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Response of Selected Horseweed (*Conyza canadensis* (L.) Cronq.) Populations to Glyphosate

CHRISTOPHER L. MAIN, THOMAS C. MUELLER,* ROBERT M. HAYES, AND JOHN B. WILKERSON

Department of Plant Sciences, The University of Tennessee, 2431 Joe Johnson Drive, 252 Ellington Plant Sciences Building, and Biosystems Engineering & Environmental Science, 309 Agricultural Engineering B, 2506 E. J. Chapman Drive, Knoxville, Tennessee, 37996

Horseweed (*Conyza canadensis* (L.) Cronq.) seed was collected in Illinois, Indiana, Kentucky, Mississippi, Missouri, and Ohio to determine susceptibility of different horseweed biotypes to glyphosate. Horseweed resistant to glyphosate was found in Mississippi, Ohio, and western Tennessee. In a separate experiment examining Tennessee biotypes, a dose response curve demonstrated that four times as much glyphosate was needed to achieve a 50% fresh weight reduction (GR_{50}) in resistant biotypes when compared to a susceptible biotype. Resistant biotypes from Tennessee displayed a GR_{50} of 1.6 kg/ha as compared to a GR_{50} of 0.4 kg/ha in a susceptible horseweed population. Although growth was reduced, the resistant plants did not completely die and could potentially produce seed. Variation in glyphosate resistance was found among the populations tested.

KEYWORDS: Glyphosate; glyphosate resistance; horseweed; *Conyza canadensis*; horseweed distribution

INTRODUCTION

Horseweed (*Conyza canadensis* (L.) Cronq.) is a winter annual or biennial in the Composite family, native to and commonly found throughout North America (*1*). Horseweed is sometimes referred to as Canada fleabane, mare's tail, or *Erigeron canadensis* L. Seed dispersal is the only mechanism of horseweed spread, and plants are capable of producing over 200,000 small, wind-dispersed seeds per plant in late summer (*2*). Plants are ruderal in nature, and seeds germinate best in early fall or spring. However, observations indicate that germination can occur throughout the year (*1*, *3*).

The first reported occurrence of glyphosate resistant horseweed was in Delaware in 2000 (*4*) followed by reports of similar resistance in west Tennessee (*5*). No-till crop production has been widely adopted in the mid-Atlantic and mid-South regions, which has favored the establishment and growth of horseweed populations. Horseweed is adapted to periodically plant-free, open, undisturbed soil (*6*), something often found in no-till crop production systems. Horseweed is less of a problem in tilled fields where fall or spring disking provides control (*7*). Glyphosate failed to control horseweed in some fields after 3 years of using only glyphosate for weed control in continuous cropping of glyphosate resistant soybeans (*4*).

The United States Department of Agriculture estimates for crop production indicate that herbicide resistant crop varieties were planted on 80% of soybean hectares and 60% of cotton hectares and 10% of corn hectares for 2003 (*8*). The use of glyphosate resistant crops for weed control is common in no-tillage farming practices. A major environmental benefit of no-till systems is reduced soil erosion. In west Tennessee, no-till systems reduce soil erosion by up to 90% (*9*). In a no-till production system, herbicides are the primary method of weed control due to the lack of soil disturbance by tillage. A no-tillage production system utilizing herbicide resistant crops and a single herbicide, such as glyphosate, could lead to selecting for herbicide resistant weed biotypes with changes at the physiological level that confer resistance to glyphosate (*5*). Use of glyphosate for preplant weed control and subsequent postemergence weed control in glyphosate resistant crops has led to the exclusive use of glyphosate on many crop areas, with the result being a decrease in the number of herbicide modes of action on those production areas. Likewise, this system can lead to weed species shifts, where species that were never controlled or were poorly controlled by glyphosate increase in relative abundance. Because horseweed is a winter annual plant that germinates primarily in late winter or early spring in this geographic area, the widespread adoption of no-till systems has greatly increased horseweed's relative abundance.

Glyphosate has a unique mode of action in plants (*10*−*12*). Glyphosate inhibits aromatic amino acid biosynthesis leading to blockage of protein synthesis and secondary metabolite production (*10*, *13*). Glyphosate is a competitive inhibitor of the enzyme 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS), which catalyzes an essential step in the aromatic amino acid biosynthetic pathway. EPSPS catalyzes the reaction of

* To whom correspondence should be addressed. Tel: 865-974-8805. Fax: 865-974-7997. E-mail: tmueller@utk.edu.

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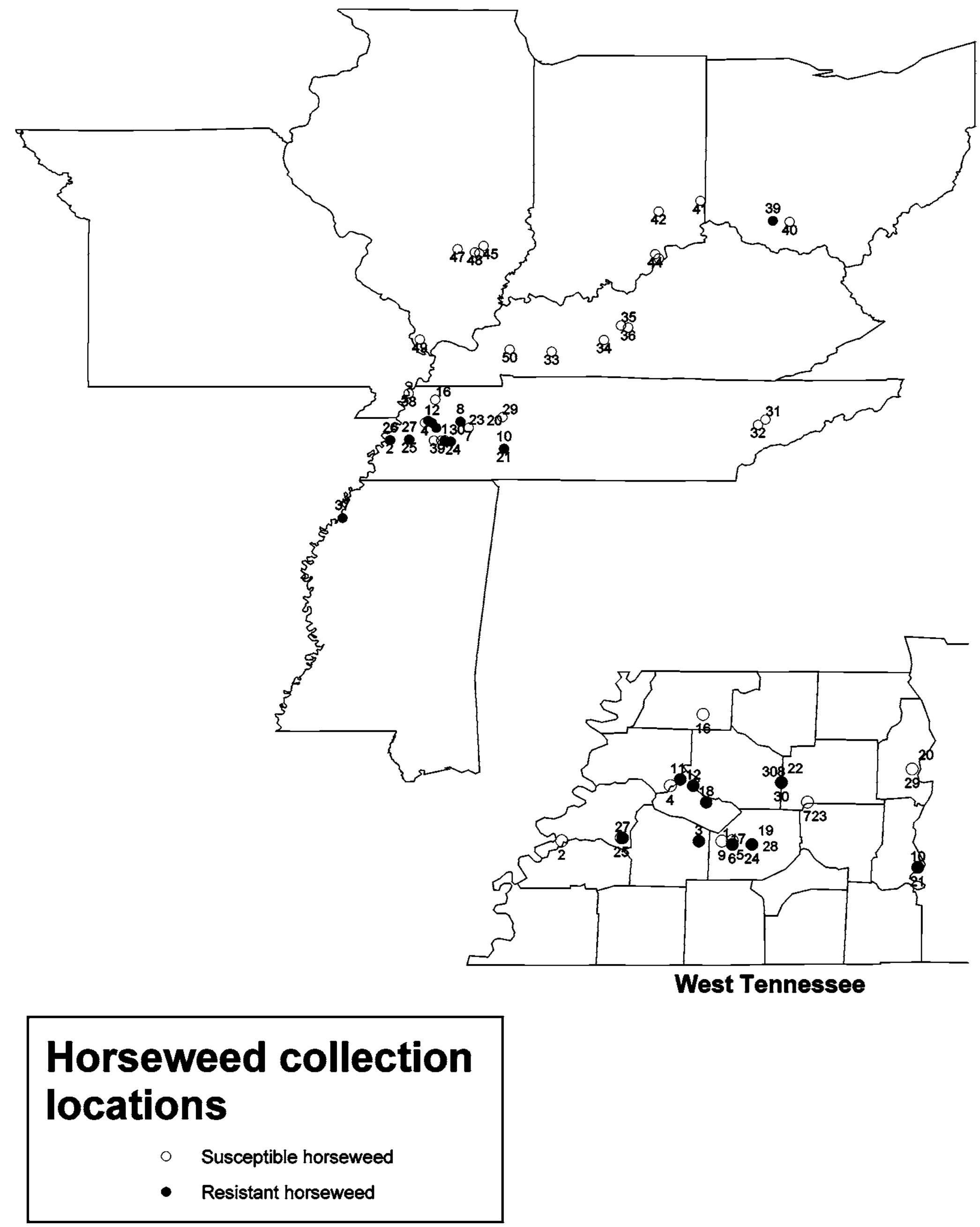

Figure 1. Location of horseweed germplasm collection and response to glyphosate in several states in the United States (location coordinates in **Table 1**).

shikimate-3-phosphate and phosphenolpyruvate to yield 5-enolpyruvylshikimate-3-phosphate (EPSP) and inorganic phosphate. EPSP is a precursor to chorismate formation, the base molecule for all aromatic amino acid formation.

To adequately discuss herbicidal effects on plants, the ambiguous terminology surrounding plant susceptibility, tolerance, or resistance to herbicides requires clarification. For the purpose of this manuscript, the following definitions will be used, and the authors acknowledge that others have expressed slightly different interpretations of these terms. Susceptibility indicates that a plant dies after application of a herbicide at normal doses (*14*). Tolerance is the ability of a plant to remain uninjured by herbicide doses normally used to control other plants. Resistance is the ability of a formerly susceptible plant population to continue to survive herbicide doses above those that were once used to control that original plant population (*14*).

Essential to the understanding and control of glyphosate resistant horseweed is determination of the extent of the geographic distribution of glyphosate resistant horseweed in the midsouth and midwest regions of the United States. Previous research has confirmed that horseweed resistance to glyphosate was located in or near Delaware (*4*) and in a single county in western Tennessee (*5*). It is suspected that glyphosate resistant horseweed has greatly spread based on field observations. These anecdotal reports of putative resistance do not confirm resistance, since they do not include susceptible horseweed plants that die from glyphosate application, so they do not provide proof of widespread glyphosate resistance in horseweed. The first research objective was to examine the potential geographic extent of glyphosate resistant horseweed. Information of this nature will allow researchers to determine patterns of distribution and extent of infestation for glyphosate resistant horseweed. Results from this research will also benefit agricultural producers by alerting them to the area of resistance and will allow them to implement alternative management options for control of this troublesome weed. The second objective of this project was to characterize the sensitivity of horseweed biotypes to glyphosate, resulting in collections of horseweed seeds with varying sensitivity to glyphosate for future study.

MATERIALS AND METHODS

Distribution of Glyphosate Resistant Horseweed. A horseweed germplasm collection of mature seed heads was conducted in the fall of 2002 at 33 locations in western Tennessee. In the late winter and early spring of 2003, additional collections were made including samples from Illinois, Indiana, Kentucky, Mississippi, Ohio, and the bootheel of Missouri. Samples were collected from two types of horseweed populations. First, samples were collected where glyphosate resistance would not be expected due to minimal selection pressure. These collection sites included pastures and roadside areas without probable glyphosate application. Second, samples were collected from putative glyphosate resistant horseweed locations in no-till production fields. Each location where seed was collected was recorded with global positioning satellite technology. Because of the presentation of a large geographic area, some of the collection points in **Figure 1** are superimposed on each other; thus, you cannot see all of the collection points on the figure. For this reason, all of the data are presented in tabular form.

Seeds were collected from a single seed head and placed directly into large paper bags. The bags were sealed and stored at −4 °C for <6 months prior to seed processing. Individual seed heads were gently homogenized, and the stems and other large plant material were removed. The resulting mixture of seeds and chaff was used in later studies. Care was taken to minimize cross-contamination of seed lots, since the small horseweed seeds were easily moved by wind currents, such as those inside an operating chemical fume hood.

The collected seed was germinated, grown in a greenhouse, and subsequently sprayed with glyphosate to determine sensitivity. To conduct the study, horseweed seed was germinated in Styrofoam float trays in soilless potting media. After germination, horseweed seedlings (1−2 true leaves, 5 mm in height) were transferred to pots containing the same growth media for the duration of the study. Each pot contained a single horseweed plant and was considered to be an individual experimental unit. All treatments were replicated four times, and the experiment was conducted twice, so the data presented are the mean of eight observations. Horseweed plants were grown under supplemental metal halide lighting (400 microeinsteins per cm) with 16 h light and 8 h dark periods. Plants were watered twice daily, and supplemental fertilizer (MiracleGro) containing macro- and micronutrients was applied weekly. The time interval from planting seeds to transplanting into cups was 4 weeks, and the time from transplanting to herbicide application was 5 weeks. Plants were watered the evening prior to glyphosate application and not watered after treatment so as not to wash off the herbicide. Watering was resumed 24 h after glyphosate application. The commercially available potassium salt formulation of glyphosate (Roundup WeatherMax) was used. Applications of 0, 0.84, and 3.36 kg/ha were applied to 5 cm diameter horseweed rosettes in 190 L/ha of water carrier applied in two passes (95 L/ha per pass) to provide complete coverage. Glyphosate at 0.84 and 3.36 kg/ha represents a 1× (normal) and a 4× dose, respectively. Previous research had indicated that a 4× glyphosate dosage (3.36 kg/ha) was a discriminating application rate to separate resistant from susceptible populations (*5*). Applications were made in an enclosed spray booth to prevent glyphosate contamination of nontarget plants. Plants were allowed to grow for 21 days after treatment (DAT) to determine glyphosate sensitivity. A visual evaluation of total plant growth decline was conducted at 21 DAT. This evaluation utilized a 0−100 scale, with 0 being no visible effects and 100 being plant death. The visual evaluation incorporated plant size, cholorsis or necrosis, and general plant vigor and robustness. Other data comparing visual evaluations and horseweed fresh weight indicated a high correlation ($R > 0.90$, analysis not shown). Fresh weight determination was problematic due to the variable growth of horseweed plants and also the small amount of plant residue of treated susceptible plants remaining 21 DAT. Visual symptoms clearly elucidated a differential response of various horseweed populations to glyphosate application. Data were subjected to analysis of variance, and means were separated by Fisher's protected LSD ($P = 0.05$). Data are also displayed graphically georeferenced to the physical location of germplasm collection. For this research, horseweed populations that had <70% injury from glyphosate at 0.84 kg/ha were considered to be resistant, and horseweed populations that had <70% injury from glyphosate at 3.36 kg/ha were denoted as highly resistant. Data were subjected to analysis of variance, and means within each herbicide rate were separated using Fisher's protected LSD test ($P = 0.05$).

Table 1. Geographic Location and Response of Horseweed to Glyphosate[a]

sample	state	latitude	longitude	glyphosate rate (kg ae/ha) control % 0	0.84	3.36	sensitivity to glyphosate[b]
1	TN	35.62	−89.95	0	13	92	R
2	TN	35.64	−89.82	0	91	97	S
3	TN	35.63	−89.12	0	55	93	R
4	TN	35.92	−89.27	0	99	99	S
5	TN	35.64	−89.95	0	95	99	S
6	TN	35.62	−89.95	0	99	99	S
7	TN	35.83	−89.57	0	99	99	S
8	TN	35.93	−89.70	0	6	32	HR
9	TN	35.63	−89.03	0	69	89	R
10	TN	35.50	−88.01	0	10	10	HR
11	TN	35.95	−89.21	0	30	70	HR
12	TN	35.92	−89.15	0	12	12	HR
13	TN	35.62	−89.85	0	99	99	S
14	TN	35.62	−89.85	0	99	99	S
15	TN	35.62	−89.85	0	99	99	S
16	TN	36.60	−89.10	0	99	99	S
17	TN	35.62	−89.85	0	99	99	S
18	TN	35.83	−89.08	0	31	70	HR
19	TN	35.62	−89.85	0	99	99	S
20	TN	36.60	−88.03	0	99	99	S
21	TN	35.50	−88.01	0	99	99	S
22	TN	35.93	−89.70	0	88	99	S
23	TN	35.83	−88.57	0	94	99	S
24	TN	35.62	−89.85	0	99	99	S
25	TN	35.65	−89.52	0	99	99	S
26	TN	35.64	−89.82	0	7	66	HR
27	TN	35.65	−89.51	0	14	31	HR
28	TN	35.62	−89.85	0	25	55	HR
29	TN	36.60	−88.03	0	94	99	S
30	TN	35.93	−89.70	0	93	99	S
31	TN	35.97	−83.85	0	99	99	S
32	TN	35.88	−83.97	0	99	99	S
33	KY	36.83	−87.25	0	99	99	S
34	KY	37.23	−86.42	0	99	99	S
35	KY	37.47	−86.15	0	99	99	S
36	KY	37.43	−86.03	0	99	99	S
37	MS	34.40	−90.57	0	26	90	R
38	MO	36.38	−89.52	0	99	99	S
39	OH	39.13	−84.73	0	3	33	HR
40	OH	39.12	−83.47	0	99	99	S
41	IN	39.45	−85.88	0	99	99	S
42	IN	39.28	−85.55	0	99	99	S
43	IN	38.53	−85.55	0	99	99	S
44	IN	38.60	−86.60	0	99	99	S
45	IL	38.73	−88.33	0	99	99	S
46	IL	38.62	−88.40	0	99	99	S
47	IL	38.38	−88.75	0	99	99	S
48	IL	38.63	−89.47	0	99	99	S
49	IL	36.90	−89.35	0	99	99	S
50	IL	37.08	−87.92	0	99	99	S
LSD (0.05)					5	6	

[a] Data represent the means of eight measurements and represent percent control. [b] S = susceptible biotype; R = resistant to glyphosate at 0.84kg/ha (70% or less control) but susceptible to glyphosate at 3.36kg/ha; HR = resistant to 3.36 glyphosate dose (70% or less control).

Table 2. Control under Greenhouse Conditions of Resistant and Susceptible Horseweed Biotypes as Affected by Increasing Glyphosate Dosage

horseweed biotype	glyphosate rate (kg ae/ha)	control % 7 DAT	control % 14 DAT	control % 21 DAT	fresh weight (21DAT) (g)	fresh weight as % of untreated (%)
susceptible	0	0 m[a]	0 k	0 j	17.2 a	100
	0.45	40 ghi	40 fgh	74 abc	5.1 efgh	30
	0.84	58 def	63 de	88 a	4.6 efg	27
	1.25	60 cde	92 ab	90 a	3.2 fghij	19
	1.68	63 cde	98 a	99 a	0.2 j	1
	2.52	83 ab	99 a	99 a	0.3 j	2
	3.36	85 ab	95 ab	99 a	1.1 ij	6
	9	90 a	98 a	99 a	0.2 j	1
resistant 1	0	0 m	0 k	0 j	14.5 ab	100
	0.45	10 lm	8 jk	11 hij	14.3 ab	99
	0.84	20 jkl	13 ijk	11 hij	13.6 ab	94
	1.25	28 ijk	18 ij	30 fghi	12.8 bc	88
	1.68	40 ghi	40 fgh	43 defg	5.2 efgh	36
	2.52	58 def	45 fg	59 cdef	3.7 fghij	25
	3.36	73 bc	58 def	75 bcde	1.2 j	7
	9	80 ab	80 bc	80 ab	1.4 j	9
resistant 2	0	0 m	0 k	0 j	13.2 abc	100
	0.45	13 lm	13 ijk	20 ghij	12.5 bc	94
	0.84	23 jkl	14 ijk	24 fghij	11.4 bcd	86
	1.25	33 hij	15 ijk	25 fghij	9.6 cde	73
	1.68	50 efg	44 fg	50 cdef	6.5 efg	49
	2.52	53 defg	50 efg	63 bcd	3.2 fghij	24
	3.36	60 cd	69 cd	75 abc	2.4 ghij	18
	9	73 bc	91 ab	89 a	1.6 j	12
LSD (0.05)		14	16	26	4	

[a] Means within a column followed by the same letter are not different according to Fisher's protected LSD, $P = 0.05$.

Dose Response of Glyphosate Resistant Horseweed. To gain an understanding of the level of glyphosate resistance in Tennessee horseweed biotypes, comparative studies utilized a stepwise rate comparison of glyphosate resistant and susceptible horseweed from normal application rates (0.45−0.84 kg/ha) of glyphosate to >10× rate of 9 kg/ha. Horseweed seed collected from two confirmed resistant biotypes and a confirmed susceptible biotype were examined (*5*). The plants were grown in a greenhouse by the previously mentioned methods. Glyphosate rates examined with each horseweed biotype included 0, 0.45, 0.84, 1.25, 1.68, 2.52, 3.36, and 9 kg/ha. Visual evaluations of plant effects were conducted 7, 14, and 21 DAT along with fresh weight determination at 21 DAT. The glyphosate dosages and procedures to determine GR_{50} values are similar to those previously used by VanGessel (*4*). Data were subjected to analysis of variance, and means were separated using Fisher's protected LSD test ($P = 0.05$).

RESULTS AND DISCUSSION

Distribution of Glyphosate Resistant Horseweed. Horseweed collected in Mississippi, Ohio, and Tennessee was determined to be glyphosate resistant (**Table 1**). Analysis of horseweed treated with glyphosate at 3.36 kg/ha indicated that population response to glyphosate varied greatly (10−99%). To account for this population variation, populations that displayed 70% or less control (four times the LSD) from a 3.36 kg/ha application were classified as highly resistant (HR, **Table 1**). Horseweed populations where control was 70% or less from 0.84 kg/ha glyphosate was considered resistant (R, **Table 1**). All other plant responses were defined as susceptible. Plants from the Mississippi location displayed resistance. Plants from one Ohio location were highly resistant, while plants from a separate site in Ohio were susceptible. There was minimal variation in plant response between the eight experimental units.

In Tennessee, seven of 32 samples were highly resistant, while two other horseweed populations were resistant. The remaining 23 samples were susceptible to both glyphosate application rates. All horseweed population samples from Illinois, Indiana, Kentucky, and the Missouri bootheel were susceptible to both glyphosate application rates.

Herbicide resistance is dynamic in the ecological plant system. This collection of samples, although covering a wide geographic area, was not exhaustive. It is quite possible that horseweed populations with glyphosate resistance could have been present in adjacent areas at the time of sampling. Additionally, in later years, new horseweed ascensions or introductions could exhibit glyphosate resistance. These data indicated that most of the populations examined in this study were susceptible to glyphosate. While some farmers use no-till production practices, the level of adoption in southern Illinois and western Kentucky is not as extensive as in western Tennessee and in the Delaware region. Greater use of tillage may reduce the incidence of glyphosate resistant horseweed, but this is only a hypothesis (*7*).

This research suggests that horseweed populations may still be segregating into those that are either glyphosate resistant or glyphosate susceptible, based on varying degrees of selection pressure. This research also demonstrated a wide geographical distribution of horseweed that is not controlled by a normal glyphosate application of 0.84 kg/ha (**Figure 1**). This spread of glyphosate resistant horseweed has been accomplished in a relatively short time period. However, >75% of the sampled horseweed seed lots were still susceptible to glyphosate. The question then arises, are these glyphosate resistant horseweed from a single source that then spreads or is glyphosate resistance developing in separate locations as unique events? The exploration of this question will be an area of future study, involving an examination of the physiological and genetic basis for the observed resistance.

Dose Response of Glyphosate Resistant Horseweed. Glyphosate produced some visual symptoms on all horseweed plants from 7 to 21 DAT (**Table 2**). This plant effect indicated that an active site was still present in even those plants resistant to glyphosate, although effects from 0.84 kg/ha provided only 10−25% control 21 DAT. Glyphosate susceptible horseweed displayed an increase in control from 7 to 14 and then to 21

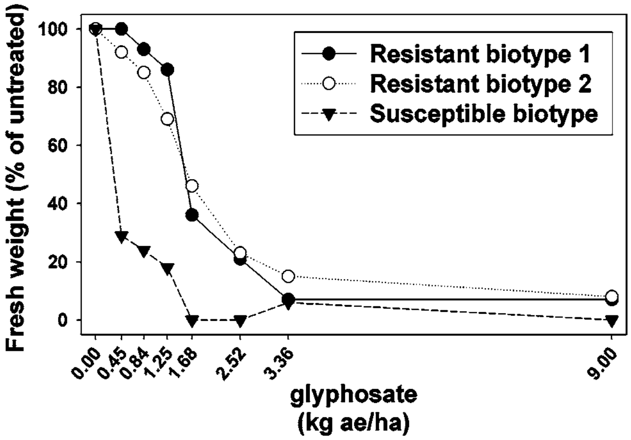

Figure 2. Horseweed biotype response to increasing doses of glyphosate. Calculated GR_{50} for resistant = 1.6 kg/ha and for susceptible = 0.4 kg/ha.

DAT, while glyphosate resistant horseweed displayed no change in control from 7 to 21 DAT (**Table 2**).

Glyphosate at 0.84 kg/ha controlled the susceptible biotype (86%), while the same application rate controlled both resistant biotypes < 20% (**Table 2**). The susceptible biotype was completely controlled (99%) by glyphosate applications of 1.68 kg/ha or greater. The resistant biotypes required 9 kg/ha glyphosate and 3.36 kg/ha glyphosate or greater to achieve the same control of biotypes I and II, respectively. While these application dosages provided statistically similar control, the plants never completely died and thus could possibly continue to grow and produce seed. The production of seed from plants treated with glyphosate has been verified to occur under field conditions (*15*).

Horseweed fresh weight decreased with increasing glyphosate application rate (**Table 2**). Susceptible horseweed displayed >70% fresh weight reduction with any glyphosate application and a calculated GR_{50} of 0.4 kg/ha (**Figure 2**). Resistant biotypes I and II required glyphosate applications of 1.6 kg/ha to achieve 50% fresh weight reduction. Analysis of the GR_{50} of resistant to susceptible populations showed a 4:1 ratio. These results are consistent with previous research by VanGessel (*4*).

There was no apparent growth reduction of horseweed plants associated with glyphosate resistance. In the absence of glyphosate application, fresh weight of susceptible horseweed (17.2 g) was similar to resistant horseweed (14.5 and 13.2 g). The two types of horseweed plants (resistant and susceptible) looked identical until they were sprayed with glyphosate.

Glyphosate resistance in horseweed and other weeds could have a detrimental impact on current cropping systems in the midsouth and midwest regions of the United States. Results from these studies suggest that resistance is becoming more widespread with resistant biotypes being found in Mississippi, Ohio, and throughout western Tennessee. Special care should be taken to control horseweed with weed management strategies other than glyphosate. It should be noted, however, that greater than 75% of the horseweed seed lots collected were still susceptible to glyphosate. Because glyphosate had activity on most of the horseweed populations, it is possible that other factors could partially explain the increase in horseweed occurrence. Environmental conditions such as wet weather, or changes in production systems such as a lack of residual soil-applied herbicides, or a decrease in tillage operations, and other soil factors may be a cause of greater horseweed germination and growth. Future research hopes to elucidate the genetic similarity/dissimilarity of the collected germplasms, possibly to determine if the ascension of glyphosate resistant horseweed is from a single source or from multiple sources.

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