Differential Responses to Preemergence and Postemergence Atrazine in Two Atrazine-Resistant Waterhemp Populations

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ABSTRACT

Waterhemp [Amaranthus tuberculatus (Moq) Sauer] is a difficult-to-control dicot weed in the United States. Atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] is commonly used for preemergence (PRE) and postemergence (POST) waterhemp control in maize (Zea mays L.). Previous research reported that atrazine metabolism via glutathione S-transferase (GST) activity contributes to atrazine POST resistance in two waterhemp populations from Illinois, designated MCR (McLean County, Illinois, resistant) and ACR (Adams County, Illinois, resistant). Objectives were to quantify responses of these populations to atrazine PRE and determine if the combination of a GST inhibitor and atrazine PRE or POST increases their control. Dose-response analyses indicated MCR was resistant to atrazine PRE relative to ACR or WCS (Wayne County sensitive; herbicide-sensitive population), despite MCR and ACR exhibiting equivalent levels of atrazine resistance POST. The ACR response to atrazine PRE (LD₅₀) was intermediate compared with MCR and WCS. Seedling survival of ACR was reduced by 4-chloro-7-nitrobenzofurazan (NBD-Cl; a GST inhibitor) and atrazine PRE more than atrazine PRE alone, but not in MCR. Atrazine following NBD-Cl applied POST inhibited seedling growth in ACR, but not in MCR. Enhanced atrazine activity with NBD-Cl further supports rapid metabolism via GSTs as the main atrazine-resistance mechanism in ACR. GST(s) that metabolize atrazine in MCR may not have been completely inhibited by NBD-Cl, indicating that similar yet distinct atrazine-resistance mechanisms exist in MCR compared to ACR. In conclusion, atrazine PRE (with or without NBD-Cl) still controls ACR when applied at typical field-use rates in maize, but the length of residual activity may be shorter than in sensitive populations.

Core Ideas

- Two waterhemp populations are resistant to atrazine POST due to increased metabolism by GST enzymes.
- Since atrazine can also be soil applied, quantifying the levels of resistance in these populations is important for weed management in maize.
- The MCR population was resistant to atrazine PRE and displayed a higher level of resistance to atrazine PRE than the ACR population.
- NBD-Cl, a metabolic inhibitor of GST enzymes, enhanced atrazine activity in the ACR population but not MCR.
- The length of residual activity of atrazine may be shorter for controlling these populations in maize compared with sensitive populations.

WATERHEMP is a difficult-to-control weed in maize, soybean [Glycine max (L.) Merr], and grain sorghum [Sorghum bicolor (L.) Moench] production, due to its competitive ability, outcrossing nature, high degree of genetic diversity, and resistance to multiple herbicide sites-of-action (Horak and Loughlin, 2000; Hager et al., 2002; Steckel and Sprague, 2004; Steckel, 2007; Heap, 2015). Atrazine is still commonly used in maize and grain sorghum for PRE or POST control of waterhemp (Anderson et al., 1996) and other annual dicot weeds, in spite of hundreds of cases of triazine resistance occurring within Amaranthus spp. worldwide (Heap, 2014, 2015). Previous research reported that elevated rates of atrazine metabolism via GST activity contribute to atrazine POST resistance within two waterhemp populations (Ma et al., 2013). The MCR and ACR are both resistant to atrazine POST, and their responses have been compared with an herbicide-sensitive population, WCS (from Wayne County, Illinois) (Patzoldt et al., 2005; Hausman et al., 2011; Ma et al., 2013).

Differences in PRE vs. POST activity of atrazine among several atrazine-resistant waterhemp populations identified from Illinois were noted in previous studies (Patzoldt et al., 2003, 2005). In particular, an atrazine-resistant waterhemp population from Illinois (different than MCR and ACR) was not resistant to atrazine PRE at a rate of 1.5 kg ha⁻¹ (Patzoldt et al., 2003). Moreover, the ACR and MCR populations were not controlled but responded equally to atrazine POST at the rate of 560 g ha⁻¹ (Hausman et al., 2011), and both populations demonstrated almost identical rates of atrazine metabolism (Ma et al., 2013) when ¹⁴C-atrazine was supplied using an excised-leaf assay (Ma et al., 2015).

In addition to waterhemp, other dicot weed populations (most notably Abluton theophrasti Medic.) have displayed non target-site resistance (NTSR) to atrazine POST (Anderson and Gronwald, 1987; Gronwald et al., 1989; Gray et al., 1996), and each has displayed enhanced rates of rapid metabolism via glutathione-atrazine conjugation (catalyzed by elevated GST activity) as the mechanism of resistance (Anderson and Gronwald, 1991; Gray et al., 1996). This is an important physiological factor to consider when studying NTSR mechanisms in weeds because GST enzymes are encoded by nuclear genes (Anderson and Gronwald, 1987; Patzoldt et al., 2003; Frova, 2006). In contrast, the target site for atrazine

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Abbreviations: ACR, Adams County resistant; GST, glutathione S-transferase; MCR, McLean County resistant; NDB-Cl, 4-chloro-7-nitrobenzofurazan; POST, postemergence; PRE, preemergence; WCS, Wayne County sensitive.

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(the D1 protein in photosystem II [PS II]) (Hess, 2000) is encoded by the plastidic gene \textit{psb}A (Hirschberg and McIntosh, 1983), which confers maternally-inherited, high-level atrazine resistance but also results in a significant fitness cost (Holt et al., 1993). Mechanisms that confer NTSR are becoming more common and problematic in grass and dicot weed populations throughout the world (Powles and Yu, 2010; Delye et al., 2011; Ma et al., 2013; Yu and Powles, 2014), potentially arising from the lack of a significant fitness cost to the plant.

Research conducted during the 1970s and 1980s directed at improving the POST activity of atrazine and control of grass weeds in maize (Thompson et al., 1971; Thompson, 1972) involved the use of herbicide synergism (Boydston and Slife, 1986). One example of utilizing an herbicide synergist is tridiphane, a GST substrate and inhibitor, to increase atrazine POST activity for enhanced control of giant foxtail (\textit{Setaria faberi} L.) in maize (Boydston and Slife, 1986). This tank-mix combination increased the margin of selectivity between grass weeds and maize when grass seedlings were approximately 2 to 7 cm tall (Boydston and Slife, 1986). The underlying mechanism for achieving selective POST synergism in \textit{Setaria} spp. is that tridiphane and the tridiphane-glutathione conjugate are potent inhibitors of GST enzymes that metabolize atrazine in \textit{Setaria} spp. and maize. However, maize has higher total levels of GST activity that detoxify atrazine in leaves and a decreased sensitivity to inhibition by the tridiphane–glutathione conjugate compared to giant foxtail (Lamoureux and Rusness, 1986). One drawback to this strategy was that \textit{Setaria} seedlings reaching heights of 8 to 10 cm or more were typically not effectively controlled by the tridiphane–atrazine POST tank mix (Boydston and Slife, 1986; Barrett, 1997). The synergistic activity of tridiphane–atrazine tank mixes was used to improve POST control of grass weeds. However, this tank mix was not examined for synergistic PRE activity in grasses or dicots (L.M. Wax, personal communication, 2015).

Tank mixing 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides with atrazine may also result in a synergistic response. Increased POST activity in \textit{Amaranthus} and \textit{Ambrosia} occurred when mesotrione (or other HPPD inhibitors) was tank mixed with atrazine or bromoxynil (Abendroth et al., 2006; Hugie et al., 2008; Woodyard et al., 2009a). In atrazine-resistant \textit{A. retroflexus} (possessing an insensitive D1 protein) (Hugie et al., 2008), PRE atrazine followed by POST mesotrione treatments demonstrated a synergistic response, whereas in atrazine-resistant \textit{A. theophrasti} possessing enhanced GST-mediated metabolism of atrazine, only the POST tank-mix displayed a synergistic response (Woodyard et al., 2009b). However, recent discoveries of mesotrione- and atrazine-resistant waterhemp populations, such as the MCR population from Illinois (Hausman et al., 2011, 2016) and a waterhemp population from Iowa (McMullan and Green, 2011), may diminish the ability to achieve POST synergism with HPPD inhibitor-atriazine combinations and, consequently, limit effective POST control options for multiple herbicide-resistant (MHR) waterhemp in maize.

More recently, another inhibitor of GST activity, 4-chloro-7-nitrobenzofurazan (NBD-Cl), as well as numerous synthetic analogs of NBD-Cl, were tested for their ability to increase the POST activity of the acetyl-CoA carboxylase inhibitor fenoxaprop-\textit{P}-ethyl and the PS II inhibitor chlorotoluron in MHR blackgrass (\textit{Alopecurus myosuroides} Huds.) populations from Europe (Cummins et al., 2013). Results from this study indicated NBD-Cl effectively increased the activity of POST herbicides that are rapidly metabolized by GST enzymes as the main mechanism conferring resistance in \textit{A. myosuroides} (Cummins et al., 2013), which could also provide a potential new option for regaining atrazine POST activity in MCR, ACR, and other MHR waterhemp populations. However, use of NBD-Cl to increase atrazine POST activity or to enhance the activity of PRE residual herbicides metabolized by GSTs has not been reported.

Since responses of MCR and ACR to soil-applied atrazine are unknown and MCR is HPPD- and atrazine-resistant due to rapid metabolism, therefore limiting the ability to utilize HPPD-atriazine synergism for its control (Hausman et al., 2011, 2016), the specific objectives of this research were to (i) quantify the responses of the Illinois waterhemp populations MCR and ACR to atrazine PRE, and (ii) determine if the combination of the GST inhibitor NBD-Cl and atrazine can increase control of MCR or ACR seedlings PRE or POST.

**MATERIALS AND METHODS**

**Atrazine Preemergence Dose-Response Analysis**

All atrazine PRE and POST studies were conducted in the greenhouse under identical conditions. Greenhouse conditions were 28/22°C day/night with a 16:8 h photoperiod. Natural sunlight was supplemented with mercury halide lamps to provide 800 µmol m$^{-2}$ s$^{-1}$ photon flux at the plant canopy level. For the atrazine PRE study, seeds were planted using the same experimental design and watering regime as reported previously for quantifying mesotrione PRE activity in MCR (Hausman et al., 2013). In summary, plastic pots (720 cm$^3$) were filled to the top with growth medium (1:1:1 mixture of soil, peat, and sand, with a pH of 6.8 and 3.5% organic matter) then tamped to produce a smooth and level planting surface. The perforated pots were allowed to soak in water for 12 h to ensure uniform moisture distribution. After soaking, 25 seeds from one of three waterhemp populations (MCR, ACR, or WCS) were sown on the surface in a 5 by 5 grid with a 1 cm spacing between the pot sides and the seeds of outside rows at a depth of 0.7 cm. The MCR and ACR seeds were from the original populations collected in the field, and the WCS population consisted of bulk seed that had been purified through several generations and confirmed to be uniformly herbicide sensitive (Hausman et al., 2011). After sowing, an additional 50 mL of the same growth medium was passed through a 3.35 mm testing sieve, spread evenly across the top, and tamped down to produce a flat surface. Pots were then surface watered using a 1.9 L min$^{-1}$ misting nozzle until the medium surface was moist to the touch. Atrazine was applied using a compressed air research sprayer (DeVries Manufacturing, Hollandale, MN 56045) fitted with a TecJet 80015 EV5 nozzle positioned 46 cm above the soil medium surface. The sprayer was calibrated to deliver 185 L ha$^{-1}$ at 275 kPa. Atrazine was applied PRE at increasing rates equally spaced along a base 3.16 logarithmic scale. The rate range of atrazine for the WCS population was 2.2 to 708 g ha$^{-1}$, 22.4...
to 2240 g ha\(^{-1}\) for ACR, and 71 to 7083 g ha\(^{-1}\) for MCR. The maximum field-use rate of atrazine PRE in maize is 2.2 kg ha\(^{-1}\).

The number of survivors were recorded at 14 d after treatment (DAT) with atrazine and converted to a percentage of the untreated controls to determine herbicide rates that decreased plant survival by 50% (LD\(_{50}\)) and 90% (LD\(_{90}\)).

**Effect of the GST Inhibitor NBD-Cl on Atrazine Activity Preemergence and Postemergence**

To determine the effect of NBD-Cl on atrazine activity PRE, ACR, and MCR seeds were planted as described above for the atrazine dose-response PRE study. The NBD-Cl was applied first at either 0 g ha\(^{-1}\) (control) or 270 g ha\(^{-1}\), and then atrazine was applied at either 0 g ha\(^{-1}\) (control) or 224 g ha\(^{-1}\) 2 d after the NBD-Cl application. This rate of atrazine PRE caused approximately 10 and 30% reductions in seedling survival in MCR and ACR, respectively, as determined in preliminary greenhouse experiments. Although WCS was completely controlled at 224 g ha\(^{-1}\), this rate was chosen to investigate the activity of atrazine with NBD-Cl in MCR and ACR.

To determine the effect of NBD-Cl on POST atrazine activity, ACR and MCR seeds were suspended in liquid agar and planted as previously described (Ma et al., 2013). In brief, seeds of all three waterhemp populations were germinated in 12 by 12 cm trays with a commercial potting medium (Sun Gro Horticulture, Bellevue, WA) in the greenhouse. Emerged seedlings (2 cm tall) were then transplanted into 80 cm\(^3\) pots in the greenhouse. When the seedlings were 4 cm tall they were transplanted into 950 cm\(^3\) pots containing a 3:1:1:1 mixture of commercial potting medium:soil:peat:sand. Slow-release fertilizer (Nutricote, Agrivert Inc., Webster, TX) was added to this mixture (5 g pot\(^{-1}\)). When ACR and MCR plants were 8 to 10 cm tall (seven to nine true leaves), they were first treated with NBD-Cl at either 0 g ha\(^{-1}\) (control) or 270 g ha\(^{-1}\) plus crop oil concentrate (COC) at 1% v/v, then atrazine was applied at either 0 g ha\(^{-1}\) (control) or 3360 g ha\(^{-1}\) plus COC at 1% 2 d after the NBD-Cl application using the spray methods described above. Plants were 10 to 12 cm tall (8 to 10 true leaves) at the time of atrazine application. This rate of atrazine POST caused an approximate 5 to 10% reduction in dry weight biomass in both ACR and MCR, as determined in preliminary greenhouse experiments. All aboveground plant tissues were harvested at 14 d after atrazine treatment (16 d after NBD-Cl), and dry weights were recorded and converted to a percentage of the untreated control to determine percent biomass reductions.

The rate of NBD-Cl (270 g ha\(^{-1}\)) chosen for whole-plant studies with atrazine was based on a rate that increased the POST activity of chlorotoluron and fenoxaprop-P-ethyl in MHR. A. myosuroides populations (Cummins et al., 2013). Fenoxaprop-P-ethyl and chlorotoluron are herbicide substrates for GST-mediated metabolism in naturally-tolerant cereal crops and NTSR grass populations (Tal et al., 1993; Reade et al., 2004; Cummins et al., 2013). Additionally, preliminary greenhouse trials demonstrated that NBD-Cl at 270 g ha\(^{-1}\) exhibited an optimal increase in atrazine activity (PRE and POST) without displaying visual injury symptoms or secondary phytotoxic effects (data not shown).

**Experimental Design, Replication, and Statistical Analyses**

All experiments were conducted within a randomized complete block design. For the atrazine PRE dose-response study, two independent studies were conducted with five and four replications, respectively. Levene’s test for homogeneity of variance was not significant; as a result, the data from both studies (nine replications in total) were pooled and analyzed by nonlinear least squares regression to estimate LD\(_{50}\) and LD\(_{90}\) values, using the dose-response curve package in R (Knezevic et al., 2007). The dose-response model utilized for the atrazine PRE study was the same as used previously to examine MCR resistance to mesotrione PRE and POST (Hausman et al., 2011, 2013).

In each atrazine PRE and POST study, which independently investigated the effects of NBD-Cl, three individual experiments were conducted with three replications per treatment per study. Levene’s test for homogeneity of variance was not significant for either study, so the data generated from each PRE and POST study (nine replications in total per study) were pooled and subjected to ANOVA. Data were compared using Proc GLM (SAS version 9.2) and mean separation was conducted using Fisher’s LSD (0.05).

**RESULTS**

**Differential Responses to Atrazine Preemergence in Three Waterhemp Populations**

Dose-response analysis indicated that MCR was resistant to atrazine PRE and displayed qualitatively different responses to atrazine PRE than ACR at rates above 70.8 g ha\(^{-1}\) (Fig. 1). These observations are quantitatively supported by LD\(_{50}\) and LD\(_{90}\) values determined for each resistant population (Fig. 2), where MCR is 3.6-fold resistant compared to ACR at both the LD\(_{50}\) and LD\(_{90}\) rates. The ACR was completely killed by atrazine PRE at the maximum field-use rate in maize of 2240 g ha\(^{-1}\) (Fig. 1 and 2). The MCR displayed several surviving seedlings at 2240 g ha\(^{-1}\) (Fig. 1) that produced viable seeds under greenhouse conditions (data not shown) and the estimated LD\(_{90}\) for MCR is about 3.9 kg ha\(^{-1}\) (Fig. 2), well above the maximum field-use rate. By contrast, WCS was sensitive to each atrazine rate examined and all seedlings were killed at a rate of 70.8 g ha\(^{-1}\). Although both MCR and ACR are resistant to atrazine POST (Hausman et al., 2011), our results indicate that the MCR population is resistant to atrazine PRE relative to ACR and WCS (Fig. 2). Moreover, ACR is less sensitive to atrazine PRE relative to WCS (Fig. 1), as determined by estimated ratios of 9.4-fold and 23-fold at the LD\(_{50}\) and LD\(_{90}\) rates (Fig. 2).

**Atrazine Preemergence Activity with the GST Inhibitor NBD-Cl**

Atrazine applied PRE significantly inhibited seedling survival of ACR compared to the treatment with only NBD-Cl (Fig. 3), and soil treated with the sequential application containing NBD-Cl (applied 2 d before atrazine) had significantly fewer seedlings at 14 DAT compared to atrazine PRE alone (Fig. 3). Conversely, when compared to the NBD-Cl only control, MCR did not display a significant reduction in seedling mortality from either atrazine PRE alone.
or the combination treatment of NBD-Cl and atrazine PRE (Fig. 3). The atrazine-sensitive WCS population was completely controlled by atrazine; therefore treatments with or without NBD-Cl are not shown.

The enhanced activity of atrazine PRE in ACR when combined with NBD-Cl (Fig. 3) is similar to results reported previously for chlorotoluron and fenoxaprop-P-ethyl applied POST in MHR *A. myosuroides* populations (Cummins et al., 2013). However, as mentioned previously, the research reported by Cummins et al. (2013) did not investigate NBD-Cl applied before or in combination with PRE residual herbicides with grass or dicot activity (Cummins et al., 2013).

**Atrazine Postemergence Activity with the GST Inhibitor NBD-Cl**

Enhanced atrazine activity following NBD-Cl POST was detected in ACR seedlings, but neither NBD-Cl nor atrazine caused a significant decrease in biomass accumulation (Fig. 4). However, results with MCR were similar to those demonstrated by the PRE study (Fig. 3) in that MCR did not display a significant effect from either atrazine PRE alone or NBD-Cl followed by atrazine PRE when compared to the NBD-Cl only treatment (Fig. 4). As in the PRE study (Fig. 3), the atrazine-sensitive WCS population was severely injured by atrazine and did not exhibit a significant difference between treatments (data not shown). As was revealed by the atrazine PRE study, the POST results with ACR (Fig. 4) are in accord with those reported previously with NBD-Cl and several POST herbicides in MHR *A. myosuroides* populations (Cummins et al., 2013).

![Fig. 1. Dose-response study of atrazine preemergence (PRE) activity on McLean County resistant (MCR) (atrazine-resistant), Adams County resistant (ACR) (atrazine postemergence [POST]-resistant), and Wayne County sensitive (WCS) (atrazine-sensitive) at 14 d after treatment (DAT). The maximum-labeled rate for an atrazine PRE application in maize (2240 g ha⁻¹) is outlined by the red box.](image1)

![Fig. 2. Dose-response analysis (LD₅₀ and LD₉₀) of atrazine preemergence (PRE) activity on McLean County resistant (MCR) (atrazine-resistant), Adams County resistant (ACR) (atrazine postemergence [POST]-resistant), and Wayne County sensitive (WCS) (atrazine-sensitive) at 14 d after treatment (DAT). The atrazine rate of 1200 g ha⁻¹ applied to MCR and ACR in this figure is not shown in Fig. 1.](image2)

![Fig. 3. Inhibitory effects of 4-chloro-7-nitrobenzofurazan (NBD-Cl) (270 g ha⁻¹) preemergence (PRE) and/or atrazine (224 g ha⁻¹) PRE on Adams County resistant (ACR) and McLean County resistant (MCR) seedling survival at 14 d after atrazine treatment. The combination treatment consisted of a PRE application of NBD-Cl followed by atrazine 2 d later, but before seedling emergence. Wayne County sensitive (WCS) is not shown because it was completely controlled by atrazine alone, and did not exhibit a significant difference between treatments with or without NBD-Cl. Error bars represent the standard error of the mean.](image3)
**DISCUSSION**

Although the MCR and ACR waterhemp populations are equally resistant to atrazine POST applications (Hausman et al., 2011), our findings demonstrate significantly different levels of sensitivity to atrazine PRE (Fig. 1 and 2). Variability in atrazine POST sensitivity was documented among dozens of waterhemp populations randomly sampled from Illinois (Patzoldt et al., 2002), but this research did not investigate atrazine PRE activity. Since atrazine possesses a high level of activity on dicot weeds PRE and POST, a major objective of our research was to determine if MCR and ACR were only resistant to atrazine POST, as reported previously for waterhemp in Illinois (Patzoldt et al., 2003), or if either or both populations were also resistant to atrazine PRE.

Several seedlings from the MCR population survived the maximum field-use rate of atrazine PRE (2240 g ha\(^{-1}\)), whereas 708 g ha\(^{-1}\) atrazine PRE completely killed the ACR and WCS populations (Fig. 1). When comparing estimated LD\(_{90}\) values, MCR is 3.6-fold resistant compared to ACR and 34-fold resistant when compared to WCS (Fig. 2). The MCR seedlings (10–15 cm tall) can survive atrazine POST rates of greater than 35 kg ha\(^{-1}\) (C.M. Evans, personal communication, 2015) suggesting that MCR is more resistant to atrazine POST than PRE, similar to the phenotype of an atrazine-resistant *A. theophrasti* population from Wisconsin (Gray et al., 1995; 1996). Several MCR seedlings survived atrazine PRE at the maximum-labeled rate (Fig. 1) and are therefore resistant. This statement is substantiated by the estimated LD\(_{90}\) value of 3.9 kg ha\(^{-1}\) for MCR (Fig. 2), which is well above the maximum field-use rate of 2.2 kg ha\(^{-1}\) PRE. An additional negative consequence of the NTSR mechanism in MCR (Ma et al., 2013) is that the effective length of soil-residual activity resulting from atrazine PRE treatments in the field will likely be significantly reduced (relative to atrazine-sensitive populations) as atrazine dissipates over time (Accinelli et al., 2001; Hausman et al., 2013). This situation is similar to soils that exhibit enhanced atrazine degradation. After repeated applications, soil microorganisms can metabolize and accelerate atrazine degradation, which reduces the period of effective weed control (Zablotowicz et al., 2006; Shaner and Hager, 2014).

The enhanced activity of atrazine with NBD-Cl in ACR (Fig. 3 and 4) strengthens the conclusion that GST-mediated metabolism is the main atrazine-resistance mechanism in ACR (Ma et al., 2013). NBD-Cl and other thiocarbamide derivatives of NBD are potent inhibitors of human GSTs by acting as “suicide substrates” (Ricci et al., 2005). The mechanism of GST inhibition in humans involves GST-catalyzed complexation of the NBD moiety with reduced glutathione, which then tightly binds to the GST active site and therefore blocks its metabolic activities in cancerous tumor cells (Ricci et al., 2005). The precise mechanism of GST inhibition by NBD-Cl, which likely resulted in the enhanced herbicide activity in grasses (Cummins et al., 2013) and waterhemp in our study (Fig. 3 and 4), remains to be determined. However, these results indicate a possible new avenue of research for regaining herbicide activity in resistant weeds by designing, synthesizing, and testing compounds that block plant GST activities with the ability to detoxify herbicide substrates. Furthermore, atrazine PRE (with or without NBD-Cl) at labeled rates could still be used in integrated weed management programs for controlling the ACR population in maize, although the length of residual activity may be reduced. By contrast, NBD-Cl plus atrazine POST would not likely be effective in controlling ACR, despite the detection of a significant increase in atrazine POST activity (Fig. 4), because the relatively high rate of atrazine used in our POST study (3360 g ha\(^{-1}\)) is not labeled for use in maize and the rate combination in our research (Fig. 4) only provided about 40% reduction in aboveground biomass.

In contrast to the high probability of NBD-Cl inhibiting GSTs in ACR, GST(s) in MCR may not have been completely inhibited by NBD-Cl at 270 g ha\(^{-1}\) PRE or POST. However, this hypothesis remains to be experimentally investigated with a range of NBD-Cl rates. The different response of MCR to NBD-Cl plus atrazine PRE and POST relative to ACR may indicate the presence of unique GST isozyme(s) in MCR, higher constitutive GST expression in MCR, or that GST activity in ACR roots is lower than in ACR leaves and MCR roots. Alternatively, since the biological half-life of NBD-Cl in waterhemp tissues is not known, it may be possible that NBD-Cl is more rapidly degraded in MCR than in ACR. Studying the expression patterns (Cummins et al., 2013; Li et al., 2013), specific activity (Gronwald et al., 1989; Anderson and Gronwald, 1991; Giménez-Espinosa et al., 1996; Gray et al., 1996), and potential inducibility (Jachetta and Radosoveich, 1981; Dean et al., 1991; Woodyard et al., 2009b) of GST(s) that metabolize atrazine in ACR and MCR leaves, stems, and roots may assist in understanding the physiological basis for different levels of sensitivity to atrazine PRE between ACR and MCR. Along these lines, current research in our laboratory is investigating the hypothesis that similar but distinct GST-based NTSR mechanisms for atrazine occur in these two waterhemp populations (Evans et al., 2013).

![Fig. 4. Influence of 4-chloro-7-nitrobenzofurazan (NBD-Cl) (270 g ha\(^{-1}\)) postemergence (POST) and/or atrazine (3360 g ha\(^{-1}\)) POST on Adams County resistant (ACR) and McLean County resistant (MCR) dry weight accumulation at 14 d after atrazine treatment. The combination treatment consisted of a POST application of NBD-Cl followed by atrazine 2 d later. All treatments contained 1% (v/v) crop oil concentrate (COC) as adjuvant. ACR and MCR plants were 8 to 10 cm tall at the time of NBD-Cl application and 10 to 12 cm tall at the time of atrazine application. Wayne County (WCS) is not depicted because it was severely injured by atrazine alone, and did not exhibit a significant difference between treatments with or without NBD-Cl. Error bars represent the standard error of the mean.](image-url)
CONCLUSIONS

The NTSR and MHR mechanisms in grass (Reade et al., 2004; Délye et al., 2011; Cummins et al., 2013) and dicot (Patzoldt et al., 2005; Ma et al., 2013; Scarabel et al., 2015) weeds are providing complex new challenges for weed management and crop production by limiting effective herbicide options available (Preston, 2004; Yu and Powles, 2014). In addition to understanding cross-resistance patterns, genetic and biochemical mechanisms, and the molecular basis for NTSR and MHR in weeds, novel management tools are needed to combat herbicide-resistant weeds or overcome the mechanism(s) that confer resistance to both PRE and POST herbicides (Preston, 2004). Long-term, diverse strategies could therefore implement utilization of herbicide synergism with metabolic inhibitors (Barrett, 1997; Ma et al., 2013), herbicides with new target sites (if and when they are discovered) (Duke, 2012), herbicide tank mixes with synergistic or complementary activity (Woodyard et al., 2009a; Evans et al., 2016), tillage, and cultural practices in an integrated manner to manage herbicide-resistant weeds.

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