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Evaluation of Glyphosate Resistant and Susceptible Horseweed©

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SUMMARY

Glyphosate resistant horseweed (*Conyza canadensis*) is a significant issue in nursery crop production, but has not been studied broadly. Our objective was to identify a glyphosate resistant horseweed population, evaluate seed viability, and determine the effectiveness of glyphosate rate on resistant and susceptible seedlings. Two populations were identified, and individual plants were treated with glyphosate or glufosinate https://agfax.com/news/2010/03/glufosinate-glyphosate-weed-resistance-0303.htm. Resistant plants survived glyphosate treatments and died following glufosinate treatments, while all treated susceptible plants died. Seeds were collected from each population and a germination test was used to evaluate viability. Seedlings were used for a whole plant assay, with glyphosate at 0, 2.2, and 11.1 L ha^{-1} (0, 1, and 5 qt ac^{-1}). Germination test results indicate that resistant plant seeds are more likely to have a higher viability. In the whole plant assay, resistant seedlings treated with glyphosate did not have significantly lower shoot weights than non-treated seedlings. However, with susceptible seedlings there was a significant decrease in shoot weight with

increasing glyphosate rates. Results suggest that glyphosate resistance is readily passed down and resistant seedlings are more likely to survive and reproduce.

INTRODUCTION

Nursery crops were valued at over $5.1 billion based on annual sales as of 2012 (USDA, 2012). Maintaining nurseries that are already in production may cost anywhere from $2000 to $3500 per acre annually until harvested; the production period can be three to five years depending on the crop (Halcomb, 2009). Weed management is a critical component for producing quality nursery crops because weeds can cause competition for light, nutrition, and water - causing a negative impact on production and profit.

Glyphosate is a non-selective post-emergent herbicide that is used to control annual and perennial weeds. Glyphosate is commonly used in field grown nursery crops to control weeds within the row and is typically applied using a shielded sprayer to prevent contact with the crop. Glyphosate is most effective on young, actively growing plant tissue. (Kleinman and Rubin, 2016). The mode of action of glyphosate is the interruption of a plant's shikimate pathway, which is a metabolic pathway important for plant growth. The specific target site for glyphosate is a metabolic enzyme called chloroplastic enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase which produces energy for the plant. When EPSP is blocked, the buildup of shikimic acid and metabolic processes failing to function leads to chlorosis and plant death (González-Torralva et al., 2017). Glyphosate resistant crops were introduced in the late 1990's (Shaner et al., 2005). Overreliance of glyphosate has caused glyphosate resistant weeds to develop, hindering the ability of the herbicide to control weeds (Kumar, et al. 2017).

Conyza canadensis (L.) Cronquist, commonly known as horseweed, is an annual weed native to much of North America (Tilley, 2012). Due to its wide native range and adaptability, horseweed has developed glyphosate resistance from the repeated use of glyphosate particularly in agronomic crops. Glyphosate resistant horseweed has been identified in at least 25 U.S. states in some 12 countries (Heap, 2017). Horseweed has several growth stages, which can be identified as the seedling, rosette and bolting stages (Shoup et al., 2012). Horseweed can germinate throughout the year (Kumar, et al. 2017), can produce 50,000 to 250,000 seeds per plant, which are easily spread by wind. As a result, glyphosate resistant horseweed has spread to areas producing other crops, including nurseries. Horseweed also plays host to pests such as tarnished plant bugs (*Lygus lineolaris*), which is a serious horticultural pest (Steckel, 2018).

The impact of horseweed glyphosate resistance in nursery crops has not been extensively evaluated. Identifying glyphosate resistant horseweed and developing management practices to prevent and control these weeds will benefit nursery producers throughout the U.S. The objective of this preliminary research project was to identify a glyphosate resistant population of horseweed, evaluate seed viability of resistant and susceptible plants, and determine the effectiveness of glyphosate rate on resistant and susceptible seedlings.

MATERIALS AND METHODS

Suspected glyphosate resistant and susceptible populations were first identified then screened for resistance. The suspected resistant population was located in a soybean field in Warren County, TN. The field had been planted in glyphosate resistant crops (soybeans) for at least 10 years. The suspected susceptible population was located at the Tennessee State University Otis L. Floyd Nursery Research Center (NRC) in McMinnville, TN, in a field that had not been treated with glyphosate in several years. Twenty individual plants were flagged in each location and were treated with either glyphosate (1.3 oz/gal; GLY-4; 41% glyphosate; Universal Crop Protection Alliance, LLC, Eagan, MN) or glufosinate (1.3 oz/gal; Finale; 11.33%

glufosinate; Bayer, Research Triangle Park, NC). One week after treatment, plants were evaluated for herbicide activity. Plants at the soybean field treated with glyphosate showed no damage or minor damage exhibited as chlorosis of the terminal leaves. At the NRC, all plants were killed (no green tissue remained on the above ground portion of the plant) with both the glyphosate and glufosinate treatments. Seeds were collected (Sept. 22, 2017) from ten non-treated plants within each population. A separate vial (7 ml polypropylene; Evergreen Scientific, Rancho Dominquez, CA) was used to store seeds from each individual plant for a total of twenty vials (ten per population), and vials were placed in the refrigerator.

To evaluate seed viability, a germination test was performed using methods described by Travlos and Chachalis (2013). Seeds were sown in petri dishes (100 x 15 mm; VWR International, Radnor, Pennsylvania) on 2 sheets of filter paper (top layer Whatman 4, bottom layer Whatman 598; Whatman Ltd., Maidstone, England) saturated with deionized water. Ten seeds from an individual plant were placed in each petri dish, and there were ten petri dishes (replications) per plant for a total of 100 seeds per plant. Petri dishes were then placed in a growth chamber at 26/21˚C day/night temperatures with a 14 h daylength. After 72 h, petri dishes were removed from the growth chamber. Seeds were determined to have germinated if a radicle and cotyledon were present and germination percentage was calculated. The experiment was conducted twice (Test 1 and Test 2).

To evaluate the effectiveness of glyphosate rate on resistant and susceptible horseweed seedlings, a whole plant assay was conducted based on methods used by Koger and Reddy (2005). Seedlings were grown from the seeds described above. Forty-eight seeds per plant were sown into 72 cell flats (2 seeds per cell; PROP-72-RD; T.O. Plastics Inc. Clearwater, MN) filled with seed starter mix (Morton's #1; McMinnville, TN). The flats were placed in a growth chamber (as described above) and were watered as needed with a handheld pump sprayer. When seedlings were at the three to five leaf stage, they were transplanted to individual 8.9 cm square containers (SVD 350; T.O Plastics Inc., Clearwater, MN) filled with substrate (1 pine bark: 1 peat moss by volume; amended per cubic yard with 4 lb Nutricote 13-11-11, 5 lb Dolomitic Lime, 1 lb Aqua Gro, 2 lb Plantex 10-5-10 Media Starter, and 0.75 lb Micromax). When seedlings reached the rosette stage (approximate width of 12 cm), they were treated with glyphosate (GLY-4 Plus) applied at 2.2 or 11.1 L ha^{-1} (1 or 5 qt ac^{-1}) using a CO_2 pressurized sprayer calibrated to deliver 33 gpa at 30 psi. At 21 days after treatment, shoot fresh weight was measured for all seedlings. Data were analyzed using the GLIMMIX procedure of SAS (Version 9.3; SAS Institute, Inc., Cary, NC). Differences between means were determined using the Shaffer-Simulated method ($P < 0.05$).

RESULTS AND DISCUSSION

Seed germination rate varied within plant populations, but overall was higher in the resistant population (Table 1). Within the resistant population germination rates ranged from 56 to 80.3% and 57.1 to 89.9%, respectively, for Tests 1 and 2. Within the susceptible population, germination rates ranged from19.0 to 72.2% and 7.7% to 79.0%, respectively, for Tests 1 and 2. When all plants within a population were averaged, resistant seeds in Test 1 had 70.2% germination compared to 52.2% within the susceptible population. For Test 2, resistant seeds had an overall germination rate of 75.6% compared to 46.4% for the susceptible seeds. In both tests, we observed the five highest germination rates occurred for resistant plants while the five lowest germination rates were from susceptible plants. In a previous study, Travlos and Chachalis (2013) found no differences in seedling vigor between the glyphosate resistant and glyphosate susceptible horseweed in their study. They had optimal germination rates (65 %) at the temperature regime of 15/25˚C and intermediate germination rate (31 to 51%) at 20/30˚C.

In the whole plant assay, resistant seedlings treated with glyphosate did not have significantly lower shoot fresh weight compared with the non-treated resistant seedlings except for Resistant Plant 4 (Figure 1a). However, susceptible seedlings treated with glyphosate had lower shoot fresh weight compared with non-treated susceptible seedlings (Fig. 1b). When shoot fresh weight was averaged for all seedlings within the resistant group, non-treated resistant seedlings had a higher fresh weight compared with glyphosate treated seedlings (Fig. 2). However, there was no difference between resistant seedlings treated with 2.2 or 11.1 L ha^{-1} (1 or 5 qt ac^{-1}) glyphosate. Within the susceptible population, the non-treated seedlings also had a higher fresh shoot weight compared with the treated seedlings, however fresh shoot weight significantly decreased with each increase in glyphosate rate.

The germination study results show that resistant plants are more likely to have a higher germination rate than susceptible plants. The results indicate that resistant plant seeds may be more likely to establish due to being able to germinate and survive more readily than susceptible plants. The whole plant assay results suggest that resistance is passed down from parent plant to seedling. This means that resistant plants that are treated with glyphosate can survive the treatment and reproduce. Future studies will include assessing more rapid laboratory methods to confirm glyphosate resistance and evaluating establishment prevention of horseweed in nursery fields.

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Table1: Mean germination percentage of seeds from resistant and susceptible horseweed (*Conyza canadensis*) populations. Seeds were collected form 10 plants within each population and the germination test was conducted twice.

	Germination Rate (%)			Germination Rate (%)	
Plant	**Test 1**	**Test 2**	**Plant**	**Test 1**	**Test 2**
Resistant 1	74.1	86.8	Susceptible1	68.1	74.0
Resistant 2	74.0	83.4	Susceptible 2	19.0	13.4
Resistant 3	77.1	79.8	Susceptible 3	30.4	34.9
Resistant 4	56.6	63.9	Susceptible 4	72.2	73.6
Resistant 5	58.5	58.0	Susceptible 5	67.3	79.0
Resistant 6	60.0	77.8	Susceptible 6	65.4	40.2
Resistant 7	75.5	89.9	Susceptible 7	56.5	68.0
Resistant 8	73.8	86.0	Susceptible 8	63.1	7.7
Resistant 9	72.0	57.1	Susceptible 9	55.7	41.6
Resistant 10	80.3	73.0	Susceptible10	24.7	31.3
All Plants	70.2	75.7	All Plants	52.2	46.4

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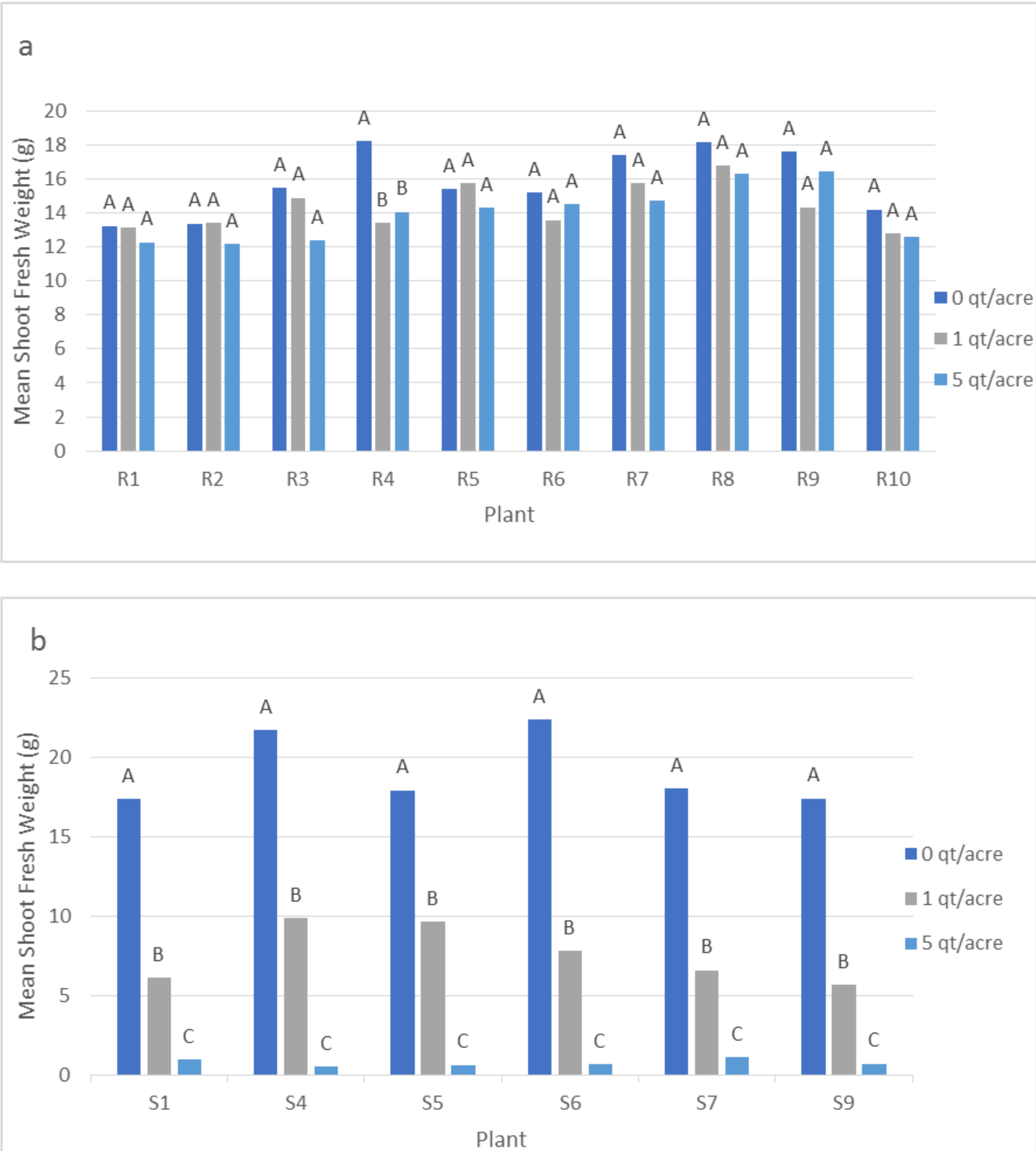

Figure 1: Mean shoot fresh weight (grams) of resistant (a) and susceptible (b) horseweed (*Conyza canadensis*) seedlings from multiple plants 21 days after glyphosate treatments at 0, 2.2, and 11.1 L ha^{-1} (0, 1, and 5 qt ac^{-1}). Columns with same letters within an individual plant are not significant at $P \leq 0.05$.

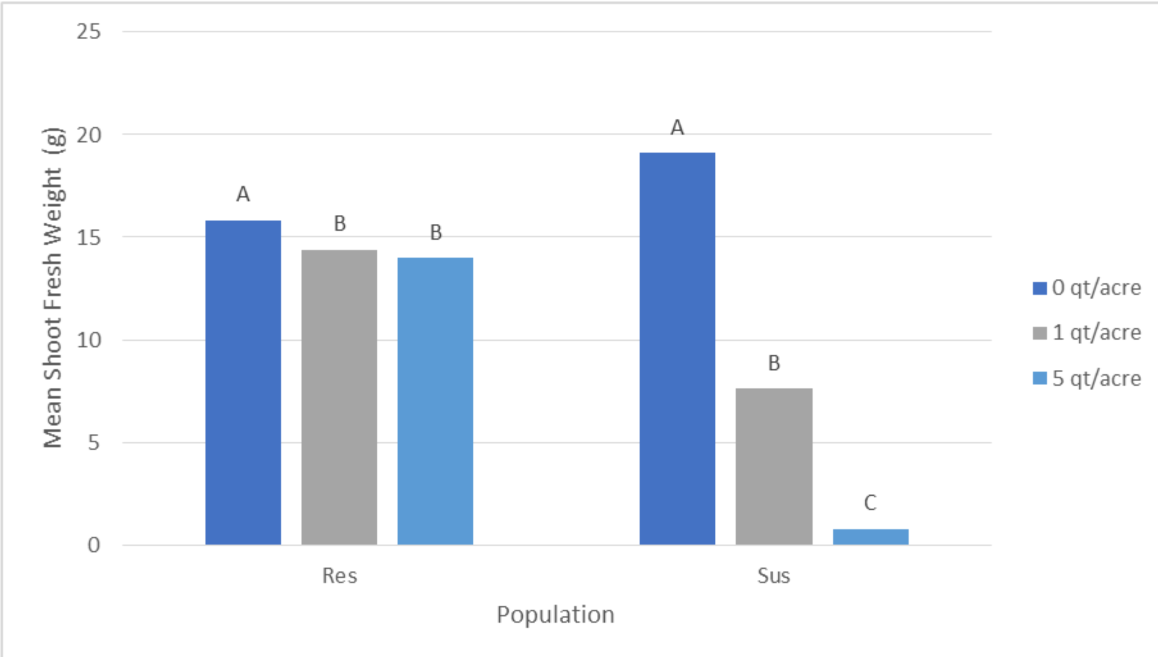

Figure 2: Total mean shoot fresh weight (grams) from all plants of resistant and susceptible horseweed (*Conyza canadensis*) populations 21 days after glyphosate treatments at 0, 2.2, and 11.1 L ha^{-1} (0, 1, and 5 qt ac^{-1}). Columns with different letters within a population are significant at $P \leq 0.05$.

Response of glyphosate-resistant horseweed [Conyza canadensis (L.) Cronq.] to a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor. D. Sarangi and A.J. Jhala. Abstract: A premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor has recently been commercialized for pre-emergence (PRE) and early post-emergence (POST) control of broadleaved and annual grass weeds in corn in the United States.Â The level of glyphosate resistance in this population ranged from 3 to 6 times that of glyphosate-susceptible (GS) horseweed population (Knezevic 2007). Greenhouse dose-response studies Greenhouse dose-response bioassay was conducted in. Glyphosate (IUPAC name: N-(phosphonomethyl)glycine) is a broad-spectrum systemic herbicide and crop desiccant. It is an organophosphorus compound, specifically a phosphonate, which acts by inhibiting the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase. It is used to kill weeds, especially annual broadleaf weeds and grasses that compete with crops. It was discovered to be an herbicide by Monsanto chemist John E. Franz in 1970. Monsanto brought it to market for agricultural use in 1974 under... Figure 2. Glyphosate-resistant (left column) and susceptible (right column) horseweed biotypes from Mississippi. Two fully expanded leaves of plants in bottom row were treated with glyphosate, and plants in the upper row were not treated. Ten microliter of glyphosate (0.84 kg ae/ha in 190 L of water; 1X field rate) solution was placed on each leaf as 20 droplets. Three weeks after treatment, the susceptible plant was killed, and the resistant plant survived, suggesting reduced translocation of glyphosate (Koger and Reddy, 2005). Tennessee (Koger & Reddy, 2005).Â The above results strongly suggest that resistance in horseweed biotypes is due to reduced translocation of glyphosate to growing parts of the plant. Goosegrass. Control of glyphosate-resistant horseweed (Conyza canadensis) with saflufenacil tank-mixtures in no-till Cotton (Gossypium hirsutum). 5. Abstract. Glyphosate-resistant (GR) horseweed management continues to be a challenge in no-till cotton systems in Tennessee and Mississippi. Field studies were conducted in 2009 and 2010 to evaluate saflufenacil in mixtures with glyphosate, glufosinate, or paraquat for control of glyphosate-resistant (GR) horseweed prior to planting cotton.Â Horseweed densities were 32 and 45 plant m2 at the 30 DAA evaluations in glyphosate and glyphosate plus flumioxazin plots, which supported the visual estimates of those two treatments providing the poorest control.