

Weed escapes and delayed weed emergence in glyphosate-resistant soybean

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Abstract

During 2001 and 2002, field experiments were conducted in soybean crops at four Minnesota locations with the aim of studying the effects of different glyphosate treatments (one-pass glyphosate, two-pass glyphosate) on weed control and weed community composition by focusing on the identity and abundance of weeds that escaped from different treatments. In addition, seedling emergence patterns of different weeds were studied to identify the influence of delayed emergence on weed escapes. Overall, 10 species were recorded as weed escapes and *Chenopodium album* L. and *Solanum ptycanthum* Dunal were present at all locations. Late weed emergence was the main reason of weed escapes with one-pass glyphosate. *C. album* showed a long period of emergence, thereby allowing the late-emerging cohorts to avoid contact with the herbicide. *S. ptycanthum* emerged late and therefore its entire seedling population escaped glyphosate treatment. These weeds showed a robust relation fecundity (seeds/m²) and plant ground cover. *C. album* ground cover of 0.1% may produce around 500 seeds/m².

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1. Introduction

During the last decade, genetically modified (GM) crops developed with the aim of incorporating different agronomic characteristics, such as tolerance to herbicides or insects resistance, have been well received and rapidly adopted by farmers in several parts of the world (Buttel, 2002). The estimated global area with GM crops for 2004 was about 81 million hectares. In the same year, soybean resistant to glyphosate was cropped in 48.4 million hectares (60% of the whole transgenic crop area) and represented 56% of the whole world area with this crop. In 2003, the area sown with crops resistant to herbicides (soybean, maize, canola and cotton) was 73% of the whole transgenic crop area. In the US there were 42.8 million hectares sown with transgenic crops representing 63% of the whole world area (James, 2003, 2004). Prior to the introduction of glyphosate-resistant

soybean, glyphosate was applied to control existing vegetation before planting or soybean emergence (Brown et al., 1987; Bruff and Shaw, 1992; Hydrick and Shaw, 1994; Wilson and Worsham, 1988). Now, it can be used postemergence in glyphosate-resistant crops, such as soybean, cotton, canola and corn (Norsworthy et al., 2001). The introduction of glyphosate-resistant crops has created new opportunities for the use of this herbicide as selective weed control in crop production, and the adoption of glyphosate-resistant soybean resulted in an important increase of glyphosate use during recent years in the USA (Tharp et al., 1999; Norsworthy et al., 2001). According to Shaner (2000); the recommendation from Monsanto in the USA includes a preplant burndown application of glyphosate followed by one or two applications of glyphosate in the crop. Thus, a weed population could be treated up to three times within one season and the label allows up to 6.72 kg a.i./ha to be applied during a season. Although glyphosate is a strong herbicide controlling many weeds at high efficacy, different weeds may escape glyphosate

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treatments. Application rate, weed age and size, spray volume, adjuvants, water quality and interactions with other herbicides affect glyphosate efficacy (Jordan et al., 1997). Control of weeds such as common lambsquarters (*Chenopodium album* L.), hemp sesbania (*Sesbania exaltata* Raf.), morningglory (*Ipomoea* spp. L.) or velvetleaf (*Abutilon theophrasti* L. Medic.) declines as the weeds become larger and must be controlled early (Mulugeta and Boerboom, 1996). On the other hand, most grasses are usually very susceptible to glyphosate (Wiesbrook et al., 2001). However, tolerance to glyphosate is not the unique reason to explain weed escapes. Other explanations for weed escapes are that some weed species continue to germinate for a long time during the growing season, including after the last glyphosate application (Hennen et al., 2002). For instance, Payne and Oliver (2000) recorded control no higher than 85% for barnyardgrass (*Echinochloa crus-galli* [L.] Beauv.) with two applications of glyphosate during the crop cycle as a consequence of late-emerging cohorts. Later application of glyphosate gave better control of large crabgrass (*Digitaria sanguinalis* L.) at the end of the season due to control of large crabgrass emerging after earlier applications (Arnold et al., 1997). This means that certain species escape the glyphosate treatment simply by avoiding the herbicide application; so timing of the glyphosate applications is the key to its efficacy.

The objectives of this research were to study: (i) the response of the weed community, in terms of weed escape, to different glyphosate treatments in soybean crops at different locations in Minnesota, (ii) the emergence dynamics of weeds to gain a better understanding of emergence patterns of specific weed species that escape glyphosate treatments, and (iii) plant fecundity of escaped plants to gain a sense of the future risk that this escape represents.

2. Materials and methods

2.1. Field experiments: treatments and locations

Five field experiments were conducted during 2001 and 2002 on glyphosate-resistant soybean crops at different locations in Minnesota (USS). Locations were the sites of the state-wide herbicide management trials at University of Minnesota Research and Outreach Centers at Morris (45:35:10 N, 095:54:49 W, 345 m elevation), Lamberton (44:13:52 N, 095:15:50 W, 351 m), Potsdam (44:09:46 N, 92:20:20 W, 347 m) and Waseca (44:04:40 N, 093:30:26 W, 351 m). In this last location, two experiments (Waseca 1 and Waseca 2) were performed in two experimental areas that had differing weed floras. At Waseca (2) data were assessed only for 2002. At each site, weed response was evaluated in three treatments each one with four replications. Plot size at each field experiment was $3 \times 6 \text{ m}^2$. The experimental treatments evaluated were: (i) the weedy check; (ii) “1-Gly,” which was glyphosate applied only once at 0.85 kg a.i./ha when the tallest weed was 15 cm and soybean was at the V3 growth stage (three nodes and three

expanded trifoliolate leaves); (iii) “2-Gly,” which is glyphosate at 0.85 kg a.i./ha applied twice when the tallest weeds was 10 cm and again before the time of soybean canopy closure. Herbicides were applied through a 3.1 m boom in 1871/ha carrier volume of water at a pressure of 207 kPa. Experimental plots were seeded using conventional planters at 30 seeds/m of soybean group maturity 0, in four rows spaced at 76 cm. Plots had been moldboard or chisel plowed and then field cultivated to prepare seedbed. The sowing was carried out between May 15 and May 25 both years. Experiments were conducted under dry land conditions and soybean received the equivalent of 15, 45 and 50 kg/ha of N, P and K at sowing.

2.2. Data collection

Density and ground cover for each weed species were assessed before the first glyphosate application, at the V2 soybean growth stage in six 0.1 m^2 quadrats randomly placed at each plot. These data were collected with the aim of knowing the initial composition of the population at different treatments. Immediately before soybean harvest, all escaped weed individuals were identified and counted in each herbicide-treated plot. In all cases, individuals were not registered before glyphosate application but recorded at preharvest, survival rate was considered as 100%.

Emergence counts of seedlings of common lambsquarter and foxtail species were made in weedy check plots from June 3 to July 12 in 2001 and from June 6 to July 12 in 2002. Four 0.1 m^2 quadrats were permanently placed in plots and seedlings were identified, counted and removed at 15 d intervals.

Immediately before soybean harvest, fecundity of escaped individuals of common lambsquarter and black nightshade (*Solanum ptycanthum*) were assessed in the following manner: (i) a 0.25 m^2 circular quadrat was placed over individual weeds or a group of specific weeds, (ii) percent of ground area of the 0.25 m^2 quadrat covered by these weeds was estimated visually, (iii) seeds on these plants were harvested by hand and the soil surface under the plants was vacuumed to collect seeds that had shattered from the plants prior to harvest, (iv) various threshers and sieves were then used to clean the seeds, (v) seeds collected from the plants and surrounding soil surfaces were combined, air-dried and weighed, (vi) total numbers of seeds produced per quadrat were calculated based on 1000-seed weights, (vii) relationships between percent ground cover and seed production were determined through regression, and (viii) unit-area seed production of escaped not dead weeds was calculated based upon the cover of these weeds at soybean harvest and the regression equation from (vii).

2.3. Statistical analyses

Data for weed density and ground cover were log and arcsine transformed, respectively, to homogenize variances.

After that, data were subjected to analysis of variance and when the F test was significant ($P < 0.05$) means were separated by Fisher's protected LSD test at 5% probability. Results are shown on the original data.

Year was included as another factor, but when there were no significant ($P < 0.05$) effects of year or year by treatment interactions, data were combined over years.

The relationships between seed production and cover were established by regression analyses. An F test was used to determine if the regression equation was significant ($P < 0.05$).

3. Results and discussion

3.1. Weed community composition

Percent ground cover for each weed species before glyphosate application was not significantly different ($P > 0.05$) among the 1-Gly, 2-Gly and weedy check treatments (data not shown). The same results were recorded in terms of weed density (Table 1). Some weed species showed significant differences between years ($P < 0.05$) and, also, certain weeds were dominant at different sites. Although Foxtail species were regarded together, green foxtail (*Setaria viridis* [L.] Beauv.) was dominant in Lamberton and in Morris. On the other hand, giant foxtail (*S. faberii* Herm.) was abundant in one of the Waseca experiments. Common ragweed (*Ambrosia artemisiifolia* L.) and waterhemp (*Amaranthus rudis* SAUER) were also dominant at Waseca experiments. Density data for waterhemp are shown included as pigweed species (AMAsp). Although common lambsquarters was present in all experiments, it was the most abundant weed only at Potsdam (Table 1).

There were 14 weed species before glyphosate application across years and treatments and nine species were recorded at preharvest across all experiments in 2001 (Tables 1 and 2). There were no weed escapes at the 2-Gly treatment neither in Morris nor in Lamberton. Common lambsquarter, pigweed, common dandelion (*Taraxacum officinale* Weber in Wiggers), smartweed (*Polygonum pennsylvanicum* L.), black nightshade and velvetleaf were recorded both at 1-Gly and 2-Gly treatments while foxtail and wild proso-millet (*Panicum sp.* L.) were only recorded at 1-Gly treatment. Although black nightshade was not commonly registered before glyphosate application, it was recorded at preharvest even for the 2-Gly treatment in Waseca and Potsdam. This means that later emergence is the key to explain the escape of this species.

In 2002, there were no survival individuals for 2-Gly treatment in Waseca 1 and Potsdam (Table 3). Ten weeds were registered as weed escapes across all experiments. At Waseca (2) experiment, velvetleaf was registered at preharvest only with one glyphosate application while common lambsquarter, waterhemp, common cocklebur (*Xanthium strumarium* L.), common ragweed, black night-

Table 1

Density (plants/m²) of weeds at different locations before glyphosate application, 20 days after sowing, when soybean was at V2 growth stage^a

Weeds	Potsdam	Lamberton	Morris	Waseca (1)	Waseca (2)
ABUTH	13	—	—	—	3.3
AMAsp	6.1	24	31	150 > 200	13
AMBEL	—	—	—	—	116
CHEAL	185	50	8.5	20.4	7.3
CIRAR	0.7	1	—	—	—
CYPES	—	—	—	0.7	—
PANsp	118	—	—	—	—
POLPY	2.5	0.8	—	—	1.5
SETsp	—	200 ^a	210 5.2	18	168
SINAR	—	—	7.8	0.13	—
SOLPT	—	0.27	—	—	—
SONAR	—	0.07	0.14	—	—
TAROF	0.3	—	—	4.3	0.07
XANST	—	—	—	—	23

^aSingle values represent means of postemergence and weedy check treatments. Where two values are present, means differed significantly ($P < 0.05$) between years and values are for each year (2001 and 2002).

Table 2

Weed escapes at preharvest as survival rate (%) related to density (plants/m²) for 1-Gly and 2-Gly treatments before glyphosate application (b.a.) on different experiments in Minnesota (2001)^a

	Weeds	One "gly" (b.a.) Surv. %	Two "gly" (b.a.) Surv. %	
Lamberton	AMBEL	0	100	0
	CHEAL	66	2.8b	75
	POLPY	6	4.8b	1.7
	SETVI	803	0.4a	803
	SOLPT	0	100a	1.7
Moris	AMAsp	23	1.5a	24
	CHEAL	28	2.14b	9
	SETVI	1492	0.6a	1113
Waseca	ABUTH	0	0	100
	AMAsp	624	0.32a	624
	CHEAL	38.7	5.83a	42.9
	SETVI	32.5	0	32.5
	SOLPT	0	100a	0
	TAROF	11.7	4b	7.5
Potsdam	ABUTH	7.8	06	12.8
	CHEAL	179	3.1b	159
	PANsp	76.2	3.9	84
	POLPY	0.42	48a	0
	SOLPT	0	100a	0
	TAROF	5.83	10a	1.7

^aValues with different letters are significantly different between locations ($P < 0.05$) for each weed species.

shade and foxtail were recorded at both treatments (data not shown).

In 2001, across all experiments, the number of species recorded at preharvest was 9 and 6 species in 1-Gly and 2-Gly treatments, respectively. In 2002, these figures were 10 and 8 species. In terms of weed density, the effect of 2-Gly was significantly ($P < 0.05$) higher than the effect of 1-Gly. In 2001, as an average of all experiments weed

Table 3

Weed escapes at preharvest as survival rate (%) related to density (plants/m²) for 1-Gly and 2-Gly treatments before glyphosate application (b.a.) on different experiments in Minnesota (2002)^a

	Weeds	One "gly" (b.a.) Surv. %	Two "gly" (b.a.) Surv. %		
Lamberton	AMAsp	36.2	0.28b	3.75	0.00
	Cheal	38.2	2.09b	65.6	0.08
	ECHsp	0	100	0	0
	Polco	1.25	4	0	100
	Polpy	1.87	21.39a	0	100
	Setvi	980	0.23	980	0
	Solpt	0.625	0	0	0
Morris	AMAsp	47.5	0.31b	53.12	0.09
	Cheal	8.75	2.28b	8.75	0
	Setvi	6.87	0	5	2
	solsp	0.625	8	0	100
Waseca	AMAsp	212.25	4.4a	139.75	0
	CHEAL	21.87	34.7a	1.875	0
Potsdam	ABUth	17.5	1.42	4.375	0
	AMAsp	11.8	0b	5	0
	CHEAL	2248	0.2b	2248	0
	PANsp	324	0	324	0
	POLPY	5	6b	6.875	0
	SOLSp	0		0	0

^aValues with different letters are significantly different between locations ($P < 0.05$) for each weed species.

density at preharvest was 1.7 and 0.3 plants/m² for 1-Gly and 2-Gly, respectively. In 2002, these figures were 2.5 and 0.03 plants/m² for the same treatments. This means that different effect between treatments is over weed density more than on number of weed species.

Peterson et al. (2002) studied the effect of glyphosate strategies on crop yield in US and found a significant correlation between percentage of maximum yield and latitude. There was not difference on crop yields between 1-Gly and 2-Gly treatment over 40°N latitude. This suggests that farmers can manage 1-Gly strategy for rich assemblages of weeds while maintaining high yields, but only at high latitudes.

Ecological reasons to explain escape from glyphosate application range from simple to complex. In-row protection from glyphosate by the crop canopy, differential tolerance according to the growth stage, environmental conditions, application technology, individual tolerance and insect disruption of glyphosate translocation within plants are some reasons that could explain the escapes for those species or individuals that were present at the time of herbicide application. For many weed species, young plants are considerably more susceptible to herbicides than older plants (Harker and Dekker, 1988). Control of lambsquarters by glyphosate was dependent on timing of application. Nevertheless, control increased as glyphosate rate increased both for early and late applications (Krausz et al., 1996). Tharp et al. (1999) registered significant differences for the GR₅₀ values of glyphosate when

velvetleaf individuals were treated at different growth stages. A theoretical example of how this influences escape from control would be a weed population wherein some individuals emerge early and are so large at the time of herbicide application that they survive exposure to the herbicide.

Although many mechanisms are reasonable to explain the escape from glyphosate, most of them assume that individuals are present at the time of the herbicide application. However, certain species with inherently late emergence or species with individuals that emerge over an extend time period, may escape control from glyphosate primarily through simple avoidance the herbicide.

Consequently, to manage the glyphosate application during the crop cycle, knowing the emergence dynamic of weeds could be an interesting strategy to avoid large effects on weed diversity.

3.2. Emergence times of weed escapes *Chenopodium album*

At soybean harvest, common lambsquarter was detected in all the experiments in both years. As expected, it was much more abundant in 1-Gly than 2-Gly plots. Indeed the 2-Gly treatment nearly eliminated common lambsquarter. In 1-Gly treatment, its densities were the highest among all species across sites (Tables 2 and 3). This suggests that common lambsquarter could increase the population size in glyphosate-resistant soybean crops.

Survival rate at one glyphosate treatment in 2001 was higher ($P < 0.05$) at Waseca than at the other locations (Table 2) These results were related to emergence dynamic of the weed that at the time of glyphosate application were significantly ($P < 0.05$) lower in Waseca than in the other sites (Fig. 1). Glyphosate at Waseca was applied on June 19 while at the other locations was carried out around ten days later. Interestingly, there were also some escapes in Morris where 100% of individuals had emerged at the time of the herbicide application (Fig. 1). So, that escapes were not due to delayed emergence but other reasons like in-row protection or individual tolerance could explain those escapes. In 2002, as in 2001, percentage of escapes was also related to emergence at the time of glyphosate application. These figures trend to be higher in Lamberton and Morris than at Potsdam where the glyphosate application were carried out with higher percentage of emergence than at the other locations (Table 3 and Fig. 1). Application dates for Lamberton, Morris and Potsdam were June 14, June 25 and June 28, respectively. At Waseca, Glyphosate was applied at the same dates that at Potsdam. The high survival rate recorded in this experiment (Table 3) suggest that there would be a tolerant population of common lambsquarter in that location.

At all locations, percent emergence was 100% at the time of the second application. However, there were lambsquarters individuals present at harvest in plots for the 2-Gly treatments in Potsdam, Waseca and Lamberton (Table 2 and 3). This means that both mechanisms may

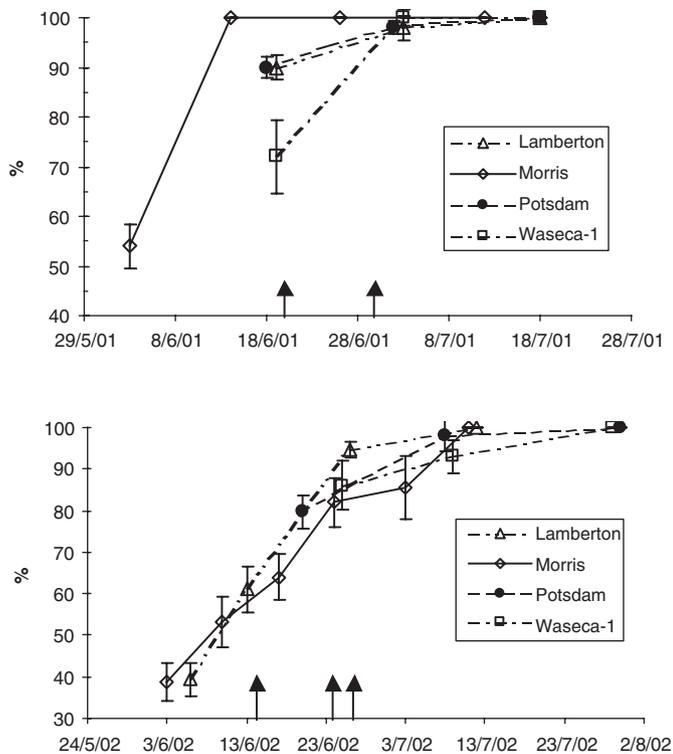


Fig. 1. Cumulative emergence of common lambsquarter (*Chenopodium album* L.) during the crop cycle at different locations in 2001 and 2002. Arrows indicate dates of 1-Gly application.

regulate the number of common lambsquarters recorded for the 1-Gly and 2-Gly treatments. However, delayed emergence showed to be more important related to the number of escaped individuals. Consequently, these results show the importance of predicting the emergence of common lambsquarters in order to decide the time of glyphosate application. Such a tool would be especially useful if the herbicide is applied only once during the cropping season. Percentage of the population emerged is related to available water and temperature conditions. Roman et al. (2000) described a mechanistic model based on the integration of hydrothermal time to predict germination and also thermal time to describe shoot elongation. These models may become important components of an integrated weed management strategy to manage time of the glyphosate application, which would aid efforts to eliminate escapes in glyphosate-tolerant crops.

3.3. Other weeds

In 2001, foxtail survival rate at preharvest in 1-Gly treatment was 0.4 and 0.6% in Lambertson and Morris, respectively. There were not survival individuals recorded at Waseca (Table 2). These results may be explained by the dynamic of weed emergence. In Waseca all foxtail individuals had emerged by the time of the first glyphosate application on June 19 (data not shown). On the other hand, there were some emergence after glyphosate application in Lambertson and Morris (Fig. 2). At Morris in 2002,

100% of the population had emerged at the time of herbicide application (June 25) and there were not survived individuals at one glyphosate application (Table 3). However, there were escape individuals at Waseca (2) where 80% of the population had emerged at the time of glyphosate application (Fig. 2).

Waterhemp was present at Waseca experiments and escaped at 1-Gly treatment both of the years (Tables 2 and 3). In Waseca, (1) higher amount of individuals had emerged in 2001 than in 2002 at the time of the glyphosate application so this may explain different survival rate between years. In addition, there were some survival individuals in two glyphosate treatments at both Waseca experiments in 2001 and at Waseca (2) in 2002. This means that not all escapes may be explained by late emergence but also individual tolerance or in crop row protection could be reasons for the escapes. Waterhemp is particularly considered as one of the tolerant weeds to glyphosate (Shaner, 2000). In addition, other reports indicate differential sensitivity to glyphosate among biotypes of common waterhemp (Smeda and Schuster, 2002). Zelaya and Owen (2002) also recorded differential responses of waterhemp to glyphosate in Iowa state.

The results showed that timing of the first and second glyphosate application is the key to its effectiveness and to the reduction of weed escapes. As emergence studies continue and emergence prediction tools improve, application dates can be determined in real time for the most effective results. Along with emergence studies, other areas need to be examined. For example, escaping glyphosate might involve the presence of high densities of the same or

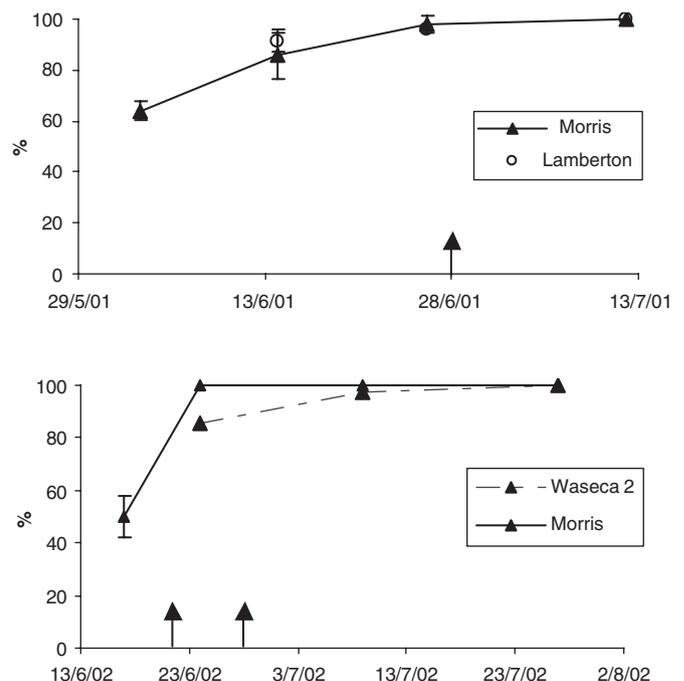


Fig. 2. Cumulative emergence of foxtail species (*Setaria* sp.) during the crop cycle at different locations in 2001 and 2002. Arrows indicate dates of 1-Gly application.

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