GLYPHOSATE-RESISTANT HORSEWEED (*Conyza canadensis* L. Cronq.) BIOTYPE FOUND IN THE SOUTHERN SAN JOAQUIN VALLEY.

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Abstract:

Glyphosate-resistant horseweed biotypes have been reported in 10 states in the US, mainly in annual row-crop systems. This study showed that glyphosate-resistant (R biotype) horseweed also exists in the Southern San Joaquin Valley and California is now the 11th state to report glyphosate-resistant horseweed. The level of resistance to glyphosate, however, was influenced by the stage of growth of horseweed at the time of glyphosate application. There was a probability of controlling some of the 'R' biotype horseweeds at the 5-8 leaf stage with a 2x or 4x rate of glyphosate. After the 18-21 leaf stage, the horseweed plants were able to survive glyphosate application rates up to 4 lb ai/ac. At later stages, even some plants of the 'S' biotype escaped the lower rates of glyphosate. Therefore, it is important to control horseweed at an early stage of growth. This is the first case of a glyphosate-resistant horseweed biotype existing in a non-crop situation. Close monitoring and an integrated weed management program will have to be implemented to manage glyphosate-resistant horseweed biotypes in the Southern San Joaquin Valley.

Introduction:

Herbicide resistance is defined by the Weed Science Society of America as "the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis." Herbicide resistance was first reported in 1957. Since then, 304 weed biotypes have developed resistance to several groups of herbicides including glyphosate (Heap, 2005).

Glyphosate [*N*-(phosphonomethyl)glyine] is a non-selective, broad spectrum, systemic, post-emergence herbicide. This herbicide kills weeds by metabolic disruptions in the plant (Franz et al. 1997). It inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) which is essential for biosynthesis of aromatic amino acids (Mueller et al. 2003). Because of the complex manipulations of the target EPSPS enzyme required for developing glyphosate-resistant crops, it was commonly believed that weeds developing resistance to glyphosate was improbable (Bradshaw et al. 1997). However, it has been repeatedly found that development of herbicide resistance in weed populations is greatly increased by repeated use of a single herbicide (Holt, 1992). Eventually, glyphosate-resistance was documented in rigid ryegrass (*Lolium rigidum* L.) in 1996 in Australia (Powles et al. 1998). Since then, 15 weed species, including horseweed

(*Conyza canadensis* L. Cronq.), are reported to have developed resistance to glyphosate (Nandula et al. 2005).

Horseweed or mare'stail is an annual plant belonging to the Asteraceae family and it is native to North America (Weaver, 2001). The first case of a glyphosate-resistant horseweed in North America was reported from Delaware in 2000 (VanGessel, 2001). Since then, nine other states in the US have reported the occurrence of glyphosate-resistant horseweed (Heap, 2005). All of these cases were from annual row-crop systems, viz. glyphosate-resistant cotton and soybean. It is believed that intense use of glyphosate in glyphosate-resistant crops has caused the evolution of several weed populations with natural resistance to glyphosate (Nandula et al. 2005). No case of glyphosate-resistant horseweed has been reported in California and in non-crop areas. Poor control of horseweed with glyphosate was reported in an irrigation canal bank in Dinuba, CA with suspected glyphosate- resistance (personal communication J. Heringer). Glyphosate had been used repeatedly at this site from the past 15 years. Therefore, the objective of this study was to confirm the existence of glyphosate-resistant horseweed in seeds collected from Dinuba, CA and to evaluate the interaction of glyphosate rate and plant growth stage.

Materials and Methods:

Horseweed seeds were collected from a suspected glyphosate-resistant population in Dinuba, CA and a suspected susceptible population in western Fresno, CA in the fall of 2004. Seeds were stored under room temperature in the lab. On April 4, 2005, seeds were planted in plastic germination trays in the lab and moved to a greenhouse soon after emergence (April 13, 2005). The seedlings were allowed to establish and then transplanted into plastic pots (6 inches deep, 4 inches wide) containing a potting mix (Promix 5, Sungro Horticulture, Canada) on May 5, 2005. The suspected glyphosate-resistant plants were designated as 'R' biotype and the suspected susceptible biotype was designated as 'S' biotype. One seedling of each biotype was planted in each pot for a total of 200 pots. Of these, 80 pots were assigned each to five greenhouse benches as five replications. The 80 pots were then separated into five groups of 'R' and 'S' biotype. The group designations were based on the plant growth stage for glyphosate application as follows:

1. Glyphosate spray at 5-8 true leaf stage of horseweed.
2. Glyphosate spray at 11-15 true leaf stage of horseweed.
3. Glyphosate spray at 18-21 true leaf stage of horseweed.
4. Glyphosate spray at bolting to 6 inch (height) stage of horseweed.
5. Glyphosate spray at 6 inch to 1 foot (height) stage of horseweed.

A fully expanded leaf was considered a true leaf. Within each group, the pots were further divided into four sub-groups, each to receive 0 (no glyphosate, control), 1x (1 lb ai/ac glyphosate), 2x (2 lb ai/ac), and 4x (4 lb ai/ac). Roundup Weathermax® (contains 5.5 lb ai/gal of glyphosate) was the herbicide used in the study. Therefore, within each

replication, there were 40 pots each of 'R' and 'S' biotype divided into five growth stages and four herbicide rates. Each pot containing a seedling was an experimental unit. The experimental design was a two factor (glyphosate rate and plant growth stage) completely randomized block with five replications. The plants were watered regularly and fertilized twice during the growing season with a commercial fertilizer (Miracle Gro, 4 g per gallon of water).

Glyphosate was applied at the designated growth stage of the plants with a CO_2 back-pack sprayer (Figure 1). The spray was discharged through a 40" boom, with a single flat-fan nozzle (TeeJet XR8002EVS) in the center and a blank at each end, 18" above the target plants. The system was pressurized to 30 psi to deliver the herbicide solution at 35 gpa (broadcast acre basis) in a 20" band. The plants were moved outside the greenhouse, sprayed, and moved back to the greenhouse after the spray dried on the leaves. The survival of each plant was evaluated weekly and data were recorded as 'alive' or 'dead'. The plants were designated as 'dead' when the above-ground plant parts started disintegrating and showed no traces of green tissue. Data were compiled as percent 'dead' or 'alive' plants and analyzed using GLM procedures in SAS. The level of significance used for the analysis was 0.05. The experiment is being repeated.

Figure 1. Glyphosate application with a back-pack sprayer.

Results and Discussion:

The 'R' and 'S' biotypes differed significantly in their ability to survive the various glyphosate treatments. A significant interaction occurred between horseweed growth stage and glyphosate application rate for both biotypes. When glyphosate was applied at the 5-8 leaf stage of horseweed, 100% of the 'R' biotype plants survived the 1x glyphosate rate (Figure 2a). Survival of the plants was reduced to 60% at the 2x rate, while none of the horseweed plants survived the 4x rate. However, none of the 'S' biotype plants survived any of the glyphosate application treatments (Figure 2a).

When glyphosate was applied at the 11-15 leaf stage of horseweed, 100% of the 'R' biotype plants survived the 1x and 2x glyphosate rates (Figure 2b) and 40% survived the 4x rate. Unlike the 5-8 leaf stage, 20% of the 'S' biotype plants survived the 1x glyphosate treatment (Figure 2b). At the 18-21 leaf stage of horseweed, 100% of the 'R' biotype plants survived the 1x and 2x glyphosate rates and 80% of plants survived the 4x rate (Figure 2c). At this growth stage, the survival of the 'S' biotype at the 1x glyphosate treatment was 40% (Figure 2c). An example of the visual damage symptoms on the horseweed plants at the 18-21 leaf stage is shown in Figure 3.

After the plants bolted, the 'R' biotype plants survived all rates of glyphosate (Figure 2d, e). Similarly, glyphosate application at a 1x rate after bolting, also increased the survival capability of the 'S' biotype and it was observed that 20% of the plants were even able to survive the 2x rate (Figure 2d, e).

These results showed that the 'R' biotype was resistant to glyphosate but the level of resistance varied with growth stage. There was a probability of controlling some of the 'R' biotype horseweed at the 5-8 leaf stage with 2x and 4x rates. At later stages, even some plants of the 'S' biotype escaped the lower rates of glyphosate. This demonstrated the importance of controlling horseweed at an early stage of growth. Therefore, when using glyphosate as a post-ement treatment, efforts should be directed to control horseweed plants very soon after emergence (before they develop more than 8 true leaves). Growers and land managers should not wait for all the horseweed plants to emerge before applying glyphosate. If the population of horseweed is to be reduced, several successive herbicide applications may have to be made to control the flushes of horseweed emergence over the growing season. An integrated weed management program should be implemented to manage the glyphosate-resistant horseweed population, including pre- and post-emergence herbicides, cultivation, and other effective methods.

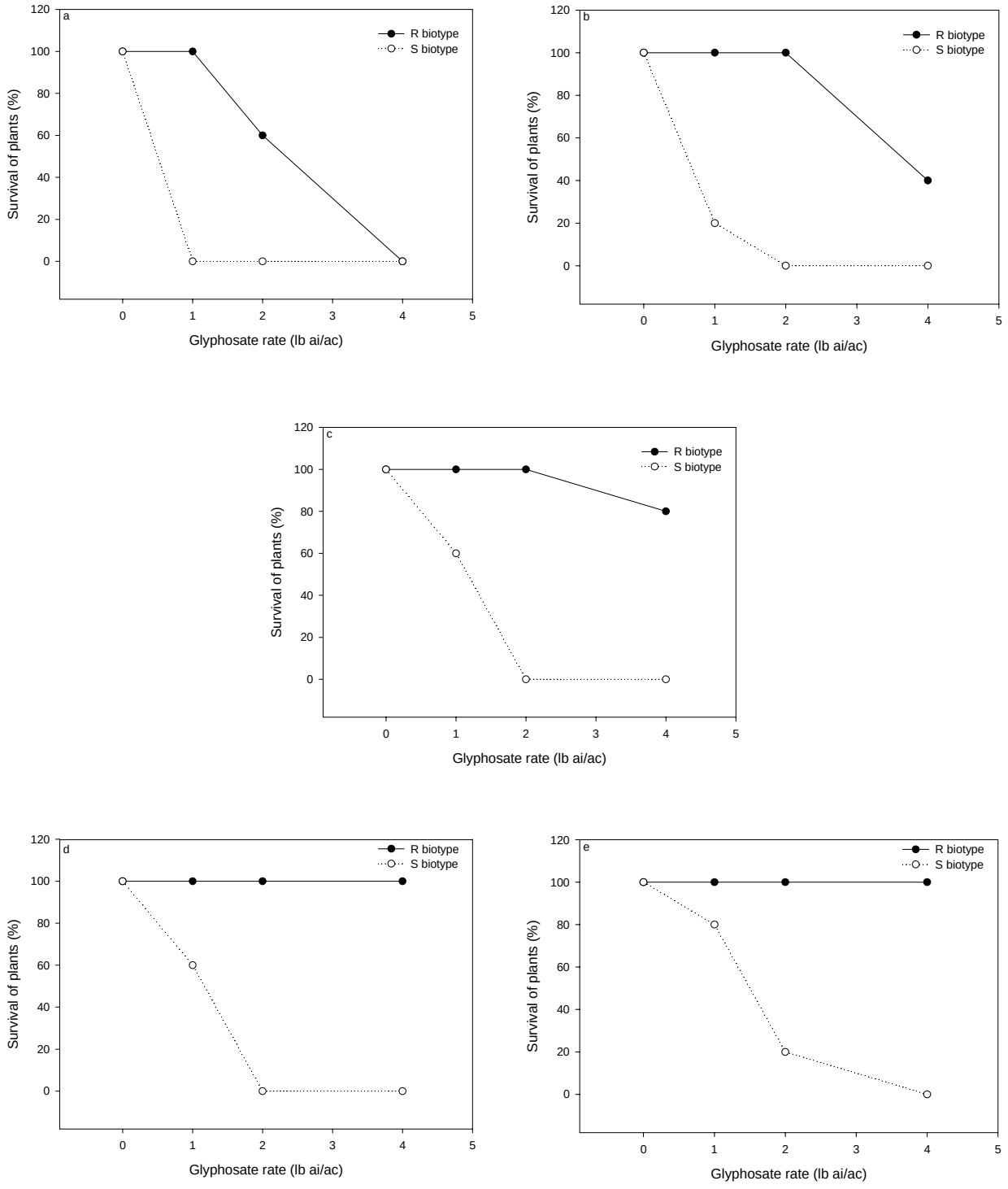

Figure 2. Horseweed plant survival under different glyphosate rates sprayed at the (a) 5-8 leaf stage, (b) 11-15 leaf stage, (c) 18-21 leaf stage, (d) bolting to 6 inch stage, and (e) 6 inch to 1 foot stage.

Figure 3. Visual damage symptoms on the horseweed plants at the 18-21 leaf stage. 'R' biotype (L) and 'S' biotype (R) sprayed at 0x, 1x, 2x, and 4x rates of glyphosate (foreground to background, respectively).

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