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Effect of Plant Nitrogen Concentration on the Response of Glyphosate-Resistant Corn Hybrids and Their Progeny to Clethodim and Glufosinate

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Rapid adoption of glyphosate-resistant (GR) corn hybrids has led to the reemergence of volunteer corn as a problematic weed in soybean and has made controlling the initial stand of corn in a replant situation more difficult. If volunteer corn in soybean or the initial corn stand in a replant situation is not controlled, yield loss can occur. Clethodim and glufosinate are often used to control GR corn in corn replant situations and in soybean. The objectives of this research were to evaluate the response of two hybrid corn varieties and their F_2 progeny to clethodim and glufosinate and to evaluate the effect of plant nitrogen (N) concentration on clethodim and glufosinate efficacy. First, a dose-response study was conducted with clethodim and glufosinate on DeKalb 60-18 and 60-18F_2, and DeKalb 63-42 and 63-42F_2 to compare the response of the hybrids and their F_2 progeny to the herbicides. DeKalb 63-42 was more tolerant to clethodim than 60-18 and 60-18F_2. No differences were found between the hybrids and their respective F_2 progeny in the response to clethodim or glufosinate. In a second dose-response study assessing the effect of N conditions on herbicide efficacy, both clethodim and glufosinate were less injurious to plants growing in low N than in high N availability.
Nomenclature: Clethodim; glufosinate; corn *Zea mays* L.; soybean *Glycine max* (L.) Merr.
Key words: Dose-response, glyphosate-resistant volunteer corn, nitrogen.

Volunteer corn (*Zea mays* L.) is a reemerging problem weed in both corn and soybean [*Glycine max* (L.) Merr.] fields and changes in cultural practices may lead to increased incidences of failed corn stands (Davis et al. 2008). In 2010, U.S. farmers planted nearly 36 million hectares of corn (U.S. Department of Agriculture–National Agriculture Statistical Service 2010), a number that is expected to increase to 38 million hectares by 2016 due to federal mandates that require greater ethanol production (Malcolm and Aillery 2009). Also, farmers in the Midwest now plant corn 2 wk earlier than farmers did in the early 1980s (Kucharik 2006). Both increased corn production and earlier planting will likely increase the occurrence of failed corn stands requiring corn replants. In Indiana, approximately 4 million hectares are in a corn:soybean rotation, which often leads to the occurrence of volunteer corn as a weed in soybean fields as well.

The presence of volunteer corn, whether it is from a failed corn stand in a corn replant situation or if it is volunteer corn in soybean, can reduce yield (Beckett and Stoller 1988; Steckel et al. 2009). In corn replant situations, competition of the replanted corn with the initial stand, if left uncontrolled, can reduce yield up to 25% (Steckel et al. 2009). Corn height variation can result in uneven competition between plants and can reduce grain yield (Carter et al. 1992; Liu et al. 2004; Nafziger et al. 1991). Nafziger et al. (1991) reported yield losses of 6 to 8% when 25% of the corn stand emerged 1.5 wk after initial corn emergence. In the same study, a 3-wk delay in planting of 25, 50, or 75% of the plants resulted in yield losses of 10, 20, or 22%, respectively. Liu et al. (2004) found that one out of six plants with a two-leaf stage delay in emergence reduced yield by 4%, and one out of six plants with a four-leaf stage delay reduced yield by 8%. In soybean, Beckett and Stoller (1988) showed yield decreases up to 25% with volunteer corn densities of five plants m^{-2}.

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Control of volunteer corn in soybean or of the initial corn stand in a corn replant situation is critical to maximize yield in either crop. As of 2010, herbicide-resistant corn was planted on 76% of Indiana corn hectares (U.S. Department of Agriculture–Economic Research Service 2010), and due to trait stacking with insect resistance traits, the majority of the 76% were GR. The GR gene is a single dominant gene and is present in 75% of GR hybrid progeny (F_2) when the hybrid is heterozygous (Magulama 2009). A greenhouse study comparing the level of glyphosate tolerance of hybrid corn to its F_2, when the F_2 was GR, showed no difference in tolerance (Paul Marquardt unpublished data). As the adoption of GR corn has increased, the presence of volunteer corn in soybean has also increased, which is likely due to farmers' sole reliance on glyphosate for weed control (Davis et al. 2008). In corn replant situations, planting GR corn is a disadvantage because nonglyphosate herbicides must be used to control corn to avoid tillage (Steckel et al. 2009). Clethodim and glufosinate, with the use of glufosinate-resistant crops, are two herbicide options for postemergence control of volunteer corn in soybean and corn replant situations (Anonymous 2008a,b). Previous research showed that natural tolerance to both herbicides may exist in corn. Pornprom et al. (2003) indicated that nonherbicide-resistant corn hybrids can have varying levels of tolerance to glufosinate. Tolerance to clethodim has not been shown in corn, but it has been shown with other acetyl-coenzyme A carboxylase (ACCase) inhibiting herbicides. For example, sethoxydim-resistant corn hybrids have been developed through conventional breeding (Parker et al. 1990). Additionally, Andersen and Geadelmann (1982) reported that F_2 generations of corn hybrids expressed dissimilar tolerances to diclofop. However, research has not been conducted on differential tolerance to either clethodim or glufosinate between hybrid corn and their F_2 progeny. Therefore, determining how different corn hybrids and their F_2 progeny respond to clethodim and glufosinate is critical to help understand how to control GR corn.

Herbicidal control of plants can also be influenced by the growing environment of the plant when the herbicide is applied. One environmental factor that has been shown to

impact herbicide efficacy is soil N availability. Tame oats (*Avena sativa* L.) were more tolerant to fluazifop and glyphosate when grown under low soil N (1.0 mol m^{-3}) compared to high soil N (10 mol m^{-3}) (Dickson et al. 1990). Additionally, fluazifop translocation was nearly three times greater with high N vs. low N. Cathcart et al. (2004) reported a 50% growth reduction (GR_{50}) of redroot pigweed (*Amaranthus retroflexus* L.) required 110.8 g ai ha^{-1} of glufosinate with low soil N (0.7 mM) compared to 39.5 g ha^{-1} with high soil N (7.0 mM). However, soil N level did not influence the response of velvetleaf to glufosinate. Poor corn stands are often a result of wet soil conditions, likely resulting in soil denitrification, and reducing soil N content (Randall et al. 2003) and resulting in a N-deficient corn stand. Also, volunteer corn in soybean is often N deficient, a result of no additional N fertilizer applied to the soil before soybean planting. To date, there are no reports on the impact corn N concentration has on clethodim or glufosinate efficacy, despite the likelihood that these herbicides will often be applied to N deficient plants.

Therefore, the objectives of this research were to (1) determine if tolerance to clethodim and glufosinate differs between hybrid corn lines and their F_2 progeny, and (2) determine if corn tolerance to clethodim and glufosinate is influenced by N availability.

Materials and Methods

Corn and Herbicide Selection. DeKalb (Monsanto Company, St. Louis, MO 63167) 60-18 and 63-42 GR hybrids and their F_2 progeny (60-18F_2 and 63-42F_2) were the corn lines used for all dose-response studies. F_2 progeny of DeKalb 60-18 and 63-42 were collected from corn fields in the fall of 2009 and stored in a cold room at 4 C.

Clethodim (Select Max, Valent USA Corporation) and glufosinate (Ignite, Bayer CropScience LP, Research Triangle Park, NC 27709) were the herbicides evaluated for the dose-response because of their ability to control GR corn in both corn replant situations and in preplant or postemergence soybean applications (Anonymous 2008a,b). Glufosinate can be applied preplant to corn and soybean regardless of the presence or absence of technology traits but will only control corn that is not glufosinate-resistant and can only be applied post crop emergence in glufosinate-resistant corn or soybean.

Clethodim and Glufosinate Dose-Response. In a greenhouse, single corn seeds were planted approximately 3.5 cm deep in 750 cm^3 Deepots (D-20, Steuwe & Sons, Corvallis, OR 97333) containing a potting soil mixture (Redi-Earth Plug and Seedling Mix, Sun-Gro Horticulture, Bellevue, WA 98008). Plants were exposed to high intensity, supplemental lighting to provide a 16-h photoperiod with day/night temperatures of 24/28 C and 18/22 C. Pots were watered daily and fertilized (Water Soluble All Purpose Plant Food [24-8-16], Scotts Miracle-Gro Products Inc., Marysville, OH 43041) weekly with a nutrient solution.

The experimental design was a 4 by 7 and 4 by 8 factorial randomized complete block design for glufosinate and clethodim, respectively, with five replications and with two experimental runs. The clethodim experiment was conducted in February and March 2010, and the glufosinate experiment was conducted in August and September of 2010. Factor 1 was assigned to one of the four corn lines (DKC 60-18, 63-42, 60-18F_2, and 63-42F_2), and factor 2 was the herbicide treatment (Table 1). Corn was at the V2 growth stage (12 to 18 cm), determined by the collar method (Ritchie et al. 1992), at the time of herbicide application. All plants were treated with herbicides applied with a track spray chamber using a XR8002 even flat-fan nozzle (TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187) at 190 L ha^{-1} carrier volume and a pressure of 275 kPa. At 14 d after treatment (DAT), plants were severed at the soil surface and dried for 7 d in a forced air dryer at 30 to 38 C. Dry weight of treated plants was measured and statistical analysis was performed on the untransformed dry weight data. Data were analyzed using a nonlinear regression model with the *drc* (dose response curve) package in R (R 2.10, Kurt Hornik, online) as described by Knezevic et al. (2007). Dose-response models were constructed using Equation 1, a four-parameter log-logistic model for clethodim, and Equation 2, a three-parameter log-logistic model for glufosinate.

Table 1. Herbicide treatments used in the clethodim and glufosinate dose-response study (Experiment 1) and in the effects of plant nitrogen concentration on glyphosate-resistant corn dose-response study (Experiment 2).

Herbicide treatment	Experiment 1		Experiment 2	
	Clethodim[a]	Glufosinate[b]	Clethodim[a]	Glufosinate[b]
	g ai ha^{-1}			
1	0	0	0	0
2	0.8	28	0.8	56
3	1.6	56	1.6	225
4	3.2	113	3.2	338
5	6.4	225	6.4	450
6	12.8	338	12.8	900
7	25.6	450	25.6	1,800
8	51.2	—	51.2	—

[a] Clethodim applied with liquid ammonium sulfate at 5% v/v and nonionic surfactant at 0.5% v/v.
[b] Glufosinate applied with liquid ammonium sulfate at 5% v/v.

$$Y = c + \{d - c/1 + \exp[b(\log(x) - \log(GR_{50}))]\} \quad [1]$$

$$Y = d/1 + \exp[b(\log x - \log(GR_{50}))] \quad [2]$$

In the four-parameter model (Equation 1), b is the slope of the curve, c is the lower limit, and d is the upper limit. In the three-parameter model (Equation 2), $c = 0$ and is not used, and the other variables are the same as in the four-parameter model. Growth reduction for dry weights were calculated as GR_{50} and GR_{90} values, indicating either a 50 or 90% decrease in the growth of plants when compared to the nontreated control, respectively. Two-tailed t-tests were performed to determine if the GR_{50} and GR_{90} values were significantly different between corn lines at $\alpha = 0.05$. For visual presentation, results of dry weights are expressed as a percent of the nontreated check. There was no interaction between experimental runs; therefore, data were pooled across runs.

Effect of Plant N Concentration on Clethodim and Glufosinate Dose-Response. Greenhouse experiments to evaluate corn response to clethodim and glufosinate under high and low N concentrations were performed on two corn hybrids and their progeny. Two corn seeds were planted 3.5 cm deep in 750 cm^3 Deepots containing baked montmorillonite clay (Turface MVP, Profile Products LLG, 750 Lake Cook Road,

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Table 2. Nutrient solution composition used to determine the effects of plant nitrogen (N) concentration on clethodim and glufosinate dose-response studies.

N[a] concentration	Ammonium sulfate	Potassium nitrate	Potassium chloride	Calcium sulfate	Calcium chloride
	mg L^{-1}				
1.0 mM	119.1	181.8	404.7	366.4	180.3
4.0 mM	476.2	727.3	—	—	478.3

[a] 1.0 mM N is equal to 50 mg N L^{-1} of solution and 4.0 mM N is equal to 200 mg N L^{-1} of solution.

Suite 440, Buffalo Grove, IL 60089) that was mixed with 0.36 g of dolomitic limestone prior to filling. Photoperiod and temperature were the same as in the previous experiment. After emergence, pots were thinned to a single corn plant per pot. Corn seedlings were watered with 50 ml of tap water in the morning and 50 ml of a nutrient solution in the evening. The nutrient solution alternated daily between an N solution and a non-N fertilizer solution. There were two N solutions, a low (1.0 mM N) and a high (4.0 mM N). The source and rates of the nutrients used for the N solutions are listed in Table 2. Calcium chloride, calcium sulfate, and potassium chloride were added at different rates to balance the calcium, potassium, and sulfate concentrations in the N solutions. The low N concentration was 50 mg N L^{-1} and the high N concentration was 200 mg N L^{-1}, while other nutrient concentrations in the N solutions were (L^{-1}): 172 mg Ca, 281 mg K, and 115 mg S. The non-N fertilizer (0-37-37) (0-37-37 Water Soluble Fertilizer, Grow More Inc., Gardena, CA 90247) solution was mixed at the concentration of 135 mg L^{-1}. Pots were flushed weekly with 250 ml water to wash out any accumulated nutrients.

The experimental design was a 2 by 4 by 7 and a 2 by 4 by 8 factorial randomized complete block design for glufosinate and clethodim, respectively, replicated three times and with two experimental runs. The glufosinate experiment was conducted from late October through November 2010, and the clethodim experiment was conducted in January and February 2011. Factor 1 was low (1.0 mM N) or high (4.0 mM N) N availability, factor 2 was assigned to one of the four corn lines (DKC 60-18, 63-42, 60-18F_2, and 63-42F_2), and factor 3 was the herbicide treatment (Table 1). Corn was at the V2 growth stage (10 to 15 cm) at the time of herbicide application, using the collar method (Ritchie et al. 1992). On the day of herbicide application, 10 plants from each N availability and each corn line were severed at the soil surface and dried at 30 to 38 C for at least 7 d. After the plants were dry, they were ground and analyzed for total Kjeldahl N (Issac and Johnson 1976). Herbicide treatment application, data collection, and data analysis were performed as described in the previous experiment except plants were collected at 10 DAT instead of 14 DAT. Dose-response models were constructed using Equation 2 as described earlier.

Corn hybrids and their F_2 progeny with the same N availability responded similarly to both herbicides. Therefore, data were pooled over corn lines for each N availability and herbicide treatment. Two-tailed *t*-tests were performed to determine if the GR_{50} and GR_{90} values were significantly different between N availability levels ($\alpha = 0.05$). Plant N concentration data were analyzed using ANOVA in SAS 9.2 (SAS Institute, Inc., Cary, NC 27513). The significance of N availability, corn line, and their interaction was tested. N availability was the only significant variable; therefore, data were combined across corn line. Means were separated using *t*-tests at $\alpha = 0.05$.

Results and Discussion

Clethodim Dose-Response. The GR_{50} and GR_{90} values for clethodim ranged from 2.6 to 3.9 and 11.7 to 18.1, respectively (Table 3). *T*-tests for all comparisons indicated two differences for the GR_{50} values and no differences for the GR_{90} values. When comparing GR_{50} values, 60-18 and 60-18F_2 were more susceptible to clethodim than 63-42. This difference observed in hybrid tolerance to clethodim supports previous research that reported differential tolerance to diclofop in F_2 corn populations (Andersen and Geadelmann 1982). However, when comparing 63-42 to 63-42F_2 and 60-18 to 60-18F_2 the GR_{50} values were not different (Table 3), indicating corn hybrids do not exhibit greater tolerance to clethodim than their F_2 progeny. GR_{90} values (11.7 to 18.1 g ai ha^{-1}) were below the labeled field use rate, 51 g ha^{-1}. Therefore, differences in hybrid tolerance may not occur at normal field use rates.

Glufosinate Dose-Response. In the glufosinate dose-response study, there were no differences in GR_{50} and GR_{90} values between corn hybrids or their F_2 progeny (Table 3). The

Table 3. Parameter estimates of dose-response curves for the four DeKalb corn lines in the clethodim and glufosinate dose-response studies. GR_{50} and GR_{90} represent herbicide dose required to cause 50 and 90% growth reduction, respectively. Numbers in parentheses are standard errors.

Herbicide	Corn line	Regression parameters[a]			GR_{50}	GR_{90}
		b	*c*	*d*	g ai ha^{-1}	
Clethodim	63-42	1.26	0.14	7.71	3.9 (0.4)	13.3 (2.9)
	63-42F_2	0.92	0.08	8.45	3.0 (0.5)	18.1 (5.0)
	60-18	1.08	0.24	8.12	2.8 (0.3)	11.7 (2.7)
	60-18F_2	1.01	0.28	8.03	2.6 (0.3)	12.6 (3.3)
Glufosinate	63-42	1.18	—	3.74	158 (11)	513 (50)
	63-42F_2	1.37	—	3.5	167 (12)	498 (49)
	60-18	1.31	—	3.52	172 (12)	519 (51)
	60-18F_2	1.52	—	3.23	186 (13)	494 (49)

[a] In the four-parameter logistic model used for clethodim, *b* is the slope of the curve, *c* is the lower limit, and *d* is the upper limit. In the three-parameter logistic model used for glufosinate, *b* is the slope of the curve and *d* is the upper limit.

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Table 4. Independent *t*-tests of GR_{50} and GR_{90} values depending on nitrogen rate based on dry weight for the effects of corn plant nitrogen concentration on clethodim and glufosinate dose-response studies. GR_{50} and GR_{90} represent herbicide dose required to cause 50 and 90% growth reduction, respectively. Numbers in parentheses are standard errors.

Nitrogen rate	% Nitrogen	$P > F$[a]	GR_{50}	$P > F$	GR_{90}	$P > F$
			g ai ha^{-1}		g ai ha^{-1}	
Clethodim						
1 mM	1.95 (0.13)	< **0.0001**	14.9 (2.9)	**0.02**	128.6 (53.5)	**0.02**
4 mM	2.78 (0.13)		7.9 (0.5)		48.7 (7.2)	
Glufosinate						
1 mM	2.22 (0.08)	< **0.0001**	612 (20)	**0.03**	1,727 (97)	**0.04**
4 mM	3.25 (0.08)		345 (4)		736 (24)	

[a] Independent *t* tests were used to determine significance between values at $\alpha = 0.05$. Significant values in bold.

GR_{90} values ranged from 494 to 519 g ai ha^{-1}, which are above the labeled field use rate of 450 g ha^{-1} in corn, but below the field use rate in soybean, 594 g ha^{-1}. Similar to the clethodim dose-response study, F_2 progeny did not respond differently to glufosinate than hybrids nor was there a difference in the response between hybrids. Our results are similar to those of Pornprom et al. (2003) who found no differences in glufosinate susceptibility among 10 North American corn hybrids. However, in this earlier reported study, two of the five hybrids locally adapted to Thailand exhibited a nearly twofold increase in tolerance to glufosinate when compared to the most susceptible hybrid. No explanation was provided on how germplasm origin affected response to glufosinate.

Effect of Plant N Concentration on Clethodim Dose-Response. At the time of herbicide application the N concentration of the low N corn plants was about 0.85% lower than that of the high N (Table 4). The low N corn expressed greater tolerance to clethodim than the high N corn when comparing GR_{50} and GR_{90} values (Table 4). The negative influence of low corn N concentration on clethodim efficacy is comparable to previous research by Dickson et al. (1990) that showed reduction in efficacy of fluazifop, another ACCase inhibiting herbicide, on tame oats grown in a low soil N environment. Dickson et al. (1990) attributed the reduction in tame oat control by fluazifop to be a result of reduced herbicide translocation, which may be the same reason for the reduced efficacy of clethodim when applied to N deficient corn. Low soil N availability reduced glyphosate efficacy on common lambsquarters (*Chenopodium album* L.) and velvetleaf (*Abutilon theophrasti* Medik.), a result of reduced carbon assimilation that subsequently reduced sugar export and glyphosate translocation (Mithila et al. 2008). Therefore, it is logical that low plant N concentration results in decreased clethodim translocation.

Effect of Plant N Concentration on Glufosinate Dose-Response. Corn plant N concentration in the low N availability was nearly 1% lower than that of the high N availability (Table 4). The low N corn GR_{50} and GR_{90} values were 1.8 and 3.3-fold greater than high N corn GR_{50} and GR_{90} values, respectively, indicating greater tolerance to glufosinate (Table 4). Redroot pigweed (*A. retroflexus* L.) growing in a low N soil showed a similar increased tolerance to glufosinate when compared to redroot pigweed growing in a high N soil (Cathcart et al. 2004).

It has not been fully addressed in the literature as to why plant N concentration impacts glufosinate efficacy, yet due to the mode of action of glufosinate it seems that there are two plausible explanations. Glufosinate inhibits glutamine synthetase resulting in ammonia accumulation in the leaf and disruption of photorespiration and photosynthesis (Tachibana and Kaneko 1986). Coetzer et al. (2001) observed that ammonia accumulation from an application of glufosinate may be responsible for photosynthetic inhibition and ultimately plant death. Glufosinate has little translocation (Bromilow et al. 1993; Steckel et al. 1997). However, glufosinate translocation was greater in sterile oat (*Avena sterilis* L.) than rigid ryegrass (*Lolium rigidum* Gaudin) and the increased translocation was the reported reason that control of sterile oat was greater than rigid ryegrass with glufosinate (Steckel et al. 1997). As mentioned earlier, a low soil N environment decreased glyphosate translocation (Mithila et al. 2008). Consequently, it is possible that glufosinate translocation would also be reduced. Therefore, reduced translocation of glufosinate may be the reason that N deficient corn plants were more tolerant. Secondly, low plant N concentrations may reduce ammonium accumulation lessening the degree by which photosynthesis is disrupted and ultimately diminishing efficacy. The increased tolerance observed between N deficient and N sufficient corn may help explain the inconsistent control of GR corn by glufosinate, especially in soybean fields where volunteer corn is likely N deficient. Steckel et al. (2009) reported that control of GR corn by glufosinate was undesirable in a corn replant situation. The glufosinate GR_{50} values were considerably different between experiments (170 g ai ha^{-1} vs. 345 g ai ha^{-1}), which supports the aforementioned variable control of corn with glufosinate. The observed variability between the experiments is likely due to environmental factors. Glufosinate efficacy on green foxtail [*Setaria viridis* (L.) Beauv.] and wild oat (*Avena fatua* L.) is significantly reduced by 40% relative humidity when compared to 95% relative humidity (Anderson et al. 1993; Ramsey et al. 2002). The first glufosinate experiment was conducted in late summer, whereas the second experiment was conducted in the late fall. The lower temperatures in the fall required supplemental heat from the greenhouse to achieve the desired temperature but the artificial heat lowered the relative humidity in the greenhouse. The reduction in relative humidity is likely the reason that the average GR_{50} value was nearly twofold in the second experiment when compared to the average in the first experiment.

The ability to consistently control GR corn, whether it is hybrid or F_2 corn, will continue to be an important issue in corn and soybean production. Our results indicate that a hybrid corn line and its F_2 progeny are equally susceptible to

clethodim and glufosinate. Further, this research suggests that both clethodim and glufosinate efficacy on corn are reduced when plants are N deficient. Therefore, it is essential that full clethodim and glufosinate rates are applied to N deficient corn to maximize efficacy.

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