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Defining the Rate Requirements for Synergism between Mesotrione and Atrazine in Redroot Pigweed (*Amaranthus retroflexus*)

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Joint action of the effects of atrazine and mesotrione can lead to synergistic herbicidal activity in broadleaf weed species. The objective of these experiments was to determine if specific rates are required to provide synergistic joint activity between mesotrione and atrazine in both triazine-sensitive (TS) and triazine-resistant (TR) redroot pigweed. Herbicide rates were evaluated in TS and TR redroot pigweed in two experiments: a dose response of mesotrione alone and in mixture with a constant rate of atrazine and a dose response of atrazine alone and in mixture with a constant rate of mesotrione. Results from these experiments revealed that synergism was detected in the TS pigweed when 56 g ai ha^{-1} mesotrione was mixed with 126 g ai ha^{-1} atrazine. In the TR pigweed, synergism was detected when mesotrione rate at 10 to 56 g ha^{-1} was mixed with a constant rate of atrazine at 126 g ha^{-1}. Additionally, when mesotrione was held constant at 10 g ha^{-1}, synergism was detected in mixture with atrazine from 31 to 3556 g ha^{-1} in TR pigweed. Furthermore, in TR pigweed, analysis of slope deviation across the dose-response curves of mesotrione with and without atrazine revealed a divergence that increased in magnitude as the rate of mesotrione increased. In other words, increased synergism was observed with increased mesotrione rate in the TR pigweed, which was also supported by biomass reduction and atrazine-like injury to the leaves. An additional experiment investigated synergism between mesotrione and bromoxynil in both TS and TR pigweed. Mesotrione at 10 g ha^{-1} was synergistic when paired with bromoxynil from 70 to 210 g ha^{-1} in both the TS and the TR pigweed.

Nomenclature: Atrazine; bromoxynil; mesotrione; redroot pigweed, *Amaranthus retroflexus* L. AMARE.
Key words: Dose response, herbicide joint action, HPPD inhibitor, photosystem II inhibitor, triazine resistance.

Previous research has shown synergistic activity of a mixture of mesotrione and photosystem II inhibitors, where herbicidal activity observed in the target plant is greater than the expected sum activity (Abendroth et al. 2006; Sutton et al. 2002). Applications of specific rates of mesotrione mixed with atrazine have demonstrated synergism on triazine-sensitive (TS) and triazine-resistant (TR) biotypes of redroot pigweed as well as several additional weed species (Sutton et al. 2002).

Synergistic herbicidal activity has the potential to reduce cost and the amount of pesticides entering the environment (Kudsk and Mathiassen 2004; Streibig and Jensen 2000). Furthermore, the application of herbicides with independent modes of action in combination (tank-mix) rather than in rotation has the potential to significantly delay the onset of herbicide resistance (Diggle et al. 2003). Therefore, using a combination of mesotrione and atrazine could increase the longevity of mesotrione use by reducing the likelihood of populations evolving resistance to 4-hydroxy-phenylpyruvate dioxygenase (HPPD) inhibitors, provide a valuable weed management tool for controlling TR weeds, and lessen rates at which the herbicides must be applied through gaining higher activity per gram of active ingredient.

Currently, there are over 300 independently documented cases of unique herbicide resistance in 200 plant species in over 280,000 locations (Heap 2007). Among these, there are over 100 cases of resistance in the *Amaranthus* spp., including multiple herbicide resistance in individual *Amaranthus* plants as well as other species (Burnet et al. 1991; Foes et al. 1998; Patzoldt et al. 2005). Widespread herbicide resistance in weeds has created great interest in the development of herbicides with novel target sites, particularly herbicides developed from natural plant products (Duke et al. 2000). The triketone herbicides, including mesotrione, were developed through the optimization of leptospermone, a natural plant product produced by the bottlebrush plant (*Callistemon citrinus* Stapf.) that exhibits herbicidal activity (Lee et al. 1998; Mitchell et al. 2001). More importantly, there are no reports of naturally occurring herbicide resistance to mesotrione or other triketone herbicides (Heap 2007). Recently, mesotrione has been used as an effective tank-mix partner with preemergence or postemergence applications in maize for controlling broadleaf weeds and grasses (Mitchell et al. 2001).

The assessment of joint activity of herbicides with independent modes of action is most commonly calculated using variants of the Multiplicative Survival Model (MSM) (Flint et al. 1988; Green et al. 1997; Kelly and Chapman 1995; Streibig et al. 1998), where the MSM typically utilizes Colby's equation for herbicide joint action through analysis of various quantitative observations, including plant biomass (fresh or dry weight), growth suppression, or percent survival (Gowing 1960; Colby 1967). Colby's equation is expressed as such:

$$E = (XY)/100 \qquad [1]$$

where values are expressed as growth (or dry weights) as a percent of control; X and Y = growth with herbicide A and B, respectively, applied at a specific rates; and E = expected growth with application of herbicides A + B in mixture.

Although Colby's equation is a commonly used method for calculating herbicide joint activity within the MSM, a statistical test for analyzing herbicide joint activity is still necessary. Among various statistical analysis methods for herbicide joint action, a model proposed by Flint et al. (1988) applies a statistical test to Colby's equation utilizing slope comparisons. Based on an analysis of variance (ANOVA) model for a factorial experiment, this method was designed for application to a wide range of growth data (Flint et al. 1988). Analysis of herbicide dose-response curves using a log-logistic model (Ritz and Streibig 2005; Seefeldt et al. 1995; Streibig and Jensen 2000) can also be used to supplement this statistical analysis. With regards to determining increased

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herbicidal activity when combining mesotrione and atrazine in our research, these experiments were designed to fit Flint's statistical method. In addition, dose-response data for the TR redroot pigweed further demonstrate the effect of a constant rate of atrazine in mixture with mesotrione.

Although previous studies have demonstrated that synergism can occur between mesotrione and photosystem II (PSII) inhibitors (Abendroth et al. 2006; Sutton et al. 2002), research reported in the literature has not explored herbicide joint action between these herbicides over a wide range of combined rates in PSII inhibitor-sensitive, -resistant, and naturally-tolerant weeds. Therefore, the objective of our research was to define the rate requirements for synergism between mesotrione and atrazine in both TS and site-of-action based TR redroot pigweed. In addition, joint activity of mesotrione combined with a different photosystem II inhibitor (bromoxynil) was also evaluated in TS and TR redroot pigweed because site of action-based triazine resistance confers negative cross-resistance (increased sensitivity) to bromoxynil, and the TS biotype has natural tolerance to bromoxynil (Corbett et al. 2004; Devine and Preston 2000).

Materials and Methods

TS (Missouri origin) and TR (Ohio origin) redroot pigweed seed were obtained through Herbiseed.[1] TR pigweed was confirmed to be resistant by modified site of action (Ser264 to Gly264) through PCR and DNA sequencing (data not shown). Seeds were sown in flats containing a 1:1:0.9 v/v/v mixture of sterilized Drummer silty clay loam (fine-silty, mixed, mesic Typic Haplaquoll):sand:peat, and germinated in a growth chamber set to temperatures of 28/22 C day/night with a 16 h photoperiod. Upon germination, plants were transferred to a greenhouse and transplanted into individual pots (10 by 10 by 12 cm square) containing soil mix supplemented with a slow-release fertilizer.[2] Plants were watered as needed. Greenhouse conditions were set to 33/20 $\pm$ 2 C day/night with a 16 h photoperiod. Natural light was supplemented with high intensity discharge metal halide lighting (800 μmol m^{-2} s^{-1} photon flux at plant canopy level).

Herbicide Treatments. *General Procedures*. Herbicide treatments were applied to redroot pigweed seedlings at 7- to 8-leaf stage (18 d after germination). Herbicides were applied using a track sprayer[3] fitted with a 80015EVS nozzle, delivering 190 L ha^{-1} at 210 kPa. Following herbicide application, plants were arranged in a completely randomized block design. There were 10 replications per treatment, and each experiment was conducted at least three times. Aboveground shoot biomass was harvested 14 d after treatment (DAT), dried at 60 C for 72 h, weighed, and recorded. Biomass data were then analyzed for evaluation of combined herbicide activity.

Data Analysis. Herbicide joint activity was assessed using a modification of Flint's method, where Colby's equation was modified to include a statistical test for herbicide joint action. Specifically, the slope of log-transformed data of a dose response of Herbicide A alone was compared with that obtained from Herbicide A paired with a constant rate of Herbicide B (Flint et al. 1988). These slope comparisons were used for data analysis of both TS and TR biotypes. To supplement joint-activity data, dose-response curves of mesotrione alone and paired with a constant rate of atrazine were analyzed for the TR pigweed only, using a log-logistic model to describe the nonlinear effects of the joint action of mesotrione and atrazine (Ritz and Streibig 2005; Seefeldt et al. 1995; Streibig and Jensen 2000). Data analysis for the joint activity between mesotrione and bromoxynil was performed using the same methodology as described above for the joint-action analysis between mesotrione and atrazine (Flint et al. 1988). Herbicide joint-action analysis was performed by a mixed procedure using SAS[4] software for slope comparisons (SAS 2004). Dose-response curve analysis was performed using the *drc* package of R,[5] a statistical program (R 2005).

Experiment 1—Mesotrione Dose-Response Mixed with GR_{50} Rate of Atrazine. Mesotrione[6] treatments ranged from 0.1 to 316 g ha^{-1}, and treatments were applied alone and in combination with a rate that reduces biomass by 50% (GR_{50} rate) of atrazine[7] held constant at 126 g ha^{-1}, including a treatment of atrazine alone at 126 g ha^{-1} and an adjuvant-only control. All treatments included crop oil concentrate[8] (COC) at 1% v/v and 28% urea ammonium nitrate[9] (UAN) at 2.5% v/v as adjuvants.

Experiment 2—Atrazine Dose-Response Mixed with GR_{50} Rate of Mesotrione. Atrazine treatments ranged from 0.03 to 3556 g ha^{-1}, and treatments were applied alone and in combination with a GR_{50} rate of mesotrione held constant at 10 g ha^{-1}, including a treatment of mesotrione alone at 10 g ha^{-1} and an adjuvant-only control. All treatments included COC at 1% v/v and UAN at 2.5% v/v as adjuvants.

Experiment 3—Mesotrione Mixed with Bromoxyni. Bromoxynil[10] was applied at rates below the field use rate of 280 g ha^{-1}, ranging from 70 to 210 g ha^{-1}. These rates of bromoxynil were applied alone and paired with mesotrione at a GR_{50} rate of 10 g ha^{-1}. For consistency in herbicide joint-action analysis, all treatments included COC at 1% v/v and 28% UAN at 2.5% v/v as adjuvants, although bromoxynil does not require adjuvants under field conditions.

Results and Discussion

Analysis of Herbicide Joint Activity between Mesotrione and Atrazine or Bromoxynil. *Triazine-Sensitive Redroot Pigweed*. In TS pigweed, synergism between mesotrione and atrazine occurred when 56 g ha^{-1} mesotrione was paired with 126 g ha^{-1} atrazine, or approximately a 1:2.25 ratio of mesotrione to atrazine, respectively (Table 1). The "Estimate" denoted in Table 1 is the change in slope between the mesotrione dose response and mesotrione in mixture with atrazine. For example, significant negative slope differences indicate synergism whereas significant positive slope differences indicate antagonism. Values that are not significant indicate additive herbicide joint action. As the rates of mesotrione increased from 3.2 to 56 g ha^{-1} with a constant rate of atrazine (126 g ha^{-1}), the level of significance indicative of synergism also increased (as determined by both lower P values and more negative estimates), suggesting that a certain threshold of mesotrione is required to provide a

Table 1. Herbicide joint activity of mesotrione and atrazine by herbicide rate in triazine-sensitive (TS) pigweed.

Herbicide rate (g ai ha^{-1})				
Mesotrione	Atrazine	P value	Estimate[a]	Joint activity
0.1	126[b]	0.94	−0.01	Additive
1	126	0.49	0.07	Additive
3.2	126	0.74	−0.04	Additive
10	126	0.52	−0.07	Additive
18	126	0.12	−0.17	Additive
56	126	0.02	−0.28	Synergistic
316	126	0.18	−0.15	Additive
10[c]	0.03	0.32	0.17	Additive
10	1	0.68	0.07	Additive
10	32	0.35	−0.16	Additive
10	100	0.85	−0.03	Additive
10	178	0.95	0.01	Additive
10	562	0.07	0.33	Additive
10	1,778	< 0.001	0.71	Antagonistic
10	3,556	< 0.001	0.72	Antagonistic

[a] Estimate, deviation of slope magnitude from parallel or assumed "additivity."
[b] Atrazine rate of 126 g ai ha^{-1} was determined to be a GR_{50} rate in the TS biotype.
[c] Mesotrione rate of 10 g ai ha^{-1} was determined to be a GR_{50} rate in the TS biotype.

synergistic joint activity with atrazine at 126 g ha^{-1} in TS redroot pigweed.

Although synergism was not detected as the rates of atrazine increased when paired with 10 g ha^{-1} of mesotrione (Table 1), the herbicide joint activity level of significance surrounding the treatment of 10 g ha^{-1} mesotrione and 32 g ha^{-1} atrazine implies that while these rates do not have significant slope changes, the negative slope differences suggest there is potential to reach synergism at this rate of atrazine. A rate of mesotrione above 10 g ha^{-1} might be required to achieve synergism with 32 g ha^{-1} of atrazine (as demonstrated by the requirement of 56 g ha^{-1} of mesotrione paired with atrazine at 126 g ha^{-1} in achieving synergism). Antagonism was detected at the highest rates of atrazine, 1,778 and 3,556 g ha^{-1}. However, Flint's statistical analysis is limited by single herbicide rates that achieve maximum biomass reduction (Flint et al. 1988). This results in a detection of a "false" antagonism at these high rates because it is no longer biologically achievable to obtain parallel slopes.

Triazine-Resistant Redroot Pigweed. Synergism of mesotrione and atrazine was more prominent in the TR pigweed than in the TS biotype. For example, treatments of mesotrione at 10, 18, and 56 g ha^{-1} combined with 126 g ha^{-1} atrazine were synergistic, representing a range of ratios from 1:13 to 1:2 g ha^{-1} of mesotrione to atrazine, respectively (Table 2). In the TR pigweed, the threshold for mesotrione necessary to achieve synergism with atrazine was 10 g ha^{-1} (Table 2), much lower than the threshold in the TS pigweed (Table 1). Furthermore, the magnitude of synergism increases as the rates of mesotrione increase relative to atrazine, as represented by larger differences in the slopes (Table 2).

When mesotrione was held constant at 10 g ha^{-1}, synergism was detected when combined with atrazine at 32 to 3,556 g ha^{-1}, representing a range of ratios of mesotrione to atrazine from 1:3 to 1:356 on a g ha^{-1} basis (Table 2). In the TR pigweed, rates of the herbicides in combination did not provide any "false" antagonism as detected in the TS biotype because injury in the TR pigweed did not surpass complete biomass reduction. As the rates of atrazine increased, P values remained relatively constant for rates detected as synergistic, ranging from 0.04 to < 0.0001, and estimates remained relatively constant as well, indicating that above 32 g ha^{-1}, additional atrazine does not increase the magnitude of the synergism (Table 2).

Based on the ratios of mesotrione to atrazine (on a g ha^{-1} basis) in treatments that provided synergism, these data suggest that not only is the dosage of each herbicide important for synergism to occur, but that there is a threshold for each herbicide and a ratio requirement of the two herbicides that must be met in order for synergism to occur. In this experiment, at least a 1:2 ratio (g ha^{-1}) of mesotrione to atrazine was necessary to achieve synergism in TR redroot pigweed (Table 2).

To further describe the synergism of mesotrione and atrazine in the TR pigweed, dose-response curves were analyzed for horizontal effects (Table 3), or the change in dosage to provide the same biomass reduction between a treatment of mesotrione alone and mesotrione combined with atrazine (Ritz and Streibig 2005). Because atrazine alone at 126 g ha^{-1} elicited neither biomass reduction nor visible injury to the TR pigweed, treatments of mesotrione and atrazine in combination were hypothesized to have the same effect as mesotrione alone. Analysis of mesotrione dose response alone vs. mesotrione mixed with atrazine demonstrate that as the estimates become more negative as mesotrione rate increases (Table 2), and dose-response curves also deviate by a greater magnitude (Table 3). These differences shown in Table 3 are exemplified by a steeper slope in the mesotrione plus atrazine dose-response curve (slope estimate, B, of 1.03) compared with a slope of only 0.86 in the mesotrione-alone dose-response curve. The estimates of the GR levels in Table 3 are expressed in g ha^{-1} mesotrione required to reach each GR level. These results show that when combined with atrazine, less mesotrione is required to reach the same level of injury as a treatment of mesotrione alone in a TR plant, demonstrating that atrazine is contributing towards biomass reduction in the TR biotype when paired with mesotrione. Furthermore, the difference between the dose-response curves continues to increase as mesotrione rate increases and the GR_{90} is approached. These data support the theory that mesotrione rate largely influences the herbicide joint activity with atrazine, and as rates of mesotrione increase, synergism

Table 2. Herbicide joint activity of mesotrione and atrazine by herbicide rate in triazine-resistant redroot pigweed.

Herbicide rate (g ai ha^{-1})				
Mesotrione	Atrazine	P value	Estimate[a]	Joint activity
0.1	126	0.39	0.07	Additive
1	126	0.30	0.08	Additive
3.2	126	0.06	−0.15	Additive
10	126	0.02	−0.20	Synergistic
18	126	< 0.001	−0.32	Synergistic
56	126	< 0.001	−0.45	Synergistic
316	126	0.20	−0.10	Additive
10	0.03	0.33	−0.13	Additive
10	1	0.56	−0.08	Additive
10	32	0.04	−0.28	Synergistic
10	100	< 0.001	−0.54	Synergistic
10	178	< 0.001	−0.56	Synergistic
10	562	0.001	−0.51	Synergistic
10	1,778	0.001	−0.48	Synergistic
10	3,556	< 0.001	−0.57	Synergistic

[a] Estimate, deviation of slope magnitude from parallel or assumed ''additivity.''

strengthens until complete biomass reduction is achieved, particularly in the TR biotype (Table 3).

Figure 1 illustrates a visual demonstration of the synergism between mesotrione and atrazine in the TR pigweed, where the untreated control (Figure 1a) and the atrazine-treated pigweed (Figure 1c) display no herbicide injury. When mesotrione is applied alone (Figure 1b), typical injury appears as bleaching of newly emerging leaf tissue. With the addition of atrazine at a rate that provides no injury alone (Figure 1c) to a mesotrione rate shown in Figure 1b, the resulting injury includes bleaching of new tissue and outer leaf margin necrosis (Figure 1d), typical of atrazine injury on a susceptible plant. These visual injury symptoms provide further verification that atrazine contributes towards biomass reduction and phytotoxicity when mixed with mesotrione in the TR biotype.

Joint Activity of Mesotrione and Bromoxynil. When mesotrione was mixed with bromoxynil, synergism was detected at all rate combinations in TS pigweed (Table 4). This demonstrates that although TS pigweed has some degree of tolerance to bromoxynil alone (Corbett et al. 2004), the mixture with mesotrione is sufficient to overcome natural tolerance and displays a synergistic joint action between these herbicides (Table 4).

In TR pigweed, mesotrione combined with bromoxynil exhibited significant synergism for all rate combinations tested. Although the TR pigweed was more sensitive to bromoxynil than the TS biotype (due to negative cross-resistance; Devine and Preston 2000), joint action of bromoxynil and mesotrione was not affected by variable bromoxynil activity in the TR vs. the TS biotype (Table 4).

In summary, mesotrione and atrazine displayed synergistic activity at one rate combination in TS pigweed and at a range of rates in TR pigweed. The rates that provide highly significant synergism in the TR pigweed do not always provide synergism in the TS biotype, and overall, higher rates of combined herbicides are required for synergism to occur in a TS biotype than in the TR biotype. These results also indicate a threshold requirement of mesotrione rate before synergism between mesotrione and atrazine is achieved. In addition to the threshold requirement, as mesotrione rates increase, synergism with atrazine becomes more prominent, especially in the TR pigweed (Table 2). Synergistic activity of mesotrione and atrazine in site of action-based TR pigweed

Table 3. Dose response parameters expressed as biomass (g) in triazine-resistant pigweed.

	Mesotrione only		Mesotrione + atrazine	
Parameter	Estimate[a]	SE	Estimate[a]	SE
D[b]	2.79	0.04	2.76	0.04
C[c]	0.33	0.1	0.35	0.06
B[d]	0.86	0.09	1.03	0.08
GR10[e]	1.22	0.29	0.86	0.15
GR20	3.15	0.48	1.9	0.24
GR30	5.91	0.69	3.21	0.33
GR40	9.9	1.1	4.94	0.45
GR50	15.9	2.06	7.33	0.66
GR60	25.52	4.15	10.89	1.08
GR70	42.76	8.88	16.75	1.95
GR80	80.25	21.52	28.33	4.11
GR90	206.9	75.44	62.46	12.18

[a] Estimate, value of listed parameters.
[b] D, upper limit (grams).
[c] C, lower limit (grams).
[d] B, slope of curve.
[e] GRx, mesotrione rate required to reduce x% biomass (g ai ha^{-1}).

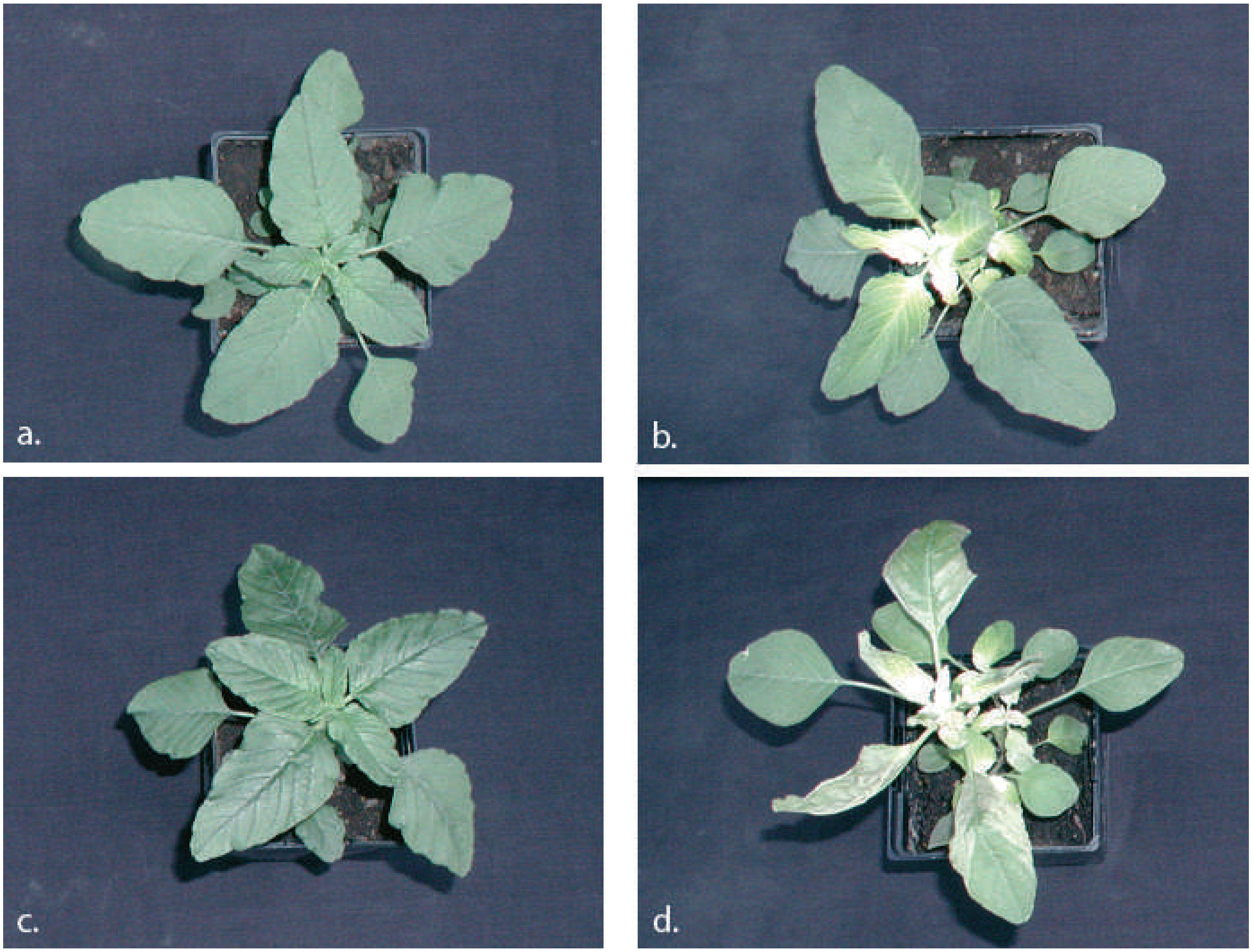

Figure 1. Triazine-resistant redroot pigweed 5 days after treatment of (a) adjuvants only (control), (b) mesotrione at 56 g ai ha^{-1}, (c) atrazine at 156 g ai ha^{-1}, and (d) mesotrione at 56 g ai ha^{-1}plus atrazine at 156 g ai ha^{-1}.

demonstrates that the addition of atrazine to mesotrione contributes significantly to biomass reduction. In addition to the biomass reduction, atrazine-like symptomology is evident when atrazine is combined with mesotrione in the TR biotype (Figure 1). Paradoxically, these results and visual observations demonstrate that atrazine is contributing to biomass reduction and phytotoxicity in a site of action-based TR plant, but only in the presence of mesotrione. This also implies that the biochemical overlap of the two herbicides potentially allows for atrazine binding at the D1 protein (i.e., reactivation of atrazine) in the TR pigweed.

TS pigweed is relatively tolerant to bromoxynil compared with other broadleaf weeds, but the site of action-based TR pigweed displays negative cross resistance to bromoxynil (Corbett et al. 2004; Devine and Preston 2000). As a result, our study examining the joint action of bromoxynil and mesotrione not only demonstrates synergism of mesotrione with a different PSII inhibitor, but also provides an example of synergism occurring in plants that are naturally tolerant to a PSII-inhibiting herbicide. Future mechanistic work will include chlorophyll a fluorescence measurement (in leaves of different maturities, under different light intensities, and in TS and TR biotypes), to investigate the separate and combined effects of atrazine and mesotrione on fluorescence induction curves (Zhu et al. 2005). In addition, greenhouse and field studies will further examine the joint action of

Table 4. Herbicide joint activity of mesotrione with bromoxynil by herbicide rate in redroot pigweed.

Herbicide rate (g ai ha^{-1})		Triazine-sensitive			Triazine-resistant		
Mesotrione	Bromoxynil	P value	Estimate[a]	Joint activity	P value	Estimate[a]	Joint activity
10	70	0.03	−0.40	Synergistic	0.04	−0.36	Synergistic
10	140	0.05	−0.35	Synergistic	0.03	−0.38	Synergistic
10	210	0.04	−0.38	Synergistic	0.01	−0.49	Synergistic

[a] Estimate, deviation of slope magnitude from parallel or assumed "additivity."

mesotrione and atrazine in nontarget site-based triazine-resistant weed biotypes, as well as whether the mesotrione-atrazine synergism occurs with sequential herbicide treatments.

Sources of Materials

[1] Herbiseed, New Farm, Mire Lane, West End, Twyford, RG10 0NJ, England.

[2] Osmocote 13:13:13 (N:P:K), The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041.

[3] Track sprayer: Allen Machine Works, 607 E. Miller Rd., Midland, MI 48640.

[4] R. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

[5] SAS; SAS Institute Inc., SAS Circle, Box 8000, Cary, NC 27512-8000.

[6] Mesotrione: Callisto®, Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC 27419.

[7] Atrazine: AAtrex Nine-O®, Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC 27419.

[8] COC: Herbimax®, Loveland Products, Inc., P.O. Box 1286, Greeley, CO 80632.

[9] UAN: N-Pak™ 28, Agriliance, P.O. Box 64089, St. Paul, MN 55164.

[10] Bromoxynil: Buctril® 4EC, Bayer CropScience, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

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