

# Open-Pollinated Transfer of Glyphosate Resistance in Horseweed (*Conyza canadensis*) in Greenhouse Isolation

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## Introduction

Horseweed (*Conyza canadensis*) has become a problematic weed in crop production fields across the United States. The fraction of the population that is resistant to the herbicide glyphosate continues to increase each year. In Indiana, farmers have ranked horseweed as a top-five problematic weed, and a field survey found 38% of the population of horseweed to be glyphosate-resistant (GR) in southeastern Indiana. A single-locus, nuclear encoded gene confers glyphosate resistance (Zelaya et al. 2004). Horseweed primarily self pollinates, but nuclear encoded paraquat resistance out-crosses at approximately 4% under field conditions (Smisek 1995). The transfer of glyphosate resistance via pollen as a mechanism of gene flow, in addition to long distance seed movement, is troubling because it could aid in the evolution of multiple resistance.

## Objective

The objective of this experiment was to quantify the potential for GR horseweed to outcross in open-pollinated populations with comingling glyphosate-resistant and susceptible biotypes.

## Materials and Methods

Known GR field populations were purified with 0.42 kg ae ha<sup>-1</sup> glyphosate and a known glyphosate-susceptible (GS) field population was used for parental plants (F<sub>0</sub>).

GR survivors and GS F<sub>0</sub> plants were grown under normal greenhouse conditions and arranged in six, 8:1 GR:GS clusters at 10-25% open flowers.

Three clusters of 572 x 502 were placed in one greenhouse, and three clusters of 572 x 524 were in another greenhouse with an additional pair for manual cross pollinations. Clusters were spaced 3-m apart, and plants were spaced 13-cm apart within a cluster.

F<sub>1</sub> seeds were collected from the seven GS parents, propagated, and sprayed with 0.84 kg ae ha<sup>-1</sup> glyphosate at 3 to 5-cm rosette width. Surviving F<sub>1</sub> plants were self-pollinated, and F<sub>2</sub> seeds were collected.

F<sub>2</sub> generation were then propagated and sprayed with 0.84 kg ae ha<sup>-1</sup> glyphosate at 3 to 5-cm rosette width. Digital images were taken at 28 DAT with a Nikon Coolpix E5700 digital camera. Data were combined over three experimental runs.

F<sub>2</sub> segregation ratios were determined by visual GR or GS assessment and images were analyzed for green pixel counts using digital imaging software, ImageJ. ImageJ is available at <http://rsbweb.nih.gov/ij/>. GR:GS ratios were subjected to chi-square goodness-of-fit analyses.

## Results



Figure 1. GR and GS F<sub>1</sub> horseweed progeny 35 DAT with 0.84 kg ae ha<sup>-1</sup> glyphosate.



Figure 2. GR and GS F<sub>2</sub> horseweed progeny 28 DAT with 0.84 kg ae ha<sup>-1</sup> glyphosate, and example of green value analysis with ImageJ software.

**Table 1** Response of first (F<sub>1</sub>) and second (F<sub>2</sub>) generation horseweed progeny to 0.84 kg ae ha<sup>-1</sup> glyphosate for open and manually cross pollinated horseweed. Green pixel value was determined by digital image analysis program.

Horseweed Biotypes	Cross pollination method	Glyphosate-resistant			Glyphosate-susceptible			% GR	Chi-square P-value <sup>1</sup>
		Green pixel #	Green pixel value	Std. Dev.	Green pixel #	Green pixel value	Std. Dev.		
<b>GS x GR</b>									
<b>Checks</b>									
GR		87	86	23	—	—	—	100	—
GS (572)		—	—	—	82	66	18	0	—
<b>F<sub>1</sub></b>									
572 x 502	open	2	185	4	182	121	14	1.1	—
572 x 524	open	11	167	13	276	106	18	4.0	—
572 x 524	manual	42	140	7	98	118	11	43	—
<b>F<sub>2</sub></b>									
572 x 502	open	59	89	16	19	67	15	76	1
572 x 524	open	92	91	12	22	65	9	81	0.20
572 x 524	manual	42	91	13	23	69	11	65	0.07

<sup>1</sup>Chi-square goodness-of-fit values for an expected 3:1 GR:GS ratio

## Conclusions

Outcrossing for the 8:1 GR:GS clusters ranged from 1.1% to 4% and segregation for F<sub>2</sub> plants fit expected 3:1 R:S ratios according to chi-square goodness-of-fit analyses. Our results confirmed that glyphosate resistance in horseweed can transfer to a closely located, putative GS biotype at low frequencies under open-pollinated greenhouse conditions.

## Literature cited

- Smisek, A. 1995. Resistance to Paraquat in *Erigeron canadensis* L. M.S. Thesis, University of Western Ontario, London, ON N6A 5B8. Pp.82-84.
- Zelaya, I. A., M. D. K. Owen, and M. J. VanGessel. 2004. Inheritance of evolved glyphosate resistance in *Conyza canadensis* (L.) Cronq. Theor. Appl. Genet. 110:58-70.