



Glyphosate Tolerance Mechanism in Italian Ryegrass from Mississippi



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Introduction

Glyphosate is a non-selective, broad spectrum, systemic, post-emergence herbicide. It has been used extensively throughout the world in both crop and non-crop lands since its commercialization in 1974. With the introduction of glyphosate-resistant (GR) crops in the mid 1990s, glyphosate is now widely used for weed control in GR crops without concern for crop injury. The widespread adoption of GR crops around the world has resulted in evolution of GR weed biotypes. To date, thirteen weed species are reported to be resistant to glyphosate (Heap 2008) including Italian ryegrass (*Lolium multiflorum* Lam.). We have previously reported a three-fold glyphosate tolerance in two Italian ryegrass populations from Mississippi (Nandula et al. 2007). In this research, we examined the role of absorption and translocation of glyphosate in the mechanism of glyphosate tolerance in these populations. The specific objectives of the research were to (1) determine absorption, translocation, and metabolite patterns of glyphosate in two tolerant (T) and one susceptible (S) Italian ryegrass populations, (2) compare epicuticular wax content for T and S populations, (3) determine efficacy of single-leaf-treated glyphosate on the whole plant control, and (4) compare shikimic acid accumulation patterns in response to glyphosate application in T and S populations.

Materials and Methods

Plant Material and Growing Conditions. Two glyphosate-tolerant Italian ryegrass populations, T1 and T2, and a susceptible S population, were included in this research. Plants were grown in a greenhouse 25/20 °C (d/s) day/night temperature under natural light.

¹⁴C-Glyphosate Absorption and Translocation. ¹⁴C-glyphosate was mixed with commercial potassium salt formulation of glyphosate to obtain a final concentration of 0.84 kg a.i./ha (1X feed rate) in 150 L of water. Each plant received approximately 5.0 kg a.i. of ¹⁴C-glyphosate in a total volume of 10 L. Treatment solutions were applied with a microsprayer to the adaxial surface of the three fully expanded leaf blades of 10- to 15-cm tall (four leaves, two to three tillers) Italian ryegrass plants as 10⁶ tiller densities. Plants were not sprayed with commercial glyphosate prior to application of ¹⁴C-glyphosate to minimize stress during the exposure period. Plants were harvested at 24 and 48 HAT and divided into treated leaf, shoot (including tillers), and roots. Standard procedures of leaf wash, oxidation, and liquid scintillation spectrophotometry were followed to determine absorption and translocation of ¹⁴C-glyphosate in Italian ryegrass plants. The experiment had five replications per treatment.

Wax extraction. Twenty-five fully expanded leaves from greenhouse-grown Italian ryegrass plants (75 d old, 30 to 40 cm tall, five to six leaves, four to eight tillers) were chosen for wax extraction. Total fresh weight and leaf area of selected leaves was recorded. Epicuticular wax was extracted using the procedure described previously (Chahal et al. 2001; Koger and Reddy 2005). Wax was extracted by immersing leaves in 400 ml HPLC-grade chloroform in a glass beaker at room temperature for 20 h in a sonicator. The chloroform-wax solution was filtered into a preweighed beaker. Chloroform was evaporated to dryness under a fume hood for 96 h. Wax mass was expressed as wax mass per unit leaf area and wax mass per unit leaf fresh weight. Three replicates of each population, T1, T2, and S, were analyzed.

Efficacy of Leaf-Treated Glyphosate on Whole Plant. Italian ryegrass plants with four leaves, two to three tillers, and that were 10 to 15 cm tall were used in this study. Treatment solutions were prepared using a commercial potassium salt formulation of glyphosate at 0.84 kg/ha in 150 L of water. Ten microliters of glyphosate solution was placed on the adaxial surface of a third fully expanded leaf as 10 tillers. Plants were harvested 3 wk after treatment and weight of green shoot biomass was recorded and expressed as percentage of shoot fresh-weight reduction compared with respective nontreated plants. There were three replications per treatment.

Shikimic Acid Bioassay with Leaf Segments. Shikimic acid assay was conducted following protocols described previously (Perez-Jones et al. 2005; Reddy et al. 2005). Italian ryegrass plants (populations T1 and S) were used. A fully expanded third leaf was excised from 10- to 15-cm tall (four leaves, two to three tillers) plants and the lowest 2.5-cm portion of the leaf divided into 0.5-cm leaf segments. A single leaf segment per tiller was placed in 100-ml water and 100 ml of glyphosate treatment solutions (0, 0.9, 1.9, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500, and 1,000 μM). There were four replications per treatment.

Shikimic Acid Bioassay with Whole Plants. Italian ryegrass plants (four leaves, two to three tillers, and 10 to 15 cm tall) from all populations were treated with a commercial potassium salt formulation of glyphosate at 0.22 kg a.i./ha in 150 L of water. Aboveground shoot biomass was harvested 1 wk after treatment, washed with running water, rinsed with distilled water to remove glyphosate-potassium remaining on the leaf surface, blotted dry with paper towels, and air dried. Shikimic acid levels were determined following published protocols (Reddy et al. 2005). There were five replications per treatment with eight plants per replication. Shikimic acid values represent average of duplicate samples per replication.

Glyphosate Metabolism. Plant growth stage, herbicide treatment, and shoot harvesting conditions were similar to those described in the 'Shikimic Acid with Whole Plants' section. Armonylglyoxyphosphonic acid (AMPA) analysis was performed according to published procedures (Reddy et al. 2005). There were five replications per treatment with eight plants per replication. AMPA values represent average of duplicate samples per replication.

Statistical Analysis. All experiments were conducted using a completely randomized design and repeated, except the shikimic acid assay with whole plants and the glyphosate metabolism experiment, both of which were performed once. All data, except the shikimic acid assay with leaf segments, were pooled. Treatment means were separated using Fisher's Protected LSD test at P = 0.05. Nonlinear regression analysis involving a sigmoidal logistic model was used to relate shikimic acid levels from leaf segments to herbicide concentration.

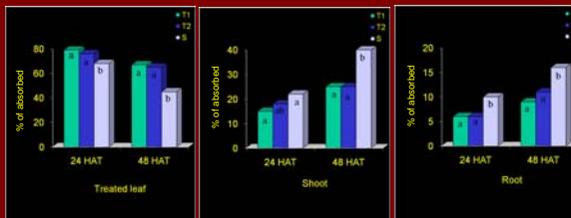


Figure 2. Distribution of ¹⁴C-glyphosate in three Italian ryegrass populations.



Figure 1. Absorption of ¹⁴C-glyphosate in three Italian ryegrass populations.

Figure 3. Epicuticular wax mass of leaves of three Italian ryegrass populations.



Figure 4. Efficacy on (left) and shoot fresh weight reduction (right) of Italian ryegrass plants (10 to 15 cm tall, four leaves, two to three tillers) by glyphosate 3 wk after treatment. A single (third fully expanded) leaf was treated with a solution of commercial potassium salt of glyphosate at 0.84 kg/ha in 150 L of water.

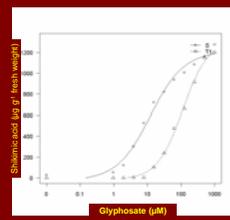


Figure 5. Levels of shikimic acid in 0.5-cm leaf segments of glyphosate-tolerant (T1) and glyphosate-susceptible (S) Italian ryegrass populations after treatment with glyphosate.

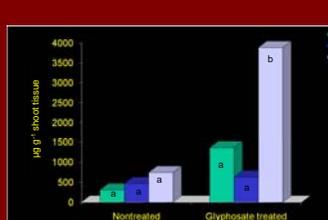


Figure 6. Accumulation of shikimic acid in Italian ryegrass plants (10 to 15 cm tall, four leaves, two to three tillers) 1 wk after treatment with glyphosate at 0.22 kg/ha.

Results

¹⁴C-glyphosate Absorption (Figure 1) and Translocation (Figure 2). The T1 population absorbed less ¹⁴C-glyphosate compared to the S population at 24 and 48 HAT, respectively. The T2 population absorbed ¹⁴C-glyphosate at levels that were similar to both T1 and S populations, but tended to be more comparable to the S population. The quantity of ¹⁴C-glyphosate that remained in the treated leaf at 48 HAT was higher in both T1 and T2 populations compared to the S population. Furthermore, the amount of ¹⁴C-glyphosate that accumulated in the shoot and root was lower in both T1 and T2 populations at 24 and 48 HAT compared to the S population.

Leaf Wax Mass (Figure 3). There were no differences in epicuticular wax mass among the three Italian ryegrass populations.

Efficacy of Leaf-Treated Glyphosate on Whole Plant (Figure 4). The S plants were killed 3 wk after treatment. In contrast, the T1 and T2 plants survived with 33 and 55% reduction in shoot fresh weight, respectively. The T1 plant recovered better compared to the T2 plant, with the latter exhibiting a higher degree of chlorosis.

Shikimic Acid Bioassay with Leaf Segments (Figure 5). Shikimic acid accumulated at higher levels in glyphosate-treated leaf segments of the S population compared to the T1 population. However, levels of shikimate were similar in the S and T1 populations at 500 and 1,000 μM glyphosate.

Shikimic Acid Bioassay with Whole Plants (Figure 6). There were no differences in shikimic acid levels between nontreated Italian ryegrass plants of the three populations. However, glyphosate-treated plants from the S population accumulated more shikimic acid than those from the T1 and T2 populations.

Glyphosate Metabolism. No degradation of glyphosate to AMPA was detected in these tolerant and susceptible Italian ryegrass populations (data not shown).

Conclusions

- Tolerance to glyphosate in the T1 population is partly due to reduced absorption and translocation of glyphosate compared to the S population. Reduced translocation of glyphosate has contributed to tolerance of the T2 population.
- Shikimate accumulation at a faster rate in leaf segments of the S population compared to the T1 population and three-fold more shikimate levels in glyphosate-treated S plants than in the T1 and T2 plants indicates alteration in the cellular transport of glyphosate conferring resistance.
- Investigations are currently underway to determine possible role of an altered target site.

Literature Cited

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