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Differential Susceptibility of Field Bindweed (*Convolvulus arvensis*) Biotypes to Glyphosate[1]

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Abstract. **Biotypes of field bindweed (*Convolvulus arvensis* L. #[3] CONAR) identified in Indiana varied widely in susceptibility to glyphosate [*N*-(phosphonomethyl)glycine] but not to 2,4-D [(2,4-dichlorophenoxy)acetic acid] or bentazon [3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] in field tests. Significant differences in injury to two of the biotypes occurred with glyphosate applied at 1.12 to 4.48 kg ai/ha in greenhouse tests. Differences of greater than 70% in injury rating, root and shoot dry weight, and shoot regrowth dry weight occurred between the two biotypes at 2.24 kg/ha glyphosate. The susceptibility of the tolerant biotype at 2.24 kg/ha glyphosate was decreased by 40% as it increased in age, while the susceptible biotype sustained complete foliar necrosis when treated at all plant ages tested. Susceptibility differences between the two biotypes could not be correlated to differences in leaf stomatal or epidermal cell number. These studies suggested that the variable control of field bindweed observed in the field may be due to the occurrence of biotypes within a given population of this weed which differ in their susceptibility to glyphosate.**

Additional index words. **N-(phosphonomethyl)glycine, herbicide tolerance, CONVAR.**

INTRODUCTION

Field bindweed is a creeping perennial weed, native to Europe and western Asia, that was first introduced into the United States along the Atlantic seaboard in the mid-1700's[4]. Field bindweed has since spread westward through the United States and presently is classified as one of the most noxious weeds found in the west and midwest regions of the United States (8).

Control of field bindweed, with its extensive perennial root system, may be best accomplished by using postemergence, translocated herbicides. Translocation of a phytotoxic herbicide concentration throughout the entire root system is necessary for total plant kill. Many field studies using translocated herbicides applied to field bindweed have resulted in variable control (5, 9, 10). Researchers in Nebraska obtained 90 to 100% control of field bindweed with glyphosate applied at 0.8 kg/ha; however, similar tests in Wyoming with glyphosate applied at 1.7 kg/ha resulted in only 13% control (5, 10). Similar variations have occurred when 2,4-D was used for field bindweed control (1, 5, 13). Variable response of field bindweed to herbicides may be related to plant age and environmental conditions (relative humidity, temperature, and soil moisture) at the time of herbicide treatment (7, 11). Another explanation for the variable response of field bindweed to herbicides could involve the occurrence of field bindweed biotypes that differ in their response to a specific herbicide treatment.

Whitworth found that clones of field bindweed which differed in leaf shape and growth vigor also varied in sensitivity to foliar 2,4-D applications (12). Whitesides, however, was unable to show differences in glyphosate susceptibility between ecotypes of field bindweed in Oregon[4].

The following research was conducted to explain the variation in control observed when postemergent herbicides were applied to field bindweed in Indiana. Field bindweed biotypes collected from a single population in Lafayette, IN, were used in these studies (3). The objectives of this research were to: a) determine whether the field bindweed biotypes differed in their susceptibility to three postemergence herbicides; b) develop a dose response to glyphosate of two biotypes which differed in their susceptibility; c) determine the effect of plant age on this observed glyphosate susceptibility pattern; and d) correlate leaf surface characteristics of these two biotypes with differences in glyphosate susceptibility.

MATERIALS AND METHODS

Field response of biotypes to herbicides. Plants used in these studies were collected in the summer of 1977 in Lafayette, IN, from a single field bindweed population and maintained under greenhouse conditions until the 1981 field study was conducted. Five biotypes were selected from the population on the basis of previous results (3) for use in the field studies. Plants of each biotype were propagated from 15-cm root sections and grown in the greenhouse for 10 weeks prior to planting in the field. The field site was treated with diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide) at 4.48 kg/ha, and the biotypes were then transplanted into individual plots consisting of three plants of the same biotype planted in a 2.0- by 0.90-m area. Plots were handweeded and irrigated as necessary. Five weeks after planting, plots of each biotype were treated with either 1.12 kg/ha 2,4-D (dimethylamine salt) in 765 L/ha of water at 345 kPa, 1.12 kg/ha bentazon plus 2.34 L/ha crop oil concentrate, or 1.68 kg/ha glyphosate in 280 L/ha of water at 276 kPa using a CO_2-pressurized backpack sprayer. In 1982 plots were established to repeat the test but because of the slow growth of the field bindweed, treatments were delayed until June 1983.

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[3]Letters following this symbol are a WSSA-approved computer code from Important Weeds of the World, 3rd ed., 1983. Available from WSSA, 309 West Clark St., Champaign, IL 61820.

[4]Whitesides, R. E. 1978. Field Bindweed: A growth stage indexing system and its relation to control with glyphosate. Ph.D. Thesis. Oregon State Univ., Corvallis, OR. 89 pp.

Visual injury ratings, based on a scale of 0 = no injury and 100 = complete foliar death, were taken 3 weeks after herbicide treatment in each year. The experimental design was a randomized complete block with four replications, and data from 1981 and 1983 were combined for analysis.

Dose response of susceptible and tolerant biotypes to glyphosate. Biotypes one and four were selected from the field experiment as the most susceptible and most tolerant biotypes, respectively, for use in greenhouse experiments. Root sections 10.0 cm in length of each biotype were planted individually in 12.5-cm plastic pots containing a 1:1 (v/v) mixture of sterilized sand and loam soil with 1% organic matter. After planting, all pots were surface watered with a nutrient solution containing 200 ppm nitrogen, 200 ppm potassium, and 92 ppm phosphorus as needed to maintain plant growth. Plants were grown in a greenhouse under temperatures of 24-C ± 5-C day and 18-C ± 2-C night. Supplemental incandescent and fluorescent lighting with an output of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (PAR) was used to maintain a 16-h day length. Plants were treated with glyphosate at 0.56, 1.12, 2.24, 3.36, or 4.48 kg/ha 8 weeks after planting. Treatments were applied in a spray chamber with 280 L/ha of spray solution. Visual injury ratings (using the same scale as previously described) were taken every 3 days for 3 weeks, after which all plant shoots were cut off at ground level and oven dried for 48 h at 70C, and dry weights were recorded (only live, green shoot tissue was harvested in all greenhouse experiments). After shoot removal, plants were allowed to regrow from the top of the root crown for 6 additional weeks so that the residual glyphosate effect on shoot regrowth between biotypes could be compared. Shoot regrowth and roots from each plant were harvested and dried, and the dry weights were recorded. The experimental design was a completely randomized design with four replications.

Influence of plant age on biotype susceptibility to glyphosate. Three ages of plants of biotypes one and four were treated with glyphosate. Plants were propagated and maintained as described for the dose response experiment. Glyphosate at 1.12 and 2.24 kg/ha was applied to the plants 30 (25-leaf stage), 60 (bud stage), or 90 (flowering stage) days after initial planting as described in the dose response experiment. Visual injury ratings were taken as previously described every 3 days for 3 weeks. The plant shoots were then separated from the roots at soil level and oven dried, and the dry weights of each were recorded.

Leaf surface studies. Leaves from 60-day-old plants of biotypes one and four were examined by use of a scanning electron microscope[5] to determine stomatal frequency and epidermal cell numbers of the adaxial leaf surface. The 10th leaf, counting from the apex, of each plant's longest shoot was sampled and used for examination. Four 3- by 4-mm sections of each leaf were individually cemented to an aluminum specimen holder with silver paint and photographed at 100× magnification. Stomatal frequency and epidermal cell numbers were determined from an 11.4- by 8.9-cm print of each section. The data of all sections from the same leaf were averaged, and these means were used for statistical analysis. Observations of the cuticle appearance between biotypes were also made at 1000× and 2000× magnification.

Stomatal penetration of glyphosate into the leaves of biotypes one and four was investigated using a fluorescent dye method described by Dybing and Currier (4). This method was modified by using a 2.24 kg/280 L of water-glyphosate concentration in the test solutions. A surfactant, X-77 (alkylarylpolyethylene glycols plus free fatty acids and isopropanol), at concentrations of 0, 0.01, 0.05, 0.1, and 0.5% (v/v) was also added to the test solutions in an attempt to increase stomatal penetration by glyphosate. Observations of stomatal penetration by the test solutions were made by examining leaf tissue under an ultraviolet light source.

All greenhouse experiments were repeated twice, and results were combined for data analysis. Analysis of variance was used for all experiments to determine the significance of treatments.

[5] AMR, Model 1200, Amray Inc., Bedford, MA.

RESULTS AND DISCUSSION

Field response of biotypes to herbicides. Results of the field experiments showed that 2,4-D consistently gave maximum control of all five field bindweed biotypes, while bentazon and glyphosate provided more variable control between biotypes (Table 1). Since 2,4-D gave complete top growth control while bentazon was essentially ineffective against the field bindweed biotypes, further work with these chemicals was discontinued. Glyphosate, however, varied from complete control of biotype one to very low control of biotypes three and four, while biotypes two and five were intermediate in susceptibility, which strongly indicated differing responses between biotypes. The injury response observed on all biotypes after glyphosate treatment was characterized by a chlorosis of the shoot meristem region approximately 1 week after treatment. Biotypes three and four did not develop any further symptoms other than slightly

Table 1. Response of field bindweed biotypes to three postemergence herbicides[a].

Herbicide	Rate	Field bindweed biotypes[b]				
		1	2	3	4	5
	(kg/ha)	(% injury)				
2,4-D	1.12	100	100	99	100	100
Glyphosate	1.68	100 a	63 b	34 cd	24 d	48 bc
Bentazon	1.12	55 a	16 b	49 a	18 b	19 b

[a]Injury ratings taken 3 weeks after treatment based on 0 = no injury and 100 = complete foliar necrosis.

[b]Means within rows followed by different letters are significantly different at the 5% level according to Duncan's multiple range test.

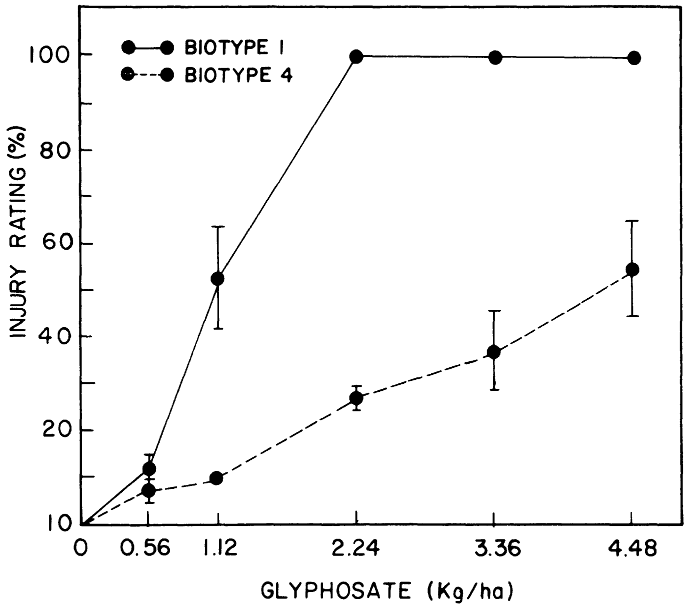

Figure 1. Dose response of two field bindweed biotypes to glyphosate. Injury ratings taken 3 weeks after treatment based on 0 = no injury and 100 = complete foliar necrosis. (Vertical bar represents standard error of the mean.)

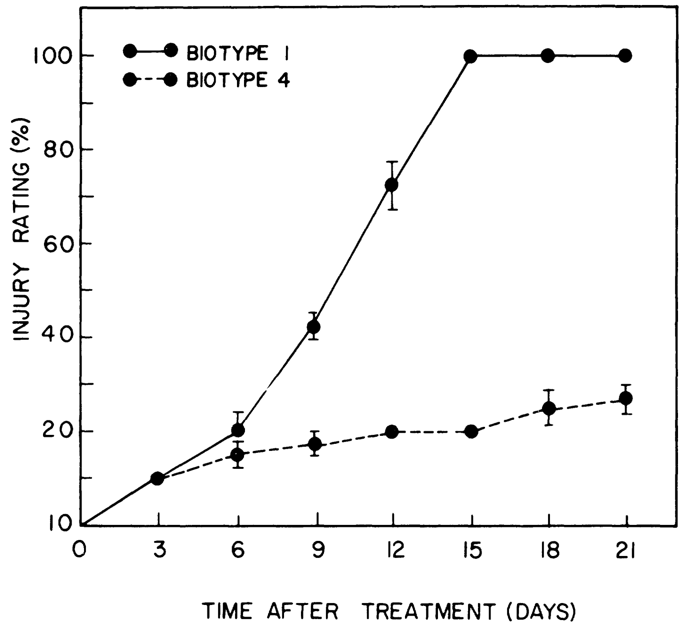

Figure 2. Time course of the response of two field bindweed biotypes to 2.24 kg/ha glyphosate. Injury ratings based on 0 = no injury and 100 = complete foliar necrosis. (Vertical bar represents standard error of the mean.)

stunted plants, while the more susceptible biotypes one, two, and five developed more severe foliar necrosis 10 to 14 days after treatment. Biotype one showed complete foliar necrosis within 3 weeks of herbicide treatment. The field tests indicated that differences in glyphosate susceptibility of field bindweed biotypes within a given population may be the cause of variable control obtained when glyphosate is applied to a field bindweed infestation.

Dose response of susceptible and tolerant biotypes to glyphosate. Glyphosate applied at 0.56 kg/ha resulted in little injury to either biotype (Figure 1). However, as the rate increased, biotype one showed injury at the 1.12 kg/ha rate and complete foliage death at the 2.24 kg/ha and higher rates. Biotype four showed minor injury at rates below 3.36 kg/ha and only 50% injury at the 4.48 kg/ha rate. The largest injury difference between the two biotypes occurred at the 2.24 kg/ha rate. From 0 to 6 days after treatment the reaction between biotypes was very similar (Figure 2). Both biotypes developed a mild chlorosis in the meristem area of the shoots and pinpoint necrotic lesions on the leaf surface where the spray solution was applied. After day 6, biotype one developed chlorotic borders around the lesions on the leaves which quickly developed into large necrotic areas that coalesced and encompassed the leaves. The leaf foliage of biotype one was completely dead within 2 weeks after treatment, while biotype four developed very little additional foliar injury beyond that observed at day 6. The chlorosis of the meristematic regions remained, but very little necrosis of the leaves occurred, and new shoots continued to develop in the leaf axils.

Although differences in injury ratings at the 1.12 kg/ha rate were observed, foliar necrosis and reduction of foliage growth were not extensive enough to significantly reduce the shoot weights of biotype one (Table 2). Dry root weights and shoot regrowth weights were signficantly different between the two biotypes at rates of 1.12 and 2.24 kg/ha gly-

Table 2. The effect of different concentrations of glyphosate on two field bindweed biotypes[a].

Glyphosate rate	Biotype	Dry shoot weight	Dry root weight	Shoot regrowth dry weight
(kg/ha)	(no.)		(% of control)[b]	
0.56	1	106 a	93 a	98 a
	4	121 a	70 a	117 a
1.12	1	64 b	22 bc	12 c
	4	72 b	68 a	41 b
2.24	1	0 c	3 c	1 c
	4	71 b	39 b	23 b
3.36	1	0 c	0 c	0 c
	4	60 b	15 bc	8 c
4.48	1	0 c	0 c	0 c
	4	61 b	10 c	7 c

[a]Plants were treated 8 weeks after planting and shoots were harvested 3 weeks later. Dry root weight and shoot regrowth dry weight were determined 6 weeks after initial determination of dry shoot weight.

[b]Means within a column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

phosate. When the initial shoot regrowth occurred on both biotypes, it was small and leaves were chlorotic at all rates of glyphosate. Within 8 weeks of treatment, however, new growth was normal in appearance for biotype four, while biotype one did not have normal growth at rates of glyphosate above 1.12 kg/ha. Biotype one was completely killed at glyphosate rates of 3.36 and 4.48 kg/ha, while biotype four showed some tolerance at these rates. To properly assess the injury of these biotypes at rates above 2.24 kg/ha, a longer regrowth period may be required. Results from these experiments showed that with all parameters measured, the 2.24 kg/ha rate of glyphosate was the rate at which consistently large and significant differences between biotypes occurred. In a population containing these two biotypes, application of 2.24 kg/ha glyphosate would be expected to cause a shift in the population to the predominantly tolerant biotype four.

Influence of plant age on biotype susceptibility to glyphosate. In these experiments, the glyphosate susceptibility differences between biotypes one and four were unaffected by plant age at 1.12 kg/ha (data not shown). However, at 2.24 kg/ha the two biotypes of field bindweed developed different patterns of susceptibility as they increased in age (Figure 3). Biotype one was extremely susceptible to the 2.24 kg/ha rate of glyphosate, as complete foliar death occurred 12 days after treatment at all plant ages. Biotype four did not exhibit the same consistent susceptibility pattern with age, as less foliar injury occurred as the plants increased in age to 60 days. The tolerance did not, however, increase further at 90 days of age. Shoot dry weight between the biotypes was also significantly different, with biotype four having greater shoot weight than biotype one at both rates and for all three plant ages (Table 3). Root dry weights at 1.12 kg/ha followed the same pattern as shoot weights at all plant ages. At 2.24 kg/ha glyphosate, only the 60-day-old plants of biotype four had root weights significantly larger than root weights of biotype one, with a similar trend on the 30- and 90-day-old plants.

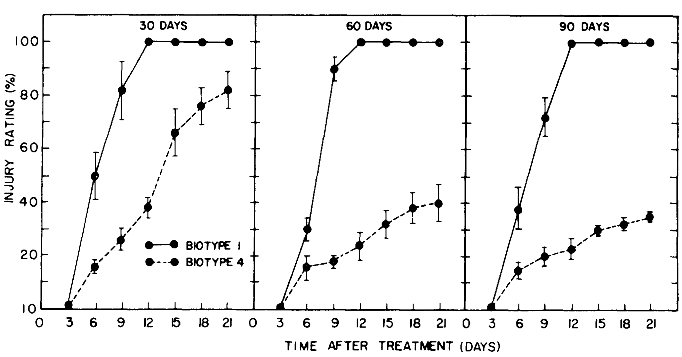

Figure 3. Time course of the response of two field bindweed biotypes to 2.24 kg/ha glyphosate at 30, 60, and 90 days after planting. Injury ratings based on 0 = no injury and 100 = complete foliar necrosis. (Vertical bar represents standard error of the mean.)

Glyphosate susceptibility of the field bindweed biotypes at different plant ages indicated that biotype four may be better controlled if treated with glyphosate 1 month (25-leaf stage) or less after initial plant emergence. However, the differences in susceptibility between biotypes, regardless of age, suggest that biotype one would be easily controlled by a single application of glyphosate at 2.24 kg/ha, but biotype four would not be killed.

Leaf surface studies. Examination of stomatal and epidermal cell number, as well as the ratio of epidermal cells/stomata, showed no significant difference between the biotypes (Table 4). Variation of both parameters was small between different samples on the same leaf; however, data between plants of the same biotype varied considerably. Observations of the cuticle formation also showed no consistent pattern of differences between biotypes one and four. Although studies have shown that variation in cuticle formation and stomata frequency can affect foliar penetration (2, 6), the results of this study indicate that differences in the leaf surface characteristics examined cannot explain the susceptibility differences between biotypes. Investigation of stomatal penetration by glyphosate into field bindweed biotype leaves using a fluorescent dye method showed that no stomatal penetration of glyphosate occurred with either biotype (data

Table 3. Root and shoot dry weights of two biotypes of field bindweed treated with glyphosate at three plant ages[a].

Glyphosate rate	Biotype	Dry shoot weight			Dry root weight		
		Plant age (days)[b]			Plant age (days)[b]		
		30	60	90	30	60	90
(kg/ha)	(no.)	(% of control[c])					
1.12	1	0 d	0 d	8 d	30 d	48 cd	38 d
	4	78 bc	92 b	133 a	119 ab	137 ab	163 a
2.24	1	0 d	0 d	0 d	10 d	53 cd	63 cd
	4	3 c	76 bc	71 bc	64 cd	132 ab	97 bc

[a]Plants were harvested three weeks after each treatment date.

[b]Plant age at spraying.

[c]Means within shoot or root weight groups followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 4. Comparison of the leaf surface characteristics of two field bindweed biotypes[a].

Biotype	Stomata	Epidermal cells	Epidermal cells/stomal pore
	(no./mm²)		
1	61	461	8
4	60	512	9

[a]No differences between means within columns were obtained at the 5% level of significance.

not shown). These observations do not, however, eliminate potential differences in the amount or rate of penetration of applied glyphosate as a possible cause of susceptibility differences between biotypes.

The results of these studies demonstrate that a population containing more than one biotype of field bindweed can respond in a variable manner when treated with glyphosate. Thus, an explanation for the reports of inconsistent field bindweed control with glyphosate (5, 9, 10) may be found in the existence of biotypes of this weed, which differ in their susceptibility to this chemical.

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