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Evaluation of the Interaction between Glyphosate and Glufosinate

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Crops transformed to provide resistance to herbicides with two different mechanisms of action provide new opportunities for control of herbicide-resistant weeds. However, unexpected interactions may develop, especially for herbicides not generally used in tank-mixtures. The objectives of this study were to evaluate weed control and determine herbicide interactions and fluorescence responses with combinations of glyphosate and glufosinate on selected weeds prevalent in Michigan cropping systems. Field studies to determine herbicide interactions resulted in synergism only at 0.84 kg ae ha^{-1} of glyphosate and 0.47 kg ai ha^{-1} glufosinate in 2008. Early synergism (7 d after treatment [DAT]) was observed in the field at several combined rates for common lambsquarters and velvetleaf in 2009, and in the greenhouse for giant foxtail. Differences between years were perhaps due to the effect of environmental conditions on herbicide absorption and translocation. Antagonism was observed in the field in 2009 for velvetleaf, common lambsquarters, and giant foxtail especially at 840 g ae ha^{-1} glyphosate and 118 g ai ha^{-1} glufosinate, 28 DAT. Antagonism was also observed in the greenhouse for giant foxtail and Canada thistle, 28 DAT. Fluorescence measurements on Canada thistle in the greenhouse showed that glufosinate and glufosinate plus glyphosate acted rapidly to quench electron transport of photosystem II (PS II) system of photosynthesis, and the fluorescence characteristics of the glyphosate and glufosinate combinations were indistinguishable from glufosinate alone.

Nomenclature: Glufosinate; glyphosate; Canada thistle, *Cirsium arvense* (L.) Scop.; common lambsquarters, *Chenopodium album* L.; giant foxtail, *Setaria faberi* Herrm.; velvetleaf, *Abutilon theophrasti* Medik.

Key words: Additive effect, antagonism, herbicide interaction, reduced rates, synergism.

Herbicides tank-mixtures may be used to prevent the development of herbicide-resistant weeds by attacking weeds at more than one lethal site of action. Tank-mixing has been shown to be more effective in reducing resistance evolution than using herbicides in a rotation (Hugh and Reboud 2009). However tank-mixtures may also result in unexpected interactions between herbicides, such as antagonism.

Interactions between glyphosate and glufosinate have been previously reported (Chuah et al. 2008; Kudsk and Mathiassen 2004). Antagonism was observed between these two herbicides in goosegrass [*Eleusine indica* (L.) Gaertn.] at all rates studied (Chuah et al. 2008). The antagonism was attributed to the fast action of glufosinate, which caused plant injury before the slower systemic glyphosate acted. Another study resulted in both antagonism and synergism between glyphosate and glufosinate (Kudsk and Mathiassen 2004).

Although little research to date has been published on the interaction of glyphosate and glufosinate, antagonism has been reported between glyphosate and other contact herbicides (Appleby and Somabhi 1978; Hayward et al. 1988; Hydrick and Shaw 1994; Lich et al. 1997; Wehtje et al. 2008). Glufosinate acts faster than glyphosate to injure the plant, much like diquat, a bipyridilium contact herbicide. In diquat plus glyphosate, early synergism was observed between the chemicals (4 DAT), but later antagonism was observed due to increased regrowth (Wehtje et al. 2008). Higher glyphosate rates were needed to compensate for the inhibition of glyphosate activity caused by the rapid plant death and retention of glyphosate in the treated leaf. An example of synergism of fast-acting herbicides is diuron and paraquat. Diuron quickly inhibits photosynthesis before paraquat can cause cell destruction and allows limited paraquat translocation to unsprayed portions of the plant (Hayward et al. 1988).

Glyphosate and glufosinate, although not primarily PS II inhibitors, ultimately cause cellular death resulting in a weakened ability of the treated plant to use or disperse light energy. Changes in fluorescence induction (Kautsky curve) have been used extensively in photosynthesis and herbicide research and are the basis for all fluorescence parameters (Abbaspoor and Streibig 2005; Christensen et al. 2003; Percival and Baker 1991). The benefits of using fluorescence include its noninvasiveness, sensitivity to many biotic and abiotic stressors, ease and efficiency, and numerous parameters to measure the status of the photosynthetic apparatus (Abbaspoor and Streibig 2005; Barbagallo et al. 2003; Frankart et al. 2003; Strasser et al. 2000). Illumination of dark-adapted unstressed leaves produces a rise in chlorophyll fluorescence emission from the ground state (F_o) to its maximum value (F_m) within 1 s. An important parameter used in fluorescence research is the Fv/Fm [Fv/Fm = (Fm − Fo)/Fm] parameter (Butler 1978). The dark adaptation of a leaf allows PS II to be fully reduced at the Q_A site on the electron transport chain and when illuminated the maximum quantum efficiency of the PS II photochemistry can be determined by Fv/Fm. This parameter is used most often in the literature to represent plant health with a value of 0.83 indicating no stress to the plant. Fv/Fm has been used to measure the effect of glyphosate on fluorescence in previous studies. Kirkwood et al. (2000) used this parameter and detected some differences from the nontreated control 1 DAT, while neither Olesen and Cedergreen (2010) nor Ralph (2000) found any effect of glyphosate on Fv/Fm.

The objectives of this research were to evaluate potential interactions among combinations of glyphosate and glufosinate in the field and greenhouse; determine if the interactions were antagonistic, synergistic or additive; and determine if herbicide interactions could be predicted by fluorescence measurements.

Materials and Methods

Field Studies. Field trials were conducted in 2008 and 2009 at the Michigan State University Agronomy Research Center

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(42°42′42″N, 84°28′13″W) in East Lansing, MI. The soil in 2009 was a sandy clay loam with 2.6% organic matter and a pH of 6.3. The soil in 2009 was a fine sandy loam with a pH of 6.9 and 2.5% organic matter. Fields preparation included fall-plowing followed by cultivation in the spring to obtain maximum weed emergence. The experiment was setup as a randomized complete block design with four replications. Treatments differed between years. In 2008, glyphosate (Roundup WeatherMax, Monsanto Co., St. Louis, MO) was applied at 0, 0.21 (0.25×), 0.28 (0.33×), 0.42 (0.5×), and 0.84 kg ae ha^{-1} (1×) alone and in combination with glufosinate (Ignite, Bayer CropScience, Research Triangle Park, NC) at 0.47 kg ai ha^{-1} (1×). In 2009, both glyphosate and glufosinate were applied at 0, 0.25×, 0.5×, and 1× rates alone and in combination with each other. All treatments, both years, included ammonium sulfate at 2.0% w/w.

Herbicide applications were made when the average weed height was 15 cm using a tractor-mounted compressed-air sprayer calibrated to deliver 180 L ha^{-1} at 210 kPa through AirMix 11003 nozzles (AirMix 11003, Greenleaf Technologies, Covington, LA). Common lambsquarters, velvetleaf, and giant foxtail were the predominant weed species in both years and were the focus in this study.

Weed control was evaluated 7, 14, and 21 DAT on a scale of 0% (no control) to 100% (complete control).

Greenhouse Studies. *Plant Material*. All plants were grown in a greenhouse in May 2009. Giant foxtail and velvetleaf from seed were grown in 9-cm pots containing a commercial potting medium (Baccto® High Porosity Professional Potting Mix, Michigan Peat Co., Houston, TX) with temperature maintained at 23 ± 3 C. Canada thistle was grown from root stock obtained in May 2008 and transplanted into soil media in 900-ml black plastic pots. All plants were from a single clone and genetically similar. Tillers from stock plants were transplanted into fresh media and pots. Canada thistle plants were selected for treatment 2 wk after transplanting. Natural light was supplemented by high-pressure sodium lamps producing a photosynthetic photon flux density of 200 mol m^{-2} s^{-1} with a photoperiod of 16 8 h light/dark. Pots were watered daily to maintain adequate soil conditions for optimum plant growth. Plants were fertilized with 50 ml of fertilizer solution containing 6 mg L^{-1} of 20% nitrogen, 20% P_2O_5, and 20% K_2O as needed. Weeds were sprayed at 10 to 12 cm height, which is considered larger than optimum size to accentuate differences between herbicide treatments. Greenhouse grown plants are typically more susceptible to herbicides; however, spraying larger plants can compensate for this. There were four replications per experiment and each experiment was repeated four times.

Herbicide Treatments. Herbicide treatments consisted of glyphosate at 0, 0.21 (0.25×), 0.28 (0.33×), 0.42 (0.5×), and 0.84 kg ha^{-1} (1×) and glufosinate at 0, 0.12 (0.25×), 0.16 (0.33×), 0.24 (0.5×), and 0.47 kg ha^{-1} (1×) each applied alone and in combination with each other. Ammonium sulfate was included at 2% w/w in all treatments.

Applications were made using a single-nozzle track sprayer with an 8001 even flat fan nozzle (TeeJet®, Spraying Systems Co., Wheaton, IL) calibrated to deliver 190 L ha^{-1} at a pressure of 210 kPa. Weed control was evaluated at 5, 7, 14, 21, and 28 DAT on a scale of 0% (no control) to 100%

Table 1. Control of velvetleaf, common lambsquarters, and giant foxtail with glyphosate and glufosinate alone and in combination from the 2009 field studies, 7 and 28 d after treatment (DAT).[a]

		Velvetleaf				Common lambsquarters				Giant foxtail			
		7 DAT		28 DAT		7 DAT		28 DAT		7 DAT		28 DAT	
Glyphosate	Glufosinate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
kg ae ha^{-1}	g ai ha^{-1}	%		%		%		%		%		%	
0	0	—	0	—	0	—	0	—	0	—	0	—	0
0.21	0	—	38	—	46	—	36	—	48	—	75	—	86
0.42	0	—	48	—	100	—	29	—	85	—	89	—	99
0.84	0	—	48	—	100	—	48	—	99	—	80	—	100
0	0.12	—	30	—	31	—	35	—	31	—	49	—	76
0	0.24	—	48	—	39	—	46	—	29	—	64	—	73
0	0.47	—	100	—	70	—	97	—	89	—	94	—	86
0.21	0.47	100	99	85	63	98	95	94	89	98	95	98	95
0.42	0.12	73	99	100	100	62	95	89	100	96	91	100	86
0.84	0.24	64	95	100	90	66	95	99	91	90	95	100	89
0.84	0.47	64	90	100	100	66	93	99	100	90	90	100	100
$LSD_{(0.05)}$[c]		8		6		7		6		7		5	

		Velvetleaf				Common lambsquarters				Giant foxtail			
		7 DAT		28 DAT		7 DAT		28 DAT		7 DAT		28 DAT	
Glyphosate	Glufosinate	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
kg ae ha^{-1}	g ai ha^{-1}	%		%		%		%		%		%	
0	0	—	0	—	0	—	0	—	0	—	0	—	0
0.21	0	—	38	—	46	—	36	—	48	—	75	—	86
0.42	0	—	48	—	100	—	29	—	85	—	89	—	99
0.84	0	—	48	—	100	—	48	—	99	—	80	—	100
0	0.12	—	30	—	31	—	35	—	31	—	49	—	76
0	0.24	—	48	—	39	—	46	—	29	—	64	—	73
0	0.47	—	100	—	70	—	97	—	89	—	94	—	86
0.21	0.47	100	99	85	63	98	95	94	89	98	95	98	95	
0.42	0.12	73	99	(+)[b]	100	100	62	95	(+)	89	100	(+)	96	91	100	86	(−)
0.84	0.24	64	95	(+)	100	90	66	95	(+)	99	91	(−)	90	95	100	89	(−)
0.84	0.47	64	90	(+)	100	100	66	93	(+)	99	100		90	90	100	100	
$LSD_{(0.05)}$[c]		8		6		7		6		7		5	

[a] Abbreviations: DAT, d after treatment; Exp., expected control from Colby's method; Obs., observed control.

[b] (+) and (−) denote a synergistic and antagonistic interactions for a given combination of glyphosate and glufosinate according to the LSD of the observed and expected values calculated using Colby's method. Observed values with a (+) or (−) are additive.

[c] LSD values may be used to compared observed values.

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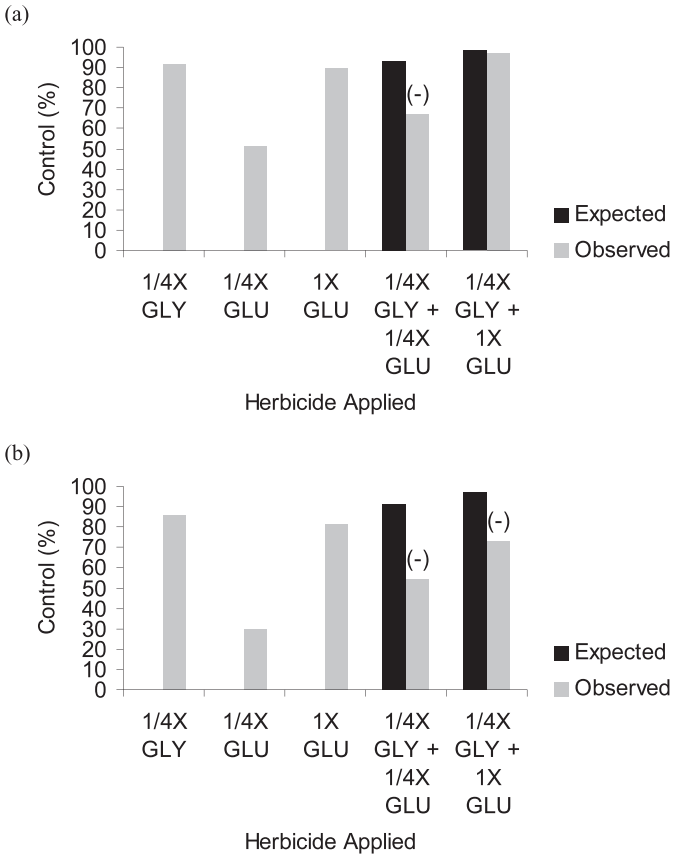


Figure 1. Glyphosate (GLY) and glufosinate (GLU) applied to Canada thistle (a) and velvetleaf. (b) Visual observations in the greenhouse 28 d after treatment. Antagonism by Colby's method indicated by a (−). LSD = 15 (a) and 10 (b).

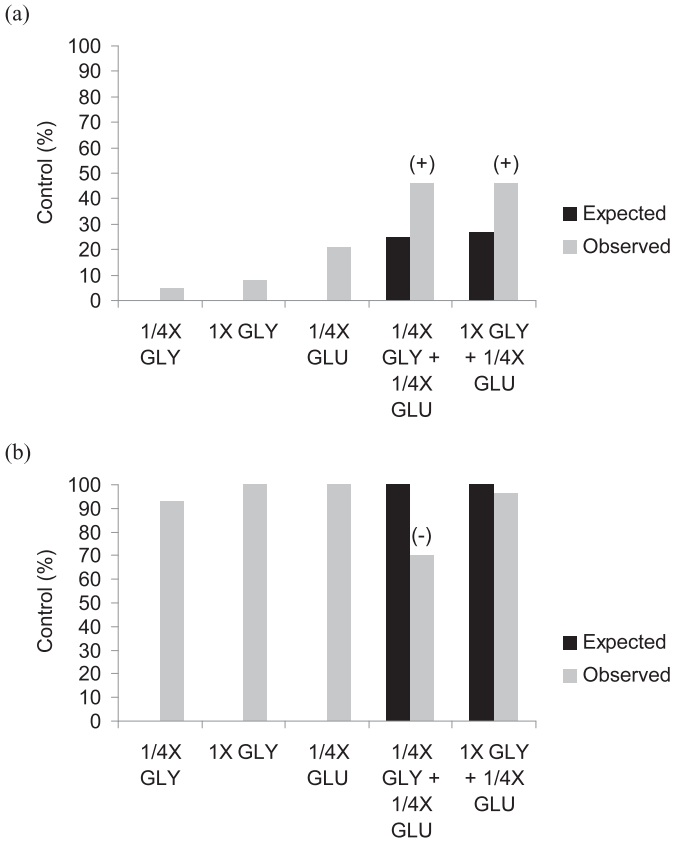


Figure 2. Glyphosate (GLY) and glufosinate (GLU) applied to giant foxtail. Visual observations in the greenhouse 5 (a) and 28 d after treatment (b). Antagonism and synergism by Colby's method indicated by a (−) or (+), respectively. LSD = 8.

(complete control). Plant height data and aboveground biomass were collected at 28 DAT. Plant samples were oven-dried at 50 C for 48 h and dry weights were recorded.

Fluorescence Studies. *Plant Material*. Plants were grown in the greenhouse as previously described. Plants were 10 to 12 cm tall at time of treatment and were randomly assigned to herbicide treatments. Treatments were replicated three times and the experiment repeated three times.

Herbicide Treatments. Herbicide treatments consisted of glyphosate at 0, 0.21 (0.25×), 0.42 (0.5×), and 0.84 kg ha^{-1} (1×) and glufosinate at 0, 0.12 (0.25×), 0.24 (0.5×), and 0.47 kg ha^{-1} (1×), each applied alone and in combination with each other. Ammonium sulfate was included at 2% w/w in all treatments. Treatments were based on preliminary results from the greenhouse studies which showed the highest observable interactions and were also the most economically interesting, such as high and a low rate combinations and low and high rate combinations.

Fluorescence Measurements. After herbicide application the plants were immediately returned to the greenhouse and prepared for fluorescence readings. Fluorescence readings were taken at 2, 4, 6, 8, 24, 48, and 72 h after treatment (HAT). The second set of fully expanded leaves above the cotyledons, with at least one more set of fully expanded leaves above, were selected for fluorescence evaluation. Leafclips (Leaf Clips, Hansatech Instruments, King's Lynn, Norfolk, U.K.) were placed in the middle of the selected leaf directly next to the midvein with the least amount of contact with any major veins to initiate dark adaptation period of 15 min. After completion of the dark adaption period, measurements of fluorescence were initiated. The positions of the leaf clips were marked before removal so that they could be returned to the exact position prior to the next series of measurements. Data were collected for 1.6 s and sorted using Handy PEA (Handy PEA, Hansatech Instruments) data management software. This experiment had four replications and was repeated in time.

Statistical Analysis. Data from the field and greenhouse experiments were subjected to ANOVA using PROC MIXED in SAS (The SAS System for Windows, Version 9.2, SAS Institute Inc., Cary, NC) with replication considered as a random effect. Data were combined over years of repetitions in time if there was no significant treatment by year or repetition interaction. Normality of the residuals was evaluated using normal probability and box plots and arc sine data transformations were conducted if there were significant deviations from normality. Homogeneity of variances was evaluated using Levene's test. Herbicide combinations were determined to be antagonistic, synergistic, or additive by comparing the observed plant responses with the expected response when the herbicides are combined. Expected values were calculated using Colby's equation; $E = X + Y - XY/100$ (Colby 1967). In the equation, X and Y are the percent growth inhibition by herbicide A and B,

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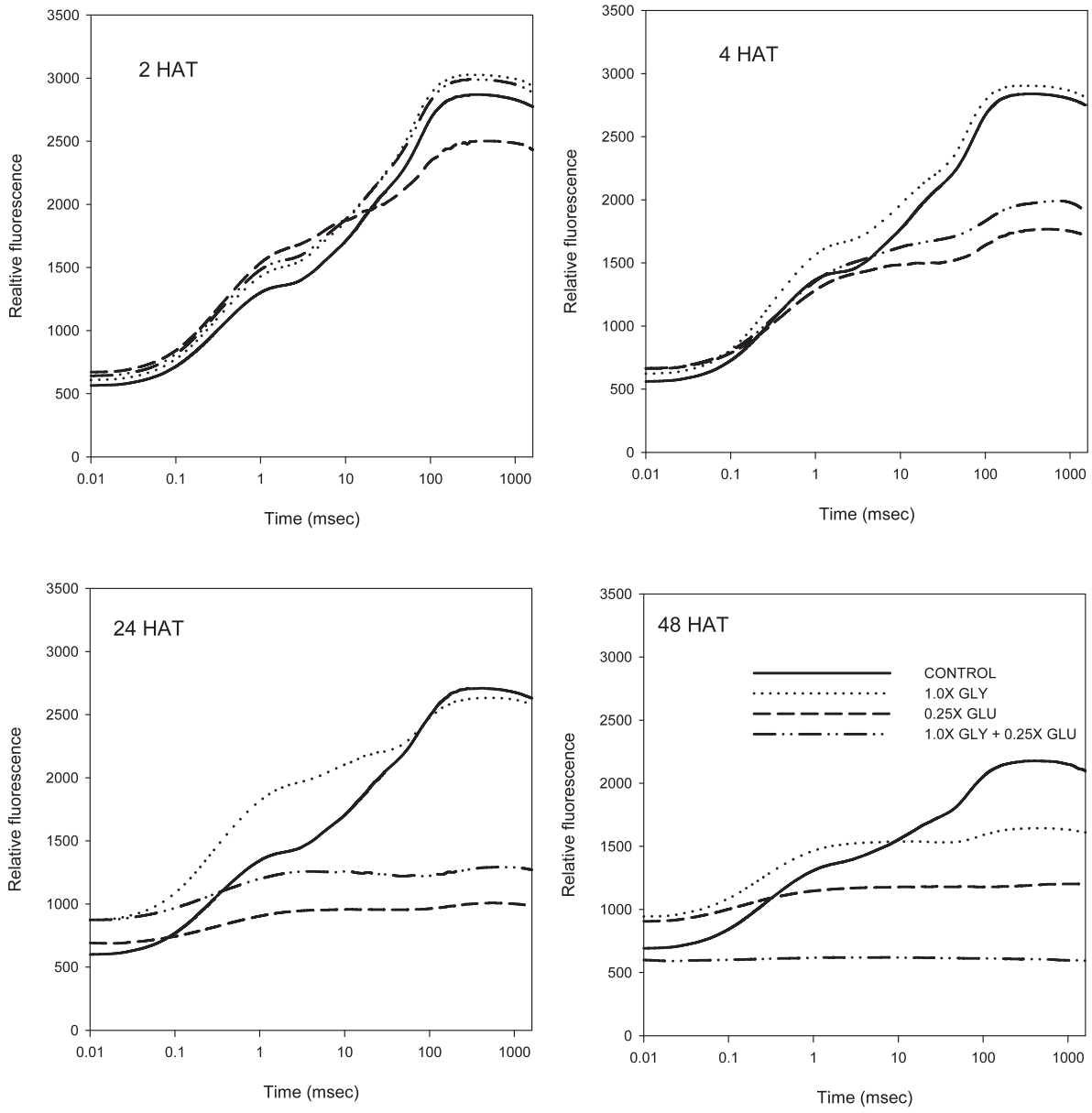


Figure 3. OJIP transients following glyphosate (GLY) and glufosinate (GLU) and combinations applied to Canada thistle at 2, 4, 24, and 48 h after treatment.

respectively, and E is the expected percent growth inhibition by herbicides A and B combined. Expected and observed responses were compared using Fisher's protected least significant difference (LSD) at $P \leq 0.05$ level of significance. Combinations were determined as antagonistic, synergistic, or additive if the observed response was less than, greater than, or similar to the expected response, respectively. In the fluorescence studies, OJIP transients and the parameters for different doses and time intervals were averaged across replicates. F_v/F_m and performance indices (PIs) were fit to linear ($y = y_o + ax$) or nonlinear decay ($y = y_o + ae^{-bx}$) response curves over time.

Results and Discussion

Field Studies. The statistical analysis did not allow combining the data over the 2008 and 2009 field seasons. There was only one significant interaction in the 2008 field study. The lowest rate of glyphosate (0.21 kg ha^{-1}) combined with glufosinate (0.47 kg ha^{-1}) resulted initially in an antagonistic response (data not shown). However, by 21 DAT giant foxtail control with the combination was equivalent to either herbicide alone. Glyphosate combinations with glufosinate produced variable results in the 2009 field season and were species dependent. Synergistic responses were observed early (7 DAT) in velvetleaf and common lambsquarters (Table 1) at 7 DAT, but at 28 DAT, all combinations were additive or antagonistic for weed control (Table 1). Glyphosate and glufosinate combined on common lambsquarters resulted in early synergism at 7 DAT, and at 28 DAT the high rate of glyphosate with the low rate of glufosinate resulted in antagonism. Where glufosinate was applied at higher rates relative to glyphosate, the antagonism was lost, which is consistent with previous research (Chuah et al. 2008; Kudsk and Mathiassen 2004). In giant foxtail, no early synergism was observed at 7 DAT with glyphosate–glufosinate combinations, but at 28 DAT, antagonism was observed with

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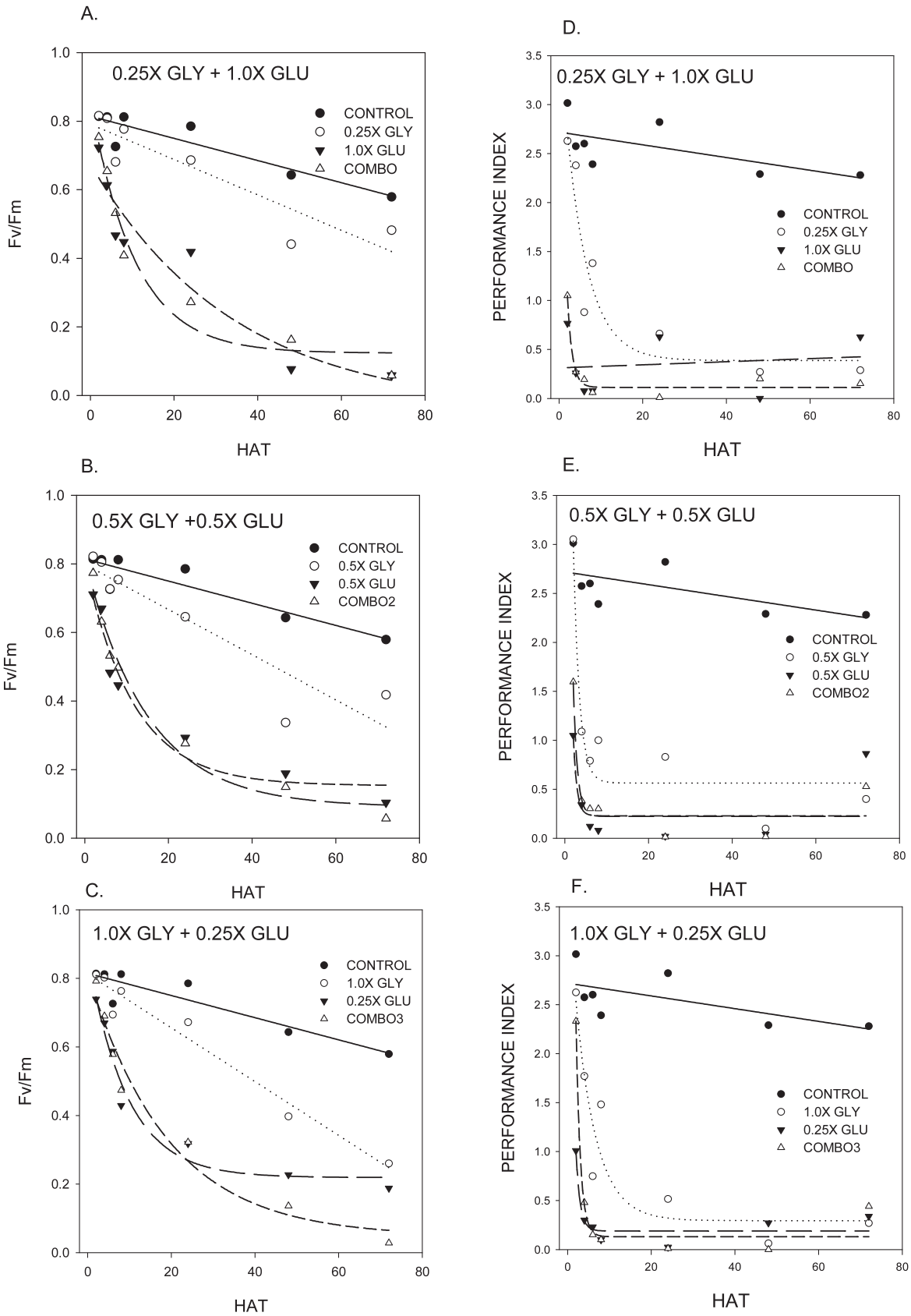


Figure 4. Time course of the change in Fv/Fm (A, B, C) and performance index (D, E, F) values following application of glyphosate (GLY) and glufosinate (GLU) and combinations (COMBO, COMBO2, or COMBO3) to Canada thistle.

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Table 2. Effects of glyphosate and glufosinate on Fv/Fm and performance index of Canada thistle. Values are the variables for a linear ($y = y_o + ax$) or nonlinear decay ($y = y_o + ae^{-bx}$) response models shown in Figure 4.

	Fv/Fm					Performance Index				
Treatment	y_o	a	b	r^2	P	y_o	a	b	r^2	P
Control	0.81	−0.003	—	0.85	0.003	2.71	−0.007	—	0.40	0.127
Glyphosate (0.25×)	0.81	−0.005	—	0.83	0.005	0.39	3.39	0.190	0.88	0.014
Glufosinate (1×)	0.12	0.742	0.095	0.96	0.002	0.31	0.002	—	0.02	0.774
Combination	−0.04	0.719	0.029	0.90	0.010	0.11	5.175	0.856	0.97	0.001
Control	0.81	−0.003	—	0.85	0.003	2.71	−0.007	—	0.40	0.127
Glyphosate (0.5×)	0.80	−0.007	—	0.85	0.003	0.56	9.614	0.679	0.91	0.008
Glufosinate (0.5×)	0.15	0.652	0.086	0.96	0.002	0.23	8.173	1.148	0.52	0.229
Combination	0.09	0.724	0.067	0.98	0.001	0.22	10.417	1.014	0.89	0.012
Control	0.81	−0.003	—	0.85	0.003	2.71	−0.007	—	0.40	0.127
Glyphosate (1×)	0.81	−0.008	—	0.96	< 0.0001	0.29	3.357	0.203	0.87	0.017
Glufosinate (0.25×)	0.22	0.653	0.107	0.96	0.001	0.19	6.013	0.993	0.89	< 0.0001
Combination	0.05	0.771	0.052	0.97	0.001	0.13	13.474	0.938	0.97	0.011

glyphosate–glufosinate combinations where below labeled rates of glufosinate were applied.

Greenhouse Studies. Glyphosate and glufosinate interacted antagonistically when applied in combination (Figure 1). This antagonism was observed at the less than 1× rates of glufosinate in combination with the range of glyphosate rates used on Canada thistle (Figure 1a). Results with giant foxtail and common lambsquarters were similar to those of Canada thistle, but the combination applied to velvetleaf resulted in antagonism across all rates of glufosinate (Figure 1b). In field experiments, results were similar to those found in the greenhouse but with less observable trends due to the complete death of many species attributable to the young growth stage at spraying (Table 1). There was significant regrowth of velvetleaf after application of combined herbicide treatments, attributed to the failure of glyphosate to reach the actively growing tissue

A second hypothesis was that early synergism between glyphosate and glufosinate would be evident in some species, but by 28 DAT the synergism may no longer be evident. This was observed for the combination of the range of glyphosate rates and the lowest glufosinate rate on giant foxtail. At the 5 DAT observations this combination showed synergism (Figure 2a), which by 28 DAT was no longer evident and in one case was replaced with an antagonistic interaction (Figure 2b). Canada thistle, common lambsquarters, and velvetleaf had observable early and late antagonism (data not shown). Field studies were not similar to the greenhouse studies. Early synergism was observed in velvetleaf and common lambsquarters, but was not seen in giant foxtail (Table 1).

Fluorescence Studies. Changes in chlorophyll a fluorescence and fluorescence parameters were examined to see if early interactions could be revealed by fluorescence analysis. Responses were similar across all species so only those for Canada thistle are shown. OJIP transients for glyphosate at 1.0× and glufosinate at 0.25× and their combination at 2, 4, 24, and 48 HAT showed that the response to glufosinate was similar with or without glyphosate (Figure 3). The responses for higher rates of glufosinate and lower rates of glyphosate and their combinations were nearly identical to Figure 3 (data not shown). Glufosinate and glyphosate combinations at all rates produced rapid decreases at the J, I, and P peaks followed by nearly complete quenching of the OJIP transient at 24 and 48 HAT (Figure 3). The interactions between glufosinate and glyphosate exhibited a character nearly identical to that of glufosinate alone. Hence, it is unlikely that fluorescence parameters would prove useful to distinguish herbicide interactions with a fast-acting herbicide like glufosinate.

Glyphosate at 1.0× had little effect on the OJIP transient compared to the control at 2 and 4 HAT. By 24 HAT, the glyphosate treatments resulted in a decrease in the P peak and an increase in the J and I peaks and the fluorescence response at Fo. Effects became more pronounced at 48 HAT with a rise in amplitude at the J peak and fall in the I peaks. Little change was observed in OJIP transients between 48 and 72 HAT (data not shown).

Fv/Fm and PI values, derived from the OJIP transients for Canada thistle, showed rapid changes shortly after application well before indications of injury or interactions appeared at the whole plant level (Figure 4; Table 2). The Fv/Fm of a healthy plant is approximately 0.83, and a value smaller than this is an indication of stress to electron transport of the photosynthetic system. Fv/Fm following glyphosate application decayed linearly throughout the time course of the experiment (Figures 4A–C). The decay in Fv/Fm was dose dependent. As glyphosate concentration increased, there was an increased decline in Fv/Fm (Table 2). There was an exponential decay in Fv/Fm following glufosinate application indicating that the response was more immediate and lethal to Canada thistle. Glufosinate alone and in combination with glyphosate resulted in similar exponential decays such that these treatments were indistinguishable. PI was a more sensitive parameter of changes in physiological responses as indicated by a steeper slope in the first few hours following treatment. The PI decreased exponentially following glyphosate treatment (Figure 4D–F). If the Fv/Fm value is used to estimate damage to PS II, the extent of damage may underestimate the change in PS II function.

Early synergism was observed in the greenhouse for giant foxtail and in the field for common lambsquarters, velvetleaf, and giant foxtail. Field studies to determine herbicide interactions generally resulted in more erratic data, possibly due to the effect of the environment on herbicide absorption and translocation. Fluorescence measurements showed that glufosinate acted rapidly to break down the PS II system of photosynthesis, and its effects overshadowed those of glyphosate. Glufosinate alone and in combination resulted in significantly lower Fv/Fm and PI values than the control or glyphosate alone. Thus it is unlikely that fluorescence

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measurements will be able to detect synergisms between herbicides when a fast-acting herbicide is used in combination with those acting more slowly. These results support those of Chuah et al. (2008) and Kudsk and Mathiassen (2004) in which an antagonistic interaction between glyphosate and glufosinate was observed on other species and was commonly attributed to the rapid action of glufosinate on the photosynthetic system, which may reduce glyphosate translocation through the plant. The results from these studies showed that the combination of glyphosate and glufosinate was antagonistic in common lambsquarters, Canada thistle, giant foxtail, and velvetleaf, although it was not indicative of the herbicide interaction.

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