Pollen-Mediated Gene Flow from Glyphosate-Resistant to Susceptible Giant Ragweed (*Ambrosia trifida* L.) under Field Conditions

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Introduction

- Glyphosate-resistance is a nuclear inherited dominant or semi-dominant trait and can be used as a selective marker to detect gene flow (Powles and Preston 2006).
- Giant ragweed is a monoecious species with facultative outcrossing nature mainly due to its prolific pollen production (≥10 million pollen grains per day per plant during the peak flowering period) and anemophilous pollination.
- Scientific literature is not available on pollen-mediated gene flow (PMGF) from glyphosate-resistant (GR) giant ragweed under field conditions.

Objective

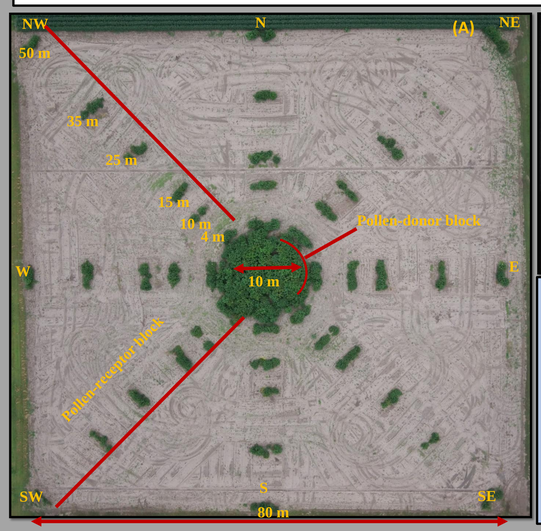
- To quantify the pollen-mediated gene flow from glyphosate-resistant to glyphosate-susceptible (GS) giant ragweed under field conditions

Hypothesis

- Pollen-mediated gene flow in giant ragweed will vary with the physical distance between glyphosate-resistant and -susceptible biotypes and wind parameters.

Materials and Methods

- **Field experiments:** Two year field experiments (2014 and 2015) were conducted at South Central Agricultural Lab (46.59^{0}N,116.14^{0}W), UNL;
- **Design:** A modified Nelder wheel design with the pollen source in the center (10 m diameter; 80 sq. m area) surrounded by pollen receptor area divided into eight directions (four cardinal directions i.e. N, S, E, and W; and four ordinal directions i.e. NE, NW, SE, and SW) (Jhala et al. 2011).
- Data for flowering synchrony between source and receptor plants and hourly weather parameters including temperature, precipitation, wind speed and wind direction were recorded throughout the duration of field experiments.
- Seeds were harvested from glyphosate-susceptible giant ragweed plants separately for each distance in all the directions from the pollen receptor area.
- **Screening:** The seedlings were grown in greenhouse and sprayed with 2× (1× = 1,260 g ha^{-1}) rate of glyphosate to determine gene flow using glyphosate-resistance as a selective marker.
- *Frequency of gene flow* = $\frac{Number\ of\ surviving\ plants}{Number\ of\ plants\ sprayed}$



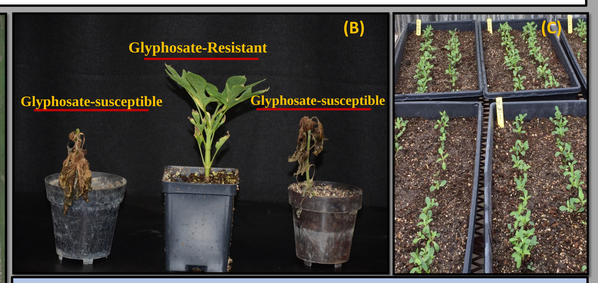


Figure 1. A). Aerial view of experimental site demonstrating pollen donor (center) and receptor plants of giant ragweed in four cardinal and ordinal directions; B). Resistant and susceptible giant ragweed plants from populations used as pollen source and receptors in this study 15 DAT with glyphosate at 2× (1× = 1,260 g ha^{-1}) rate; C). Growing seedlings for screening.

- **Statistical analysis:** Akaike's Information Criterion (*AIC*) was used to select the best model for analyzing the PMGF between GR and GS giant ragweed using the Generalized Nonlinear Model (package *gnm*) in R.
- The best fit to data was provided by a double exponential decay model:

$$logit(p_i) = \beta_0 + exp[\beta_1 + \gamma_1 \times distance] + \exp\left[\beta_2(direction{:}\,year) + \gamma_2(direction{:}\,year) \times distance\right]$$

- where p_i is the frequency of gene flow of the i^{th} observation; β_0 is overall intercept; β_1, β_2 are the intercepts for the first and second instances, respectively; and γ_1, γ_2 are the decay rates where $\gamma_1 > \gamma_2$.
- Parameters β_2 and γ_2 vary with the direction and the year (Sarangi 2016).
- The distance where the frequency of gene flow was reduced by 50% (O_{50}) and 90% (O_{90}) of the frequency predicted at closest distance were estimated from the final model using the prediction function in R.

Results and Discussion

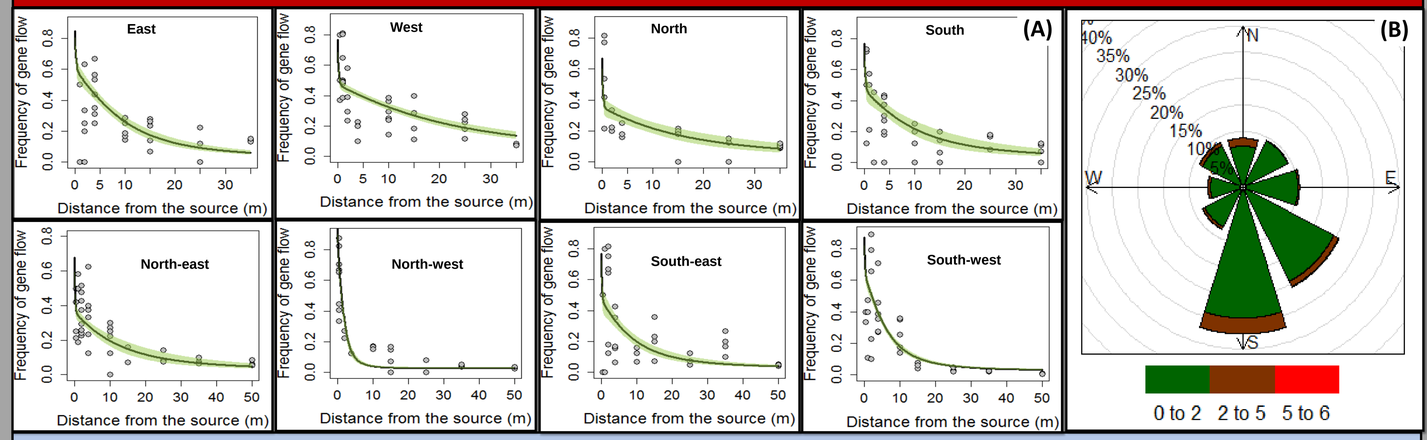


Figure 2. A). Pollen-mediated gene flow from glyphosate-resistant to -susceptible giant ragweed varied with distance (m) and directions in 2014, B). Wind rose plots displaying wind speed (m s^{-1}) and wind frequency (%) in different directions during flowering period in 2014.

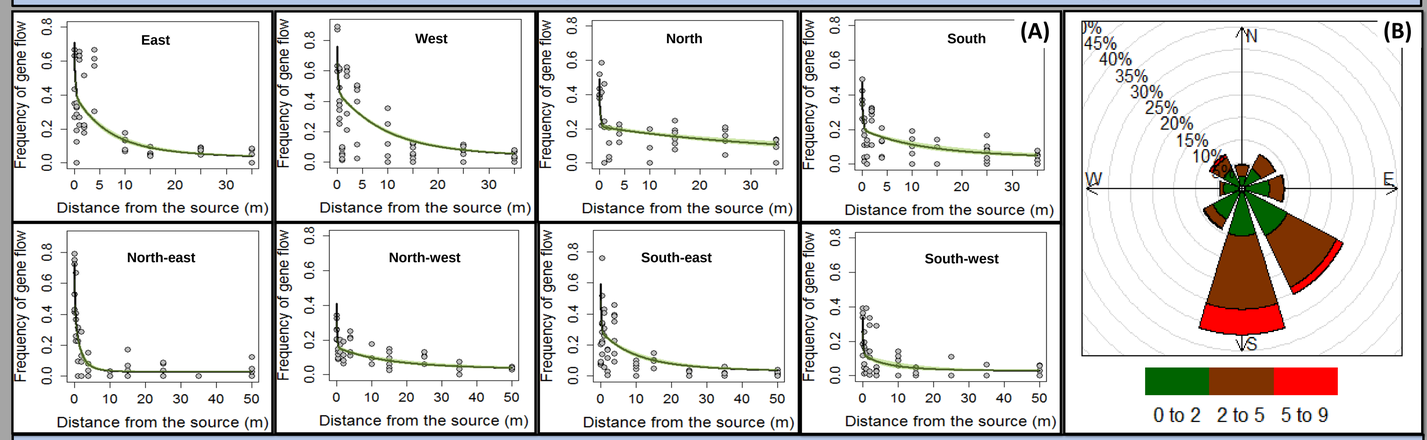


Figure 3. Pollen-mediated gene flow from glyphosate-resistant to -susceptible giant ragweed varied with distance (m) and directions in 2015. B). Wind rose plots displaying wind speed (m s^{-1}) and wind frequency (%) in different directions during flowering period in 2015.

Table 1. Estimates of the distances where the frequency of gene flow reduced by 50% (O_{50}) and 90% (O_{90}) in 2014 and 2015.

Direction	2014				2015			
	O_{50}	CI	O_{90}	CI	O_{50}	CI	O_{90}	CI
	m							
N	1.3	0.4;4.1	45.6	32.1;64.4	0.4	0.3;0.5	106.5	79.3;142.2
S	2.8	0.8;4.3	28.6	20.5;40.1	0.4	0.3;0.4	37.1	27.3;49.6
E	4.5	3.4;5.4	27	22.7;32.2	1.1	0.8;1.5	19	16.6;21.6
W	7.0	5.8;8.3	49.5	42.7;57.5	2.4	2;2.8	26.4	24;29.1
NE	1.3	0.5;2.6	35.1	25.4;48.3	0.5	0.4;0.6	4.4	3.6;5.4
NW	1.4	1.3;1.6	4.9	4.2;5.6	0.3	0.3;0.4	46.9	34.5;63.5
SE	2.5	0.7;3.9	25.5	20.2;32.3	0.6	0.4;0.7	29.4	24.5;35.3
SW	5.1	4.7;5.4	17.5	16.1;19.0	0.3	0.2;0.3	26	13.6;53.6

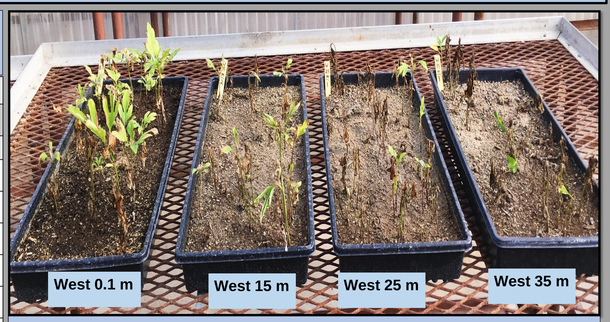


Figure 4. Screening plants from four distances in the west arm for glyphosate-resistance with 2x rate of glyphosate at 21 DAT.

- Initiation of flowering was observed on July 25 and July 30 in 2014 and 2015, respectively.
- The protrusion of stigmas in the female flowers occurred on average 3 to 5 days prior to the pollen shed from male flowers on the same plant.
- The highest frequency of gene flow (0.43 to 0.60) was observed at ≤ 0.5 m distance from the pollen source and the frequency of gene flow reduced rapidly with increasing distances from the pollen source; however, low level of gene flow (0.03 to 0.04) was detected even up to the 50 m distance (Figures 2 and 3).
- PMGF varied with the wind direction (Figures 2 and 3) and reduced by 50% (O_{50}) at ≤ 7 m distance from the pollen source, whereas 90% reduction (O_{90}) occurred at < 107 m distance (Table 1).
- Earlier studies reported 31 and 5% gene flow between ALS-resistant giant ragweed and susceptible common ragweed at 5 and 60 m respectively (Volenberg et al. 2005).

Conclusions and Future Direction

- The results suggested that PMGF plays a significant role in the dispersal of GR alleles in giant ragweed, causing an increase in the frequency of GR giant ragweed plants within field populations along with the potential to introduce GR alleles into nearby field or non-crop populations.
- The future studies should evaluate the duration of pollen viability, pollen aerodynamics and the landscape-level dissemination of the GR trait in giant ragweed.

Literature Cited

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- Powles SB, Preston C (2006) Weed Tech 20: 282-28
- Sarangi (2016) Ph.D. dissertation UNL. 84 p
- Volenberg DS et al. (2005) NCWSS Proc.