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## Inheritance of glyphosate resistance in rigid ryegrass (*Lolium rigidum*) from California

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Glyphosate resistance was found in a rigid ryegrass population in northern California. A sample of the resistant plants were collected and grown under greenhouse conditions. The objective of this study was to evaluate glyphosate resistance in the progeny of the collected plants by recurrent selection, obtain the homozygous resistant and sensitive lines to establish dose-response curves, and to determine the inheritance of glyphosate resistance in rigid ryegrass. Diverse levels of resistance were observed in the first generation with survival of 89, 59, 45, and 9% from glyphosate at 1x, 2x, 4x, and 8x respectively, where x = 1.12 kg ha<sup>-1</sup> isopropylamine salt of glyphosate. Clones of plants that died from 1x were allowed to produce seed and were further subjected to recurrent selection to generate the most sensitive plants (S lines), which died from 0.125x glyphosate. The most resistant plants (R lines) were generated from the survivors receiving 8x glyphosate. The ratio between I<sub>50</sub> rates for the glyphosate resistant and the glyphosate sensitive plants was > 100-fold. The R and S lines were crossed reciprocally and F<sub>1</sub> progeny of both (R × S) and (S × R) showed intermediate resistance. These survived up to 2x glyphosate. The F<sub>2</sub> progeny were generated by intercrossing of F<sub>1</sub> plants. The ratio of sensitive, intermediate, and resistant plants in the F<sub>2</sub> population before the treatment of glyphosate at 0.125x followed by 8x was 1 : 16, 14 : 16, and 1 : 16 respectively, which corresponded to the Mendelian segregation ratio of two genes. The results indicated that the inheritance of glyphosate resistance in rigid ryegrass from California appeared to be nuclear, incompletely dominant, multigenic, and pollen-transmitted with no indication of maternal inheritance.

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

**Key words:** Glyphosate resistance, inheritance.

After glyphosate use for more than 30 yr, some species of weeds resistant to glyphosate have been reported in several countries (Heap and LeBaron 2001). Glyphosate resistance has been reported in rigid ryegrass in Australia (Powles et al. 1998; Pratley et al. 1999) and California (Simarmata et al. 2001), goosegrass (*Eleusine indica*) in Malaysia (Lec and Ngim 2000; Tran et al. 1999), horseweed (*Conyza Canadensis*) in Delaware (VanGessel 2001), Italian ryegrass (*Lolium multiflorum*) in Chile (Perez and Kogan 2003), hairy fleabane (*Conyza bonariensis*) and buckhorn plantain (*Plantago lanceolata*) in South Africa (Heap 2004).

Rigid ryegrass found in California was most likely introduced from the Wimmera-Mallee area of Victoria, Australia, where this grass was commonly naturalized (Anderson and Sharp 1995; Sawyer and Keeler-Wolf 1997; Skinner and Pavlik 1994). After natural selection for 13 generations at Pleasanton, CA, Wimmera ryegrass was released in 1962 (PI 11419) by the California Agricultural Experiment Station, Davis, CA, and the Plant Material Center, Soil Conservation Service, Lockeford, CA (Hickman 1993; Skinner et al. 1994). It was used as a forage and cover crop for revegetation of disturbed areas and for wildfire burn rehabilitation (Sawyer et al. 1997). The current distribution of rigid ryegrass in the United States includes Oregon, California, Arizona, Texas, Louisiana, and Missouri (USDA 2000).

Breeding methods used for the first released rigid ryegrass cultivar in the United States were natural selection and roughing (Anderson and Sharp 1995). These methods iden-

tified and disposed of abnormal plants, and those with desirable characteristics were used as parents to generate the following cycles. The breeding method described by Corkill (1956) is ideal for self-incompatible plants. The procedures are most likely similar to a poly-cross breeding cycle used in the Institute of Grassland and Environment Research (IGER) breeding program (Thorogood 2003). Individual plants that have specified phenotypic characteristics were selected from the population and used for parents of the new generation. Although Corkill (1956) found that six was the optimum number of plants to obtain and retain in the subsequent generations, this number would be dependent on the heterozygosity and heterogeneity for any character.

The genus *Lolium* includes five species worldwide. They can be separated into the allogamous (self-incompatible) group including *Lolium perenne* L. (perennial ryegrass), *L. multiflorum* Lam. (annual ryegrass), and *L. rigidum* Gaud. (rigid ryegrass) and the autogamous (self-compatible) group including *L. temulentum* L. and *L. remotum* Schrank (Thorogood 2003).

Allogamous plants are self-incompatible and naturally out-cross within or between species in the same genus (Terrel 1968; Thorogood 2003). The ploidy level of ryegrass is diploid and has 14 chromosomes (2n = 14). The origin of rigid ryegrass has been proposed to be from European countries such as Corsica, Italy, and France (Clayton and Revoize 1986; Terrel 1968).

Inheritance of herbicide resistance in weeds may influence

were resistant (survived 8x glyphosate). These numbers were tested against the hypothesized distribution ratio of (1/4) : (2/4) : (1/4), and (1/16) : (14/16) : (1/16) associated with inheritance by one or two genes, respectively. Values of the  $\chi^2$  ( $\chi^2 = 0.8$ ;  $0.75 < P < 0.90$ ) with the tested ratio (1/16) : (14/16) : (1/16) indicated that at least two genes were involved in the inheritance of glyphosate resistance (Table 3). The involvement of multiple genes for glyphosate resistance in the rigid ryegrass collected from California is similar to the results reported by Feng et al. (1999) and Pratley et al. (1999) for rigid ryegrass from Australia but different from the inheritance study published by Lorraine-Colwill et al. (2001), who concluded that the inheritance of glyphosate resistance in rigid ryegrass involved a single semidominant gene.

In summary, rigid ryegrass is an allogamous species that naturally outcrosses and is self-incompatible. The glyphosate-resistant ryegrass population from northern California appeared taxonomically to be *L. rigidum*. However, the possibility of past hybridization with other *Lolium* species was not eliminated. Recurrent selections through eight and four generations were necessary to generate the homozygous-resistant and -sensitive parent, respectively, for the genetic inheritance study. Ratio of glyphosate  $I_{50}$  between resistant and sensitive was > 100-fold. This ratio appeared to be the highest reported for glyphosate-resistant weeds. The inheritance of glyphosate resistance in rigid ryegrass from California appeared to be nuclear, semidominant, multigenic, and pollen-transmitted with no indication of maternal inheritance (Table 3). Based on the results obtained, high-application rates of glyphosate may control the heterozygous population decreasing potential spread of the resistant population as discussed by Diggle and Neve (2001).

### Sources of Materials

<sup>1</sup> BACTO professional planting mix, Michigan Peat Co., P.O. Box 980129, Houston TX 77098.

<sup>2</sup> ROUNDUP ULTRA herbicide, Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198.

<sup>3</sup> TeeJet 8001E, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

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