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Glyphosate resistance in *Sorghum halepense* and *Lolium rigidum* is reduced at suboptimal growing temperatures

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Abstract

BACKGROUND: Glyphosate resistance in populations of the C_4 perennial *Sorghum halepense* (Johnsongrass) and C_3 annual *Lolium rigidum* (rigid ryegrass) has evolved and been documented in many cropping areas around the globe. In *S. halepense* and in the majority of reported cases in *L. rigidum* the glyphosate resistance trait has been associated with a mechanism that reduces glyphosate translocation within plants. Here, the significant decrease in the glyphosate resistance level when resistant plants of *S. halepense* and *L. rigidum* are grown at suboptimal cool temperature conditions is reported.

RESULTS: Lowering temperature from 30 to 19 °C in *S. halepense* and from 19 to 8 °C in *L. rigidum* significantly reduced both plant survival and above-ground biomass produced by glyphosate-resistant plants. Thus, glyphosate resistance parameters significantly decreased when glyphosate-treated resistant plants of both species were grown under non-optimal temperature conditions. The results suggest that the resistance mechanism against glyphosate damage is less efficient at optimal growing temperatures.

CONCLUSION: It is possible to increase the control of glyphosate-resistant *S. halepense* and *L. rigidum* populations by treatment with glyphosate during growing conditions at suboptimal low temperatures. Conversely, glyphosate failure will continue to occur on glyphosate-resistant populations treated during periods of higher temperatures.

Supporting information may be found in the online version of this article.

Keywords: herbicide resistance management; resistance mechanism; resistance factor; reduced glyphosate translocation; temperature effect

1 INTRODUCTION

Glyphosate resistance evolution in weeds is a current threat to the economy and efficiency of weed management programmes in modern agriculture.[1,2] In subtropical cropping areas of Argentina, populations of the C_4 grass *Sorghum halepense* (Johnsongrass) have evolved glyphosate resistance.[3] Similarly, glyphosate resistance evolution has been documented in C_3 *Lolium rigidum* (rigid ryegrass) populations from temperate areas of Australia.[4] In these specific populations of both species, a similar glyphosate resistance trait involving reduced glyphosate translocation into meristematic tissues has been identified.[5,6]

Reports on the effects of temperature on glyphosate resistance levels in weeds are lacking. A reduction in the glyphosate resistance level in the C_3 annual *Conyza canadensis* when grown at cold temperatures (<10 °C) has been recently documented.[7] Reduced glyphosate translocation due to increased Vacuolar glyphosate sequestration has been identified as the resistance mechanism in this *Conyza* population,[8] and this mechanism has been shown to operate efficiently only under warm temperature conditions (>20 °C).[7] Given the major physiological contrasts and distinct distribution areas associated with *S. halepense* and *L. rigidum*, it is likely that both species have evolved glyphosate resistance under different agroecological conditions. There is no current understanding of the effect of environmental temperature on the phenotypic level of glyphosate resistance in these species. Here, we evaluated the effect of temperature on the phenotypic level of glyphosate resistance in particular tropical C_4 *S. halepense* and temperate C_3 *L. rigidum* populations, from Argentina and Australia respectively, that exhibit a resistance mechanism involving reduced glyphosate translocation. The quantitative level of glyphosate resistance in both species and its dependence on temperature are reported. In these populations, glyphosate resistance was very apparent at optimal growing temperature conditions, but significantly less evident at suboptimal low temperatures.

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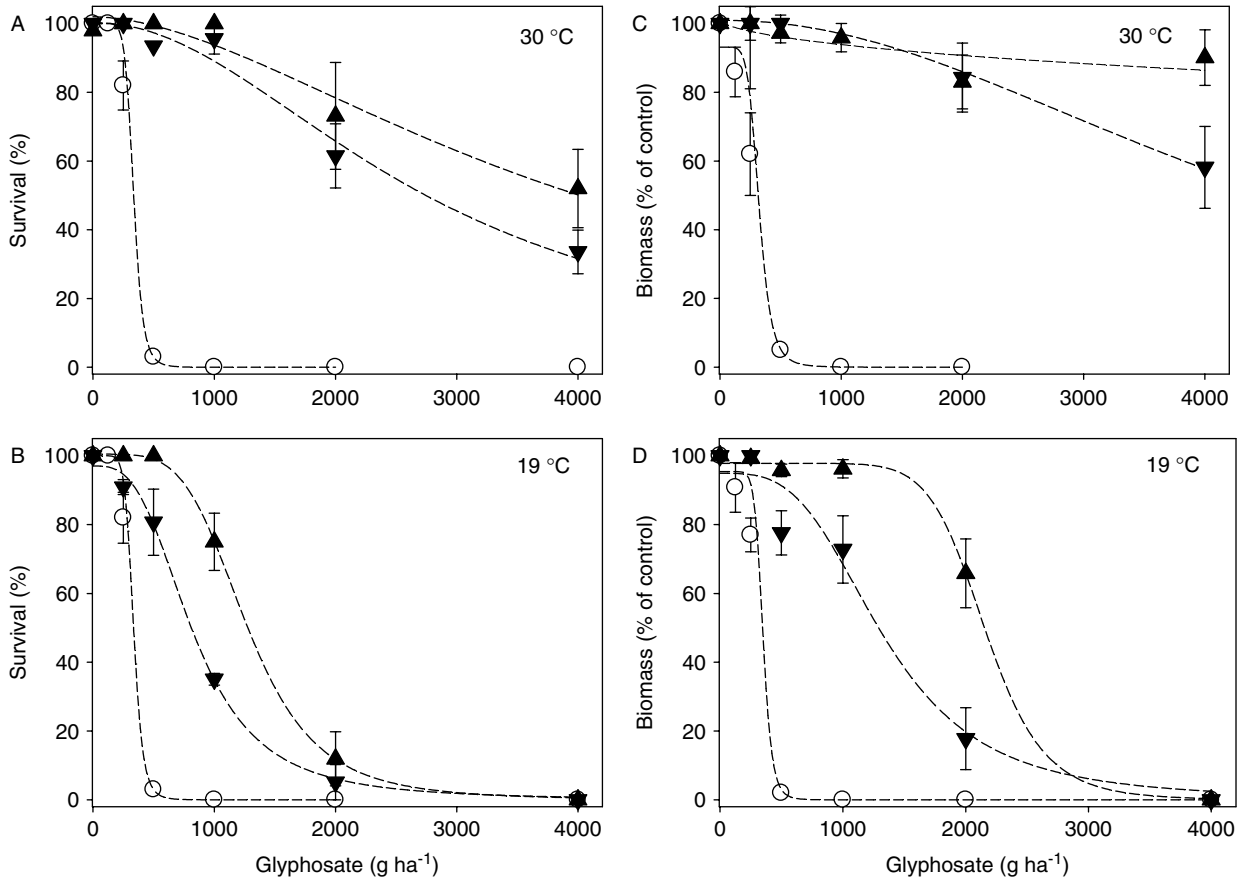

Figure 1. Plant survival (A, B) and above-ground biomass (C, D) in glyphosate-resistant (R_1 ▼, R_2 ▲) and glyphosate-susceptible (S ○) *Sorghum halepense* populations in response to increasing glyphosate rates when grown at 30 and 19 °C. Dashed lines represent predicted values derived from non-linear regression analysis. Symbols denote mean ($n = 3$) ± standard error of the mean.

2 MATERIALS AND METHODS

2.1 Plant species and populations

Two glyphosate-resistant C_4 *S. halepense* populations (R_1, R_2) that evolved glyphosate resistance in transgenic glyphosate-resistant soybean fields in subtropical Salta Province, Argentina, were used in this study.[3] Two glyphosate-resistant populations of the C_3 temperature zone grass *L. rigidum*, NLR_{70} from an orchard[4] and $WALR_{50}$ from a grain farm in Australia, were used.[9] Known glyphosate-susceptible (S) *S. halepense* and *L. rigidum* populations (VLR_1) were used as reference.

2.2 Assessment of the glyphosate resistance level at different temperatures

2.2.1 Growth conditions and glyphosate treatment

To overcome seed dormancy, seeds of the *S. halepense* populations were immersed in sodium hypochlorite bleach (chlorine 5.5% w/v) for 8 h and then washed with tap water. Then, *S. halepense* and *L. rigidum* seeds were incubated and germinated on 0.7% (w/v) agar at 12 hourly alternating 30/20 °C (*S. halepense*) or 25/15 °C (*L. rigidum*) with a 12 h photoperiod and a light intensity of 100 µmol photons m^{-2} s^{-1}. Seedlings (2 cm height) were transferred into plastic pots (17 cm diameter) at a rate of up to 18 plants per pot containing organic soil and washed river sand (2:1 v/v). *Sorghum halepense* plants were grown either at an optimal temperature (30 °C) or at a temperature suboptimal but still allowing growth (19 °C). Similarly, *L. rigidum* plants were grown at optimal (19 °C) and suboptimal (8 °C) temperatures. For all experiments there was a 12 h photoperiod and a 220 µmol m^{-2} s^{-1} light intensity.

At the four-leaf stage (*S. halepense*) and two-leaf stage (*L. rigidum*), plants were treated with increasing rates of a commercial glyphosate formulation (potassium salt), using a laboratory moving boom sprayer equipped with two Tee-jet fan nozzles, with a total output volume of 112 L ha^{-1} water at a pressure of 200 kPa. After glyphosate treatment, plants were immediately returned to the controlled environment maintained at the corresponding temperature.

2.2.2 Assessment of plant traits and glyphosate resistance parameters

Glyphosate effect on plant survival and growth was determined 6 weeks after treatment. Above-ground biomass of surviving plants was harvested and dried at 70 °C for 72 h and then weighed. Based on the parameter estimates of the non-linear regression model (see below), the amount of glyphosate to reduce 50% plant mortality (LD_{50}) and above-ground biomass compared with untreated plants (GR_{50}) was calculated. Quantitative differences in glyphosate resistance level in terms of either survival or biomass traits between resistant and susceptible populations grown at different temperatures were calculated as the resistance factor $RF = X_{50(R)}/X_{50(S)}$, where X_{50} denotes LD_{50} or GR_{50} non-linear regression estimates from the glyphosate resistant (R) and susceptible (S) populations.

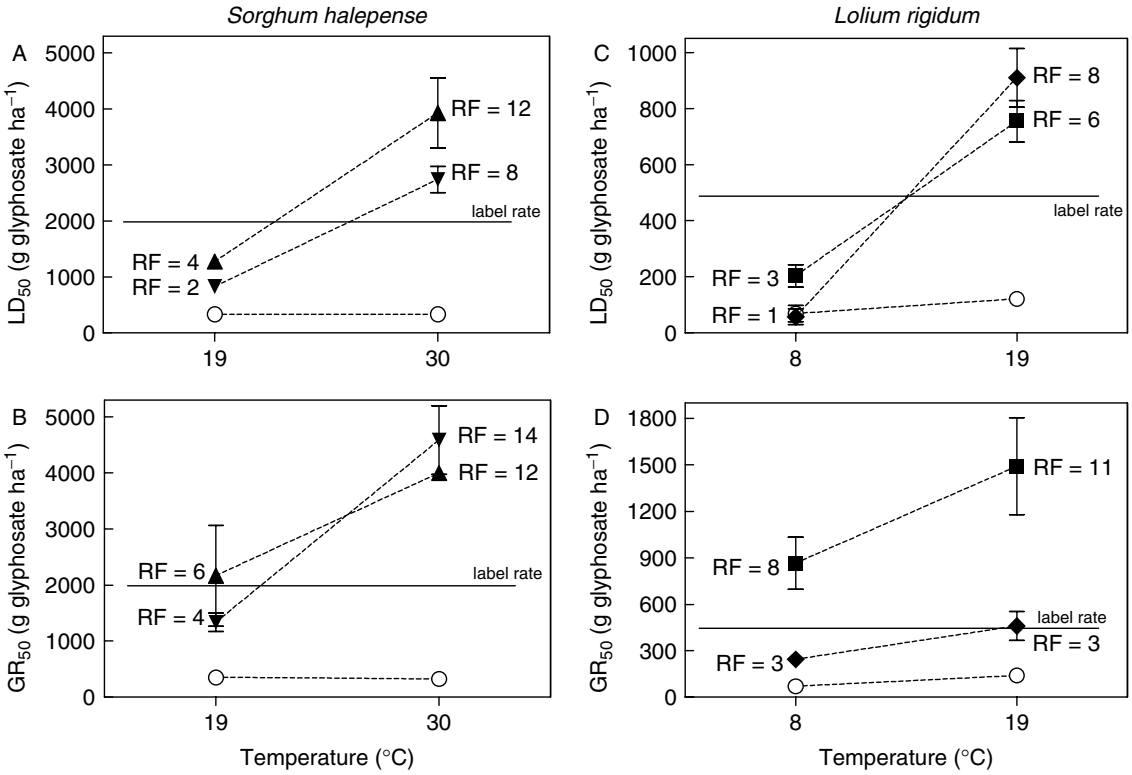

Figure 2. Changes in LD_{50} and GR_{50} non-linear regression estimates for *Sorghum halepense* (A, B) and *Lolium rigidum* (C, D) populations. Glyphosate-resistant (R_1 ▼, R_2 ▲) and glyphosate-susceptible (S ○) *Sorghum halepense* populations grown and glyphosate treated at 30 or 19 °C. Glyphosate-resistant (NLR_{70} ■; $WALR_{50}$ ◆) and glyphosate-susceptible (VLR_1 ○) *Lolium rigidum* populations grown and glyphosate treated at 8 and 19 °C.

2.3 Experimental design and statistical analysis

In a completely randomised experimental design with three replicates per treatment, a multifactorial analysis of variance (ANOVA) was performed to account for the effects of temperature, glyphosate resistance mechanism and glyphosate herbicide on plant survival and above-ground biomass attained by populations of both species (Statgraphics Centurion XV, v.15.2; StatPoint Inc., 2007). Non-linear regression analysis was carried out to estimate a number of glyphosate resistance parameters (LD_{50}, GR_{50}) for plant populations grown at different temperatures. The observed plant survival and biomass data were fitted to a three-parameter logistic model: $y = a/[1 + (x/x_0)^b]$, where y denotes survival or biomass of plants at glyphosate rate x, a is the maximum plant survival or biomass attained and b is the slope at x_0 which represents LD_{50} or GR_{50} resistance parameters (SigmaPlot v.12; Systat Software Inc., 2011). Experiments were replicated in time for both species, and similar results obtained.

3 RESULTS

Regardless of the temperature condition and plant species, the regression model described well the changes in plant survival and biomass at increasing glyphosate doses ($P < 0.001$; $R^2 \geq 0.71$). Overall, quantitative estimations of the GR_{50} resistance parameter were generally higher than LD_{50} values, denoting the ability of surviving plants to recover from glyphosate damage.

Results from ANOVA indicated that the quantitative level of glyphosate resistance exhibited by *S. halepense* and *L. rigidum* significantly interacted with the growth temperature conditions ($P < 0.001$).

3.1 C_4 tropical *Sorghum halepense*

The effect of glyphosate on the tropical C_4 grass *S. halepense* resistance to glyphosate was found to be strongly temperature dependent (30 °C versus 19 °C). Temperature had a remarkable effect on the level of glyphosate resistance and consequently on the magnitude of the LD_{50} and GR_{50} regression estimates (Fig. 1). When grown at 30 °C, individuals of both R_1 and R_2 populations exhibited on average 70% and 50% survival when treated, respectively, with 2000 and 4000 g glyphosate ha^{-1} (Fig. 1A). However, when R_1 and R_2 plants were grown at 19 °C, the plants were markedly more susceptible to glyphosate (Fig. 1B). The effect of contrasting temperatures on plant biomass was equally. For instance, when grown at 30 °C and treated with 2000 g glyphosate ha^{-1}, individuals from the R_1 and R_2 populations survived glyphosate treatment and produced 85% of the biomass of untreated plants (Fig. 1C). However, when treated with the same glyphosate rate but grown at 19 °C, the R_1 and R_2 plants were strongly affected by glyphosate and produced only 20 and 65% biomass of untreated plants (Fig. 1D). No temperature effect was evident with glyphosate-susceptible plants grown at 30 °C versus 19 °C, as estimated LD_{50} and GR_{50} parameters were similar under both growing temperature conditions (Fig. 1) (supporting information Table S1).

As a result of the significant changes in survival and biomass traits of glyphosate-resistant *S. halepense* plants but not of susceptible individuals at 30 °C versus 19 °C, a temperature glyphosate resistance factor (RF) was calculated. For plant survival, the average $RF_{(LD50)}$ for both R_1 and R_2 populations was 10 at 30 °C, but decreased to only 3 at 19 °C (Fig. 2A). When above-ground plant biomass was considered, the R_1 population showed an $RF_{(GR50)}$ of 14 at 30 °C versus 4 at 19 °C (Fig. 2B). Given the high

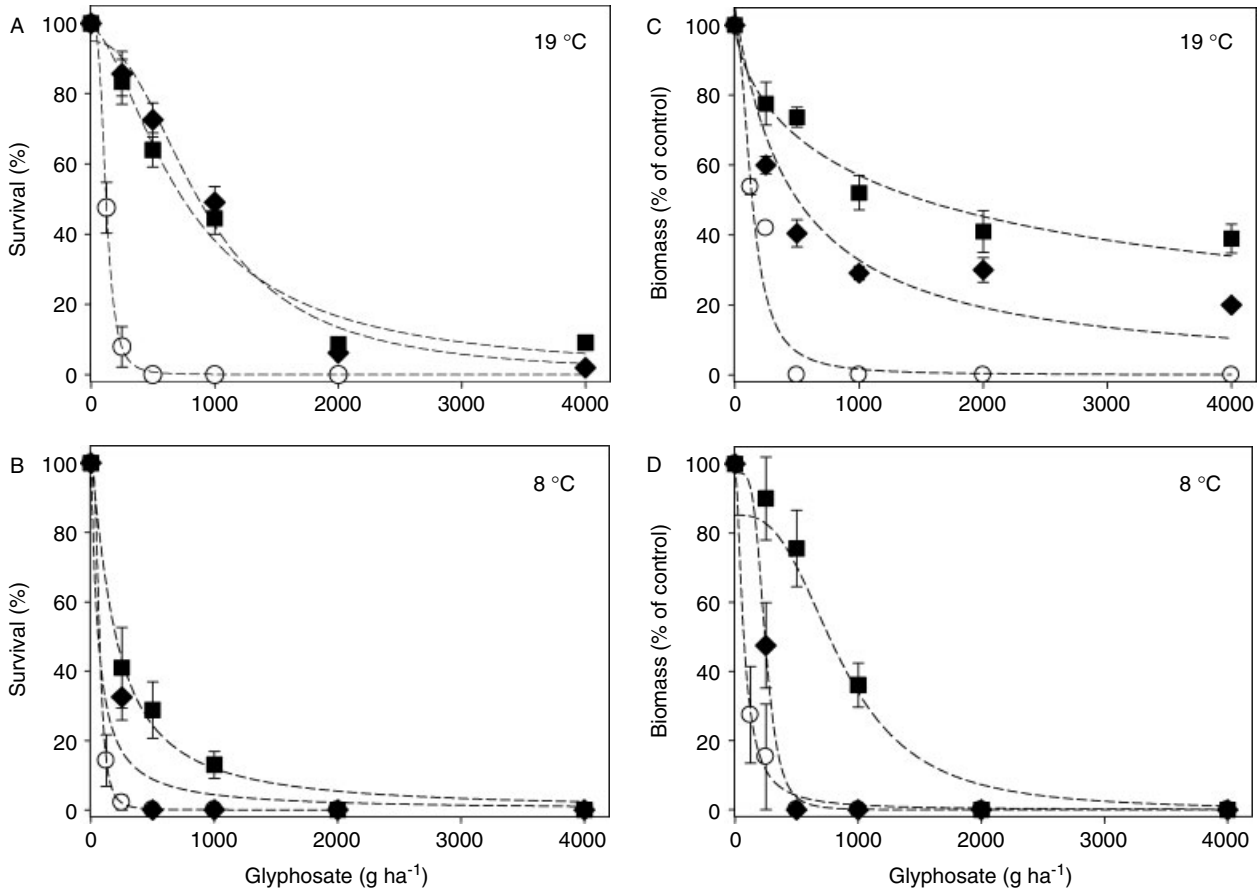

Figure 3. Plant survival (A, B) and above-ground biomass (C, D) in glyphosate-resistant (NLR_{70} ■; $WALR_{50}$ ◆) and glyphosate-susceptible (VLR_1 ○) *Lolium rigidum* populations in response to increasing glyphosate rates when grown at 19 and 8 °C. Dashed lines represent predicted values derived from non-linear regression analysis. Symbols denote mean ($n = 3$) ± standard error of the mean.

biomass production attained by the R_2 population when grown at 30 °C and treated with very high glyphosate rates (Fig. 1C), regression analysis was not significant ($P = 0.1$). As a conservative estimation of the GR_{50} parameter, the highest glyphosate rate (4000 g ha^{-1}) was used. Thus, the $RF_{(GR50)}$ associated with the R_2 population varied from 12 at 30 °C to 6 at 19 °C (Fig. 2B).

3.2 C_3 temperate *Lolium rigidum*

The level of glyphosate resistance displayed by the two resistant populations NLR_{70} and $WALR_{50}$ possessing the reduced glyphosate translocation mechanism depended on temperature ($P < 0.001$). Resistant plants grown and treated with glyphosate at 19 °C showed significantly higher survival rates than when grown at 8 °C (Figs 3A and B). At the glyphosate recommended field rate (500 g ha^{-1}), individuals of both $WALR_{50}$ and NLR_{70} resistant populations exhibited survival rates of about 80% when grown at 19 °C (Fig. 3A). However, when grown at 8 °C there was 0 and 30% survival respectively (Fig. 3B). For the $WALR_{50}$ population low temperature removed the glyphosate resistance status.

This temperature effect did not occur in glyphosate-susceptible plants, with only small differences between plants grown at 19 °C versus 8 °C (Figs 3A and B). The susceptible population exhibited only a small decrease in LD_{50} from 122 g ha^{-1} at 19 °C to 70 g ha^{-1} at 8 °C (supporting information Table S1). Overall, a more pronounced decrease in the LD_{50} parameter of both resistant populations versus the susceptible population at 8 °C versus 19 °C accounted for a significant reduction in $RF_{(LD50)}$ estimates (Fig. 2C).

The biomass production of glyphosate-resistant plants when exposed to glyphosate selection was also affected by the growing temperature conditions (Figs 3C and D). Individuals of both resistant populations treated with glyphosate produced much less biomass when grown at 8 °C versus 19 °C (Figs 3C and D) (supporting information Table S1).

Similarly to the effect of glyphosate on survival, observed biomass responses to glyphosate between glyphosate-resistant and glyphosate-susceptible plants significantly contributed to the decline of both $RF_{(LD50)}$ and $RF_{(GR50)}$ estimates upon a temperature change from 19 to 8 °C (Figs 2C and D).

4 DISCUSSION

4.1 Reduced glyphosate resistance at low temperature

Tropical *S. halepense* and temperate *L. rigidum* are species with contrasting temperature optima for growth, photosynthetic pathway, genetic ploidy, reproductive biology and dispersal strategies.[10,11] In spite of these biological contrasts, the use of glyphosate resistance evolution in the studied populations of both species resulted in a resistance mechanism that restricts glyphosate translocation within plants, thus minimising glyphosate damage.[5,6] The level of glyphosate resistance in the studied populations of both species was found to be strongly

temperature dependent. A significant reduction in both the growth and survival of both species in response to glyphosate was evident when plants were grown at suboptimal versus optimal growing temperature conditions.

The biochemical basis of the temperate dependence of glyphosate resistance reported here remains unknown. This phenomenon has been observed in glyphosate resistant *C. canadensis* populations with reduced glyphosate translocation due to enhanced rates of vacuolar glyphosate sequestration.[7,8] It is possible to speculate that the temperature dependence of glyphosate resistance in the populations of *S. halepense* and *L. rigidum* studied here is a result of inefficiency of the glyphosate resistance mechanism at low temperature.[7]

4.2 Evolutionary significance of results

The results suggest that, with all other factors equal, the selection pressure and consequently the rate of glyphosate resistance evolution in *S. halepense* and *L. rigidum* populations exhibiting restricted glyphosate translocation patterns[6,12,13] will be maximised under optimal growth temperature conditions. Cool suboptimal temperature conditions for both grass species are likely to pose limits on glyphosate resistance evolution at least for plants with the reduced glyphosate translocation mechanism. This may be the case for *S. halepense* in Argentina, where many populations have evolved glyphosate resistance in subtropical agricultural areas but not in temperate–cold cropping areas. The results reported here make evident the need for incorporation of the glyphosate resistance factor and its dependency on environmental temperature conditions in models for an accurate prediction of glyphosate resistance evolution in invasive weed species.

4.3 Implications for management of resistance

The temperature dependence of glyphosate resistance in both *S. halepense* and *L. rigidum* is an ecological response that can be exploited for resistance management. At cool suboptimal temperatures, glyphosate-resistant populations showed resistance parameters (LD_{50}) that were not high enough to endow significant plant survival rates when exposed to the glyphosate field rates currently employed (Fig. 2). The present results are consistent with the observation of substantial reductions in plant survival of glyphosate-resistant *S. halepense* field stands when treated with glyphosate during the period from early autumn to mid-autumn in Argentinian cropping areas (Balbi MC *et al.*, unpublished), and with experimental studies on glyphosate-resistant *L. rigidum* treated during the Australian wintertime.[4]

The present results make it possible to infer that it is possible to increase the control of glyphosate-resistant populations by treatment with glyphosate during growing conditions at suboptimal low temperatures. Conversely, glyphosate failure will continue to occur on glyphosate-resistant populations treated during months of higher temperatures.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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