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Potential basis of glyphosate resistance in California rigid ryegrass (*Lolium rigidum*)

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Glyphosate-resistant rigid ryegrass has been identified in California, but research has yet to elucidate the resistance mechanism. The objectives of this study were to examine the differences between sensitive and resistant rigid ryegrass in absorption and distribution of glyphosate, in vivo and in vitro absorption by chloroplasts, and shikimic acid accumulation after glyphosate treatment. Foliar absorption and distribution of ^{14}C-glyphosate did not differ 1 to 3 d after treatment (DAT) between the susceptible (S) and resistant (R) biotypes. Absorption of ^{14}C-glyphosate by isolated chloroplasts also did not differ between the S and R biotypes. After foliar application of ^{14}C-glyphosate, chloroplasts were isolated from treated leaves from both biotypes. Accumulation of ^{14}C-glyphosate in the chloroplasts did not differ between the two biotypes. Shikimic acid level increased significantly in the S biotype after treatment with glyphosate at 2.24 kg ai ha^{-1} to levels 10-fold greater than in the R biotype 11 DAT. Shikimic acid in the germination media at 2 to 5 mM did not affect seed germination of S and R biotypes but drastically decreased the length of coleoptiles of both at 5 DAT. Thus, biotype differences in sensitivity or metabolism of shikimic acid do not explain differences in sensitivity to glyphosate.

Nomenclature: Glyphosate; rigid ryegrass, *Lolium rigidum* Gaud. LOLRI.

Key words: Glyphosate resistance, shikimic acid, chloroplast, absorption, translocation.

Glyphosate is the world's most widely used herbicide. It is a foliar nonselective herbicide and has no activity in the soil (Ahrens 1994; Baird et al. 1971). It can be used preplant to control emerged weeds in a no-tillage planting system or postemergence by spot and direct application to control an extensive range of weeds (Ahrens 1994), as well as to control weeds in glyphosate-resistant crops (Padgette et al. 1996).

Glyphosate in susceptible plant species inhibits biosynthesis of the aromatic amino acids tryptophan, tyrosine, and phenylalanine (Siehl 1997). In the shikimate pathway, glyphosate competes with substrate phosphoenolpyruvate (PEP) for the binding site of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; E.C. 2.5.1.19). Glyphosate is the only herbicide reported to inhibit EPSPS (Steinrucken and Amrhein 1980).

Metabolism of glyphosate in higher plants is very limited and not well understood. Glyphosate is not readily metabolized if applied at phytotoxic rates (Sandberg et al. 1980). Coupland (1985) reported that glyphosate metabolism to aminomethyl phosphonic acid (AMPA) is slow.

Since being introduced almost 30 yr ago, glyphosate has been intensively used for controlling weeds, at times without rotation with other methods. This condition may lead to the selection of resistant weed species (Bradshaw et al. 1997; Dyer 1994; Kishore et al. 1992). Glyphosate resistance has been reported in rigid ryegrass in Australia (Powles et al. 1997, 1998; Pratley et al. 1999) and California (Simarmata et al. 2001), in goosegrass (*Eleusine indica*) in Malaysia (Lee and Ngim 2000; Tran et al. 1999), and in horseweed [*Conyza canadensis* (L.) Cronq.] (VanGessel 2002).

Tran et al. (1999) reported that the mechanism of glyphosate resistance in Malaysian goosegrass populations is target site based. They showed that EPSPS was not inhibited by glyphosate in the R biotype. However, the basis of glyphosate resistance in rigid ryegrass from Australia is not clearly understood. Uptake, translocation, and metabolism were not different between the resistant (R) and susceptible (S) biotypes (Feng et al. 1999; Lorraine-Colwill et al. 1999). The sensitivity of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAHPS; E.C.4.1.2.15) and EPSPS within the plastid cell was also similar for the R and S biotypes. Lorraine-Colwill et al. (1999) proposed that possible differences in glyphosate transport into or accumulation of glyphosate in the chloroplast might explain the differential basis of sensitivity.

Lorraine-Colwill et al. (1999) reported that shikimic acid accumulated in leaf tissue of glyphosate-susceptible biotype after glyphosate application. Shikimic acid accumulation has been identified in glyphosate-susceptible corn (Singh and Shaner 1998) as well as glyphosate-sensitive cotton (Pline et al. 2002). Singh and Shaner (1998) stated that shikimic acid accumulation may be used as a method to determine whether a plant species is resistant to glyphosate. It may be important as a means of quickly identifying and characterizing glyphosate-resistant weed biotypes to avoid their spread and to facilitate their effective management. Harring et al. (1998) stated that this assay was also useful to evaluate the efficacy of different glyphosate formulations.

The objectives of this research were to determine the role of absorption, translocation, and metabolism of ^{14}C-glyphosate in glyphosate-resistant rigid ryegrass from California, to study the movement of ^{14}C-glyphosate into the chloroplast, to investigate shikimic acid accumulation in response to glyphosate and AMPA, and to test for differential phytotoxicity of shikimic acid to rigid ryegrass biotypes.

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