NATURAL TOLERANCE AND RESISTANCE TO GLYPHOSATE IN GRASS WEEDS: A CASE OF STUDY

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INTRODUCTION

Avena sterilis and *Lolium rigidum* are economically important grass weeds in Europe, where glyphosate has been used to control these species for over three decades. Glyphosate is among the most widely used herbicides for weed control in various cropping systems. This compound is a broad-spectrum herbicide, acting exclusively via foliar uptake, belonging to the group of the non-selective inhibitors of amino acid synthesis.

Were carried out assays of dose-response, metabolism, absortion-traslocation of ^{14}C-glyphosate and EPSPS Gene Sequencing to determine the physiological and molecular basis explaining the differences in their response to glyphosate. Seed samples were collected from fields with a long history of glyphosate use (Exposed -"E") and from areas that had not been so far exposed to herbicide (UnExposed-"UE").

MATERIALS AND METHODS

Plant material

L. rigidum E sample was collected in a vineyard at Saint Pons (South of France). *A. sterilis* E sample was collected from an olive tree field in the province of Ferreira (center of Portugal).

The seeds were disinfected with 1 mM $CaCl_2$ and planted individually in pots of 0,2 L of substrate (2:1 soil:peat). Growing conditions were: day/night temperature of 28/20 ºC and a photoperiod of 12 h light, constant relative humidity of 80%.

Dose–response assays

The glyphosate (Roundup Energy SL, 450 g ae L-1 as isopropylamine salt) applications were made at the BBCH 13-14 stage (Hess *et al.,* 1997).The dose used were: 0, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 g ae ha-¹ at 200 kPa with a spraying nozzle Tee Jet 8002 E VS at a height of 50 cm. The experiment was arranged in a completely randomized design using four replicates (each replicate with three plants) per dose. Fresh weight of plants was measured 21 days after treatment. Data were expressed as a percentage of fresh weight compared to the non-treated control plants.

Metabolism study

Plants of both *A. sterelis* and *L. rigidum* biotypes were treated at a glyphosate dose of 300 g ae ha-1 as described in the dose-response assays section, and other plants were kept without treatment as non-treated controls. At 96 HAT, following the methodology described by Rojano-Delgado *et al*., (2010) glyphosate and its metabolites, i.e. AMPA (aminomethylphosphonic acid), glyoxylate, sarcosine and formaldehyde were determined by reversed-polarity capillary electrophoresis.

Absorption and translocation of 14C-glyphosate

Following the methodology described by González-Torralva *et al.,* (2012).

EPSPS Gene Sequencing

To amplify the EPSPS gene, primers previously designed by Perez-Jones *et al.,* (2007). (forward: 5' AGCTGTAGTCGTTGGCTGTG 3'; reverse: 5' GCCAAGAAATAGCTCGCACT 3'). These primers expand a 543bp fragment of the EPSPS gene that contains the mutation site described as conferring resistance to glyphosate in Lolium spp. The EPSPS DNA and the predicted peptide sequences were searched through the Gen-Bank database using the program BLAST (basic local alignment search tool) (Altschul *et al*., 1990) on the website (http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

Dose–response assays

The ED50 was significantly higher for the E population of *L. rigidum* as compared to its UE counterpart. The resistance factor [ED_{50} (E)/ED_{50} (UE)] was 5.8. By contrast, E and UE populations of *A. sterilis* displayed a similar sensitivity to glyphosate. However, both E and UE populations of A. sterilis exhibited higher ED50 values (283.7 and 297.1 g ae ha $^{-1}$, respectively) than L. rigidum UE, which means that they were more than 3.8 times less sensitive (Figure 1).

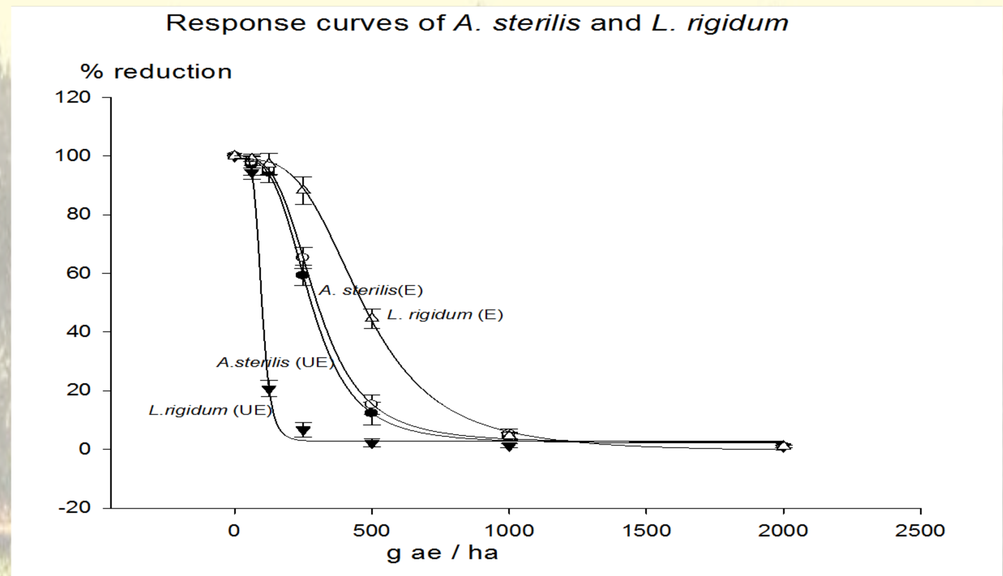

Figure 1. Dose-response curve of biotypes E and UE to glyphosate of *A. sterilis* and *L. rigidum*. Vertical bars represent ±standard error of mean.

Metabolism study

At 96 HAT, glyphosate was metabolized to a low extent (< 15 % of absorbed) in both species. There were no significant differences in the amounts of AMPA and glyoxylate measured in *A. sterilis* plants from the E and UE populations. By contrast, the differences were significant in plants from the E and UE *L. rigidum* populations. However, the amounts of non-metabolised glyphosate was similar across species and populations. These results suggest that glyphosate metabolism is not likely involved in the resistance of the E population of *L. rigidum* and in the tolerance of *A. sterilis* (Figure 2).

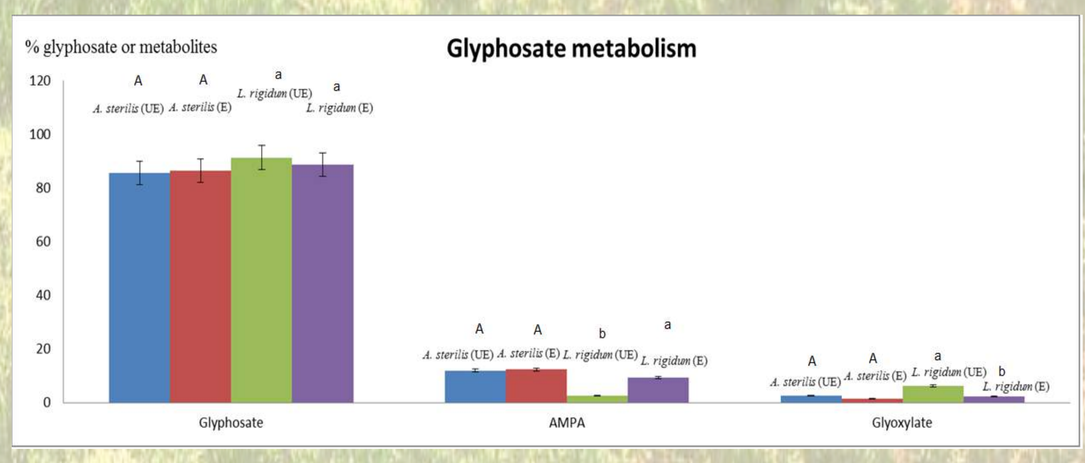

Figure 2. Metabolites of the glyphosate found in biotypes E and UE to glyphosate of *A. sterilis* and *L. rigidum*. Vertical bars represent ±standard error of mean.

Absorption and translocation of 14C-glyphosate

The two species absorbed 60 to 85% of applied 14C-glyphosate. Absorption decreased in the following order: *L. rigidum* UE >*A. sterilis* E and UE >*L. rigidum* E. The uptake difference between the two *L. rigidum* populations was significant

Species	Population	Absorption (%)[a]	Translocation (%)[a,b]		
			treated leaf	rest of shoots	Roots
A. sterilis	E	70.32 ± 1.4 bc	68.64 ± 0.2 a	18.68 ± 2.3 a	12.85 ± 2.5 b
	UE	74.75 ± 2.1 bc	65.72 ± 1.4 a	20.78 ± 1.2 a	13.69 ± 0.8 b
L. rigidum	E	60.27 ± 4.6 c	71.88 ± 2.8 a	20.41 ± 1.6 a	7.87 ± 2.0 b
	UE	85.83 ± 6.2 ab	39.14 ± 1.0 b	34.26 ± 0.3 a	26.89 ± 3.8 a

Means within a column followed by the same letter were not significantly different at the 5% level as per Tukey´s test. Mean values ±standard errors of mean.

EPSPS Gene Sequencing

Results showed a high homology with the EPSPS gene from *L. rigidum* (GenBank: AAK20397.1) and *Eleusine indica* (GenBank: AJ417033.1). Comparison of the EPSPS gene fragment between E and UE biotypes showed a mutation in the resistant biotype at position 106 in the protein amino acid sequence.

DISCUSSION

The results can be used to warn growers about the higher risk of evolved glyphosate resistance in *L. rigidum* as well as to explain why *A. sterilis* is difficult to control because of its natural tolerance to glyphosate.

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