyphosate-dependent cropping systems.

Acknowledgments

We thank Marcelo González, support staff from INTA, for his valuable assistance in preparing the containers and collecting data. We also extend our gratitude to Professor Daniel Tuesca for his insightful feedback and collaboration in reviewing this work.

Funding

This research was funded by INTA through doctoral training support.

Competing Interests

The authors declare none.

References

Asaduzzaman M, Koetz E, Wu H, Hopwood M, Shephard A (2021) Fate and adaptive plasticity of heterogeneous resistant population of *Echinochloa colona* in response to glyphosate. Sci Rep 11(1):14858. 10.1038/s41598-021-94370-7

Barroso AAM, Michelon TB, da Costa Aguiar Alves PL, Han H, Yu Q, Powles SB, Vila-Aiub MM (2022) Challenging glyphosate resistance EPSPS P106S and TIPS mutations with soybean competition and glyphosate: implications for management. Pest Manag Sci 78:4764-4773. 10.1002/ps.7096

Baucom RS (2019) Evolutionary and ecological insights from herbicide-resistant weeds: what have we learned about plant adaptation, and what is left to uncover? New Phytol 223(1):68-82. 10.1111/nph.15723

Cao J, Yao X, Wei X, Zhang R, Liu J, Li C (2024) The influence of timing and planting proportion on the intraspecific competitiveness ability of drunken horse grass (*Achnatherum inebrians* (Hance) Keng) by fungal endophyte infection. Plant Soil Environ 70:268-276. 10.17221/9/2024-PSE

Chauhan BS, Kaur V, Salgotra R (2022) Genetic diversity and population structure analysis to study the evolution of herbicide resistance in *Echinochloa colona* ecotypes in Australia. Acta Physiol Plant 44:32. 10.1007/s11738-022-03366-x

Cheng JK, Cao MY, Yang HR, Yue MF, Xin GR, Chen BM (2022) Interactive effects of allelopathy and arbuscular mycorrhizal fungi on the competition between the invasive species *Bidens alba* and its native congener *Bidens biternata*. Weed Res 62:268-276 10.1111/wre.12534

Damalas CA, Koutroubas SD (2024) Herbicide resistance evolution, fitness cost, and the fear of the superweeds. Plant Sci 339:111934. 10.1016/j.plantsci.2023.111934

Goh SS, Vila-Aiub MM, Busi R, Powles SB (2016) Glyphosate resistance in *Echinochloa colona*: phenotypic characterisation and quantification of selection intensity. Pest Man Sci 72:67-73. 10.1002/ps.4005

Hu X, Liu R, Mao H, Xu Y, Chen B, Li Y, Yang X (2023) Inter-species investigation of biological traits among eight *Echinochloa* species. Plants 12:3085. 10.3390/plants12173085

Lau JA, Funk JL (2023) How ecological and evolutionary theory expanded the ‘ideal weed’concept. Oecologia 203(3):251-266. 10.1007/s00442-023-05397-8

Li HY, Guo Y, Jin BY, Yang XF, Kong CH (2023) Phytochemical cue for the fitness costs of herbicide-resistant weeds. Plants 12(17):3158. 10.3390/plants12173158

Liu R, Singh V, Abugho S, Lin H, Zhou X, Bagavathiannan M (2022) Morphophysiological diversity and its association with herbicide resistance in *Echinochloa* ecotypes. Weed Sci 70(1):26-35. 10.1017/wsc.2021.64

Mahajan G, Kaur V, Thompson M, Chauhan BS (2020) Growth behavior and glyphosate resistance level in 10 populations of *Echinochloa colona* in Australia. PLoS Onee 15(1):e0221382. 10.1371/journal.pone.0221382

Matloob A, Chauhan BS (2021) Utilization of the neighborhood design to evaluate suitable cover crops and their density for *Echinochloa colona* management. PLoS One 16(7):e0254584. 10.1371/journal.pone.0254584

Mollaee M, Mobli A, Chauhan BS (2020) The response of glyphosate-resistant and glyphosate-susceptible biotypes of *Echinochloa colona* to carbon dioxide, soil moisture and glyphosate. Sci Rep 10(1):329. 10.1038/s41598-019-57307-9

Mutti NK, Mahajan G, Chauhan BS (2019) Seed-germination ecology of glyphosate-resistant and glyphosate-susceptible biotypes of *Echinochloa colona* in Australia. Crop Pasture Sci 70:367-372. 10.1071/CP18444

Oreja FH, Vera ACD, Kruk BC, Fuente EB, Scursoni JA (2024) Survey of major weed problems, management practices and herbicide use in extensive row crops from Argentina. Adv Weed Sci 42:e020240050. 10.51694/AdvWeedSci/2024;42:00015

Picapietra G, Acciaresi HA (2022) Overcoming seed dormancy of junglerice (*Echinochloa colona*). Chilean J Agr Animal Sci 38(2):154-163. 10.29393/chjaa38-15osgh20015

Radosevich SR, Holt JS, Ghersa CM (2007) Ecology of weed and invasive plants, 3rd ed. Wiley, New York, 454 p.

Romillac N, Piutti S, Slezack-Deschaumes S, Gaba S (2023) Intraspecific functional trait variation in weeds: A strategy in response to competition with crop and weed plants. Weed Res 63(5):283-296. 10.1111/wre.12592

Roush ML, Radosevich SR (1985) Relationships between growth and competitiveness of four annual weeds. J Appl Ecol 22:895-905. 10.2307/2403238

Satorre EH, de la Fuente EB, Mas MT, Suárez SA, Kruk BC, Guglielmini AC, Verdú AM (2020) Crop rotation effects on weed communities of soybean (*Glycine max* L. Merr.) agricultural fields of the Flat Inland Pampa. Crop Prot 130:105068. 10.1016/j.cropro.2019.105068

Shabbir A, Chauhan BS, Walsh MJ (2019) Biology and management of *Echinochloa colona* and *Echinochloa crus-galli* in the northern grain regions of Australia. Crop Pasture Sci 70:917-925. 10.1071/CP19261

Shaner DL (2014) Lessons learned from the history of herbicide resistance. Weed Sci 62(2): 427-431. 10.1614/WS-D-13-00109.1

Shrestha A, De Souza L, Yang P, Sosnoskie L, Hanson B (2018) Differential tolerance of glyphosate-susceptible and glyphosate-resistant biotypes of junglerice (*Echinochloa colona*) to environments during germination, growth, and intraspecific competition. Weed Sci 66(3):340-346. 10.1017/wsc.2017.79

Snaydon RW, Satorre EH (1989) Bivariate diagrams for plant competition data: modifications and interpretation. J Appl Ecol 26(3):1043-1057. 10.2307/2403711

Swanton CJ, Nkoa R, Blackshaw RE (2015) Experimental methods for crop-weed competition studies. Weed Sci 63(sp1):2-11. 10.1614/WS-D-13-00062.1

Tillería SG, Pandolfo C, Presotto A, Ureta S (2024) No fitness cost in *Brassica rapa* L. (wild turnip) populations associated with transgenic glyphosate and non-transgenic AHAS-inhibiting herbicides resistance. Crop Prot 177:106527. 10.1016/j.cropro.2023.106527

Tomiolo S, Damgaard CF, Gay L, Ronfort J, Ehlers BK (2022) A plant growth model to test for changes in plant–plant interaction over a growing season: the case of kin competition. Oikos 2022(12):e09358. 10.1111/oik.09358

Vulchi R, Guan T, Clark T, Brim-DeForest W (2024) Phenotypic characterization of *Echinochloa* species in California rice. Weed Res 64:456-466. 10.1111/wre.12667

Weiner J (2004) Allocation, plasticity and allometry in plants. Perspect Plant Ecol Evol Syst 6(4):207-215. 10.1078/1433-8319-00083

Table 1. Junglerice height (PLH), aboveground dry matter (ADM), seed number (SEN), seed weight (SEW), hundred-seed weight (HSW), and tiller number (TIL) per plant, as influenced by the significant interaction between junglerice density (ECHCO) and soybean inclusion (GLYMA). Different letters indicate significant differences (Fisher LSD, α = 0.05).

ECHCO	GLYMA	PLH		ADM		SEN		SEW		HSW		TIL[*]	
------ pl pot^{-1} ------		cm		cg pot^{-1}		No pl^{-1}		cg pl^{-1}		cg 100^{-1}s^{-1}		No pl^{-1}	
2	0	42.7	c	76	C	431	bc	29	b	6.7	c	13	a
	1	49.2	a	95	A	476	a	43	a	9.1	ab		
4	0	46.3	ab	86	B	391	c	32	b	8.5	b	11	b
	1	48.0	a	97	A	454	ab	41	a	9.3	ab		
8	0	42.4	c	52	D	228	d	22	c	9.8	a	8	c
	1	44.5	bc	54	D	248	d	24	c	9.8	a		

[*] Due to the non-significant interaction, TIL average values are shown for each junglerice density.

Table 2. Junglerice seed number (SEN), seed weight (SEW), and hundred-seed weight (HSW) per plant, as influenced by the significant interaction between the proportion (PROP) of junglerice glyphosate-resistant (R) and -susceptible (S) and junglerice density (ECHCO). Different letters indicate significant differences (Fisher LSD, α = 0.05).

PROP[*]	ECHCO	SEN		SEW		HSW[+]	
	pl pot^{-1}	No pl^{-1}		cg pl^{-1}		cg 100^{-1}s^{-1}	
S	2	347	d	23.9	c	8.1	b
	4	408	cd	30.0	b		
	8	233	e	22.1	c		
S:R	2	442	c	33.5	b	8.0	b
	4	408	cd	31.9	b		
	8	251	e	21.5	c		
R:S	2	519	a	41.7	a	9.4	a
	4	420	c	40.3	a		
	8	225	e	23.4	c		
R	2	505	ab	45.2	a	10.0	a
	4	454	bc	44.4	a		
	8	425	e	24.4	c		

[*] Proportion of EC-S and EC-R individuals per pot: pure EC-S (S), pure EC-R (R), EC-S from the mixture (S:R), and EC-R from the mixture (RS).

[+] Due to the non-significant interaction, HSW average values are shown for each proportion.

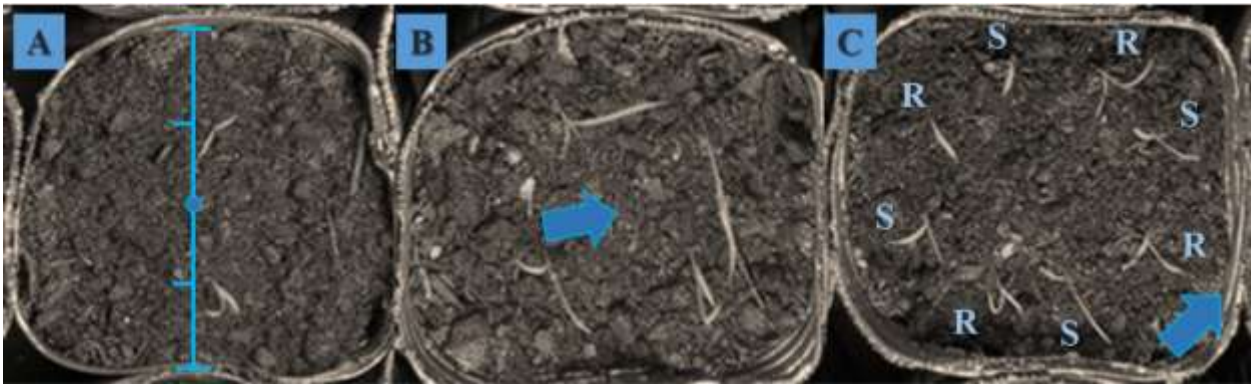

Figure 1. Description of pot procedure: A) Seedlings arranged equidistantly between the pot margin and center; B) soybean planted after junglerice transplanting in the center of the pot; C) in mixtures EC-S and EC-R, seedlings were intercalated, and the margin of the pot was labeled to identify EC-R individuals.

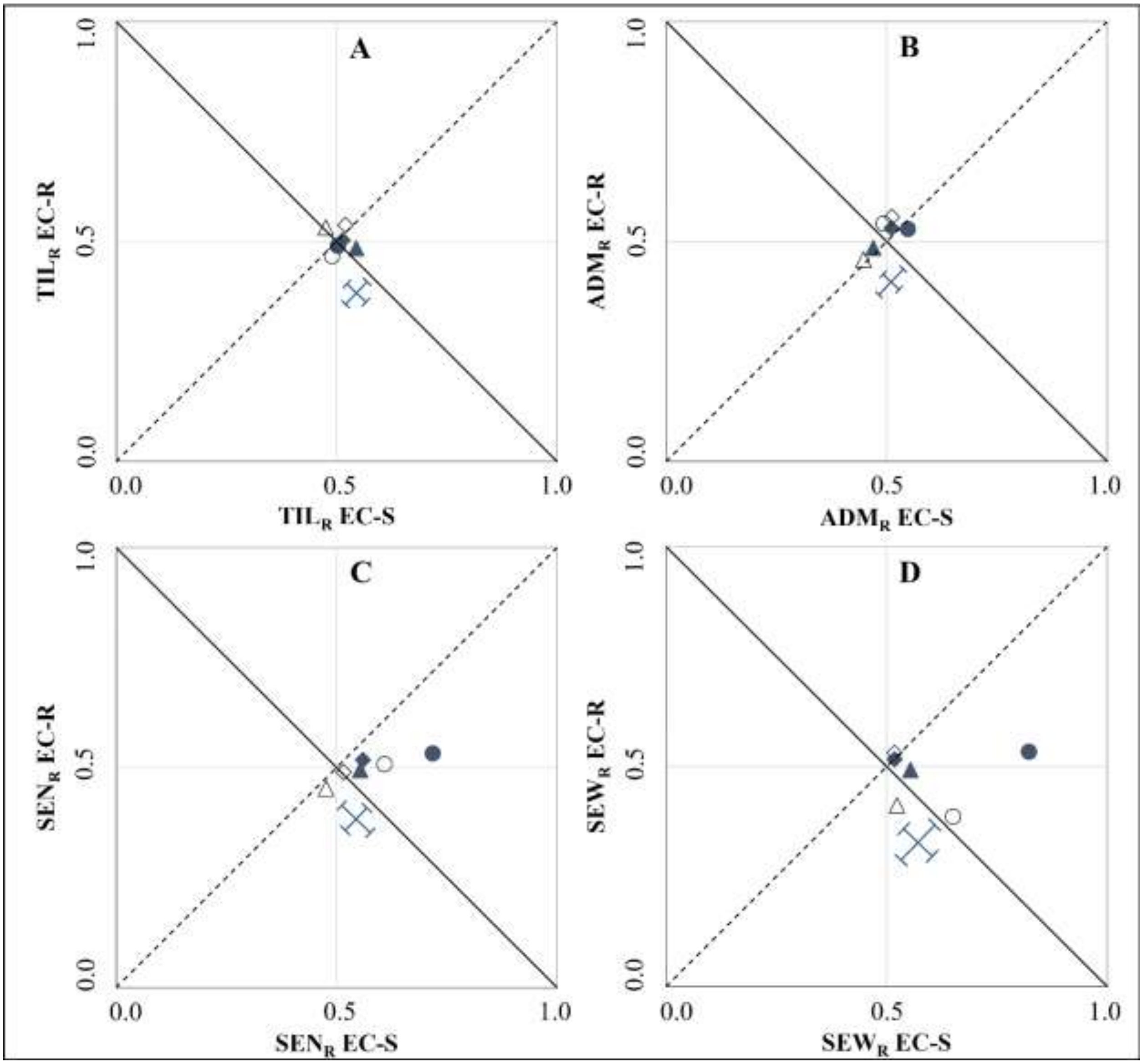

Figure 2. Bivariate plot of relative yield as proposed by Snaydon and Satorre (1989), adapted to the following relative variables: a) total number of tillers (TIL_R), b) aboveground dry matter (ADM_R), c) seed number (SEN_R), and d) seed weight (SEW_R) from glyphosate-susceptible junglerice (EC-S, x-axis) and -resistant (EC-R, y-axis) at densities of 2 plants pot^{-1} (circles), 4 plants pot^{-1} (triangles), and 8 plants pot^{-1} (diamonds), grown without (empty symbols) and with (filled symbols) soybean. Note: The solid diagonal line represents RYT = 1.0, and the dashed diagonal line represents AGR = 0.

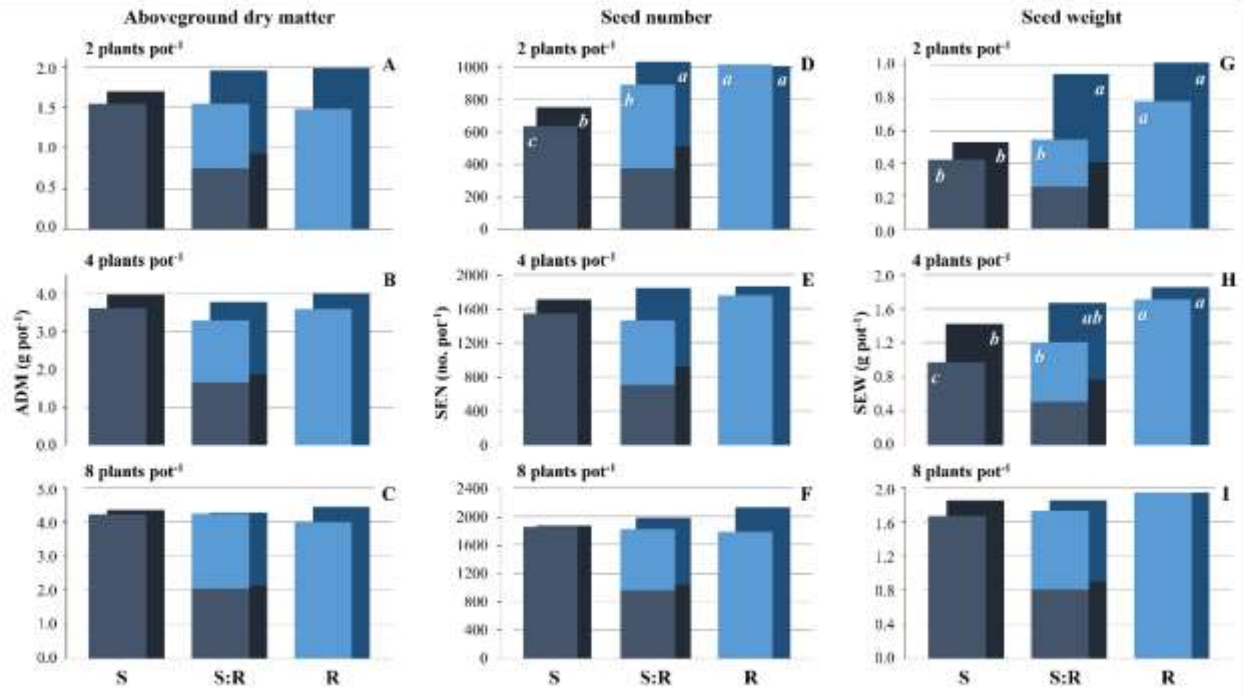

Figure 3. Junglerice aboveground dry matter (ADM; A, B, and C), seed number (SEN; D, E, and F), and seed weight (SEW; G, H, and I) at (from top to bottom) 2, 4, and 8 plants pot^{-1}, for susceptible individuals (S, blue color), resistant individuals (R, cyan color), and the mixture (S:R), grown without (front bars) or with (back bars) one soybean plant. Different letters indicate significant differences between S and R combinations (LSD Fisher, α=0.05).

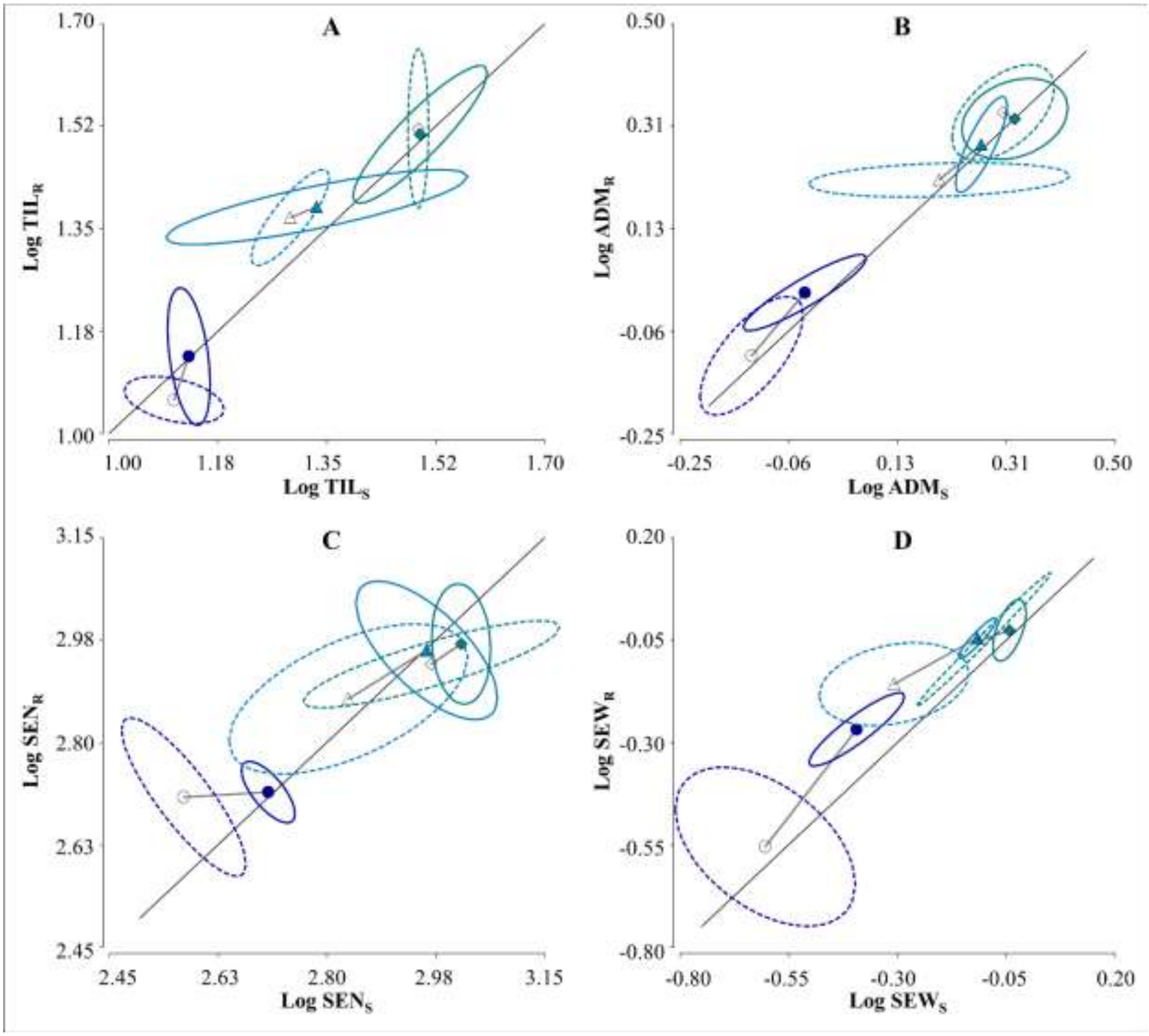

Figure 4. Bivariate double logarithmic plot of yield per pot, based on Snaydon and Satorre (1989), adapted to the following variables: A) total number of tillers (TIL), B) aboveground dry matter (ADM), C) seed number (SEN), and D) seed weight (SEW) from glyphosate-susceptible junglerice (S, x-axis) and resistant (R, y-axis), at 2 plants pot^{-1} (circles), 4 plants pot^{-1} (triangles), and 8 plants pot^{-1} (diamonds), grown without (empty symbols) and with (filled symbols) one soybean plant. Note: The solid diagonal line represents equality of the variable between S and R individuals, and the ellipses represent the 95% confidence area for the bivariate comparison.