Weed Technology

www.cambridge.org/wet

Research Article

Cite this article: Drake Copeland J, Montgomery GB, Steckel LE (2019) Evaluation of the time-of-day effect of herbicides applied POST on protoporphyrinogen IX oxidase–resistant and –susceptible Palmer amaranth (*Amaranthus palmeri*). Weed Technol. **33**: 651–657. doi: 10.1017/wet.2019.43

Received: 29 October 2018
Revised: 10 May 2019
Accepted: 16 May 2019
First published online: 22 July 2019

Associate Editor:
William Johnson, Purdue University

Nomenclature:
acifluorfen; fomesafen; glufosinate; lactofen; metribuzin; paraquat; Palmer amaranth; *Amaranthus palmeri* S. Watson

Keywords:
PPO-resistant Palmer amaranth; PPO-susceptible Palmer amaranth; application time of day

Author for correspondence:
Lawrence Steckel, Department of Plant Sciences, University of Tennessee, 605 Airways Boulevard, Jackson, TN, 38301.
E-mail: lsteckel@utk.edu

Evaluation of the time-of-day effect of herbicides applied POST on protoporphyrinogen IX oxidase–resistant and –susceptible Palmer amaranth (*Amaranthus palmeri*)

J. Drake Copeland[1], Garret B. Montgomery[2] and Lawrence E. Steckel[3]

[1]Former Graduate Research Assistant, Department of Plant Sciences, University of Tennessee, Jackson, TN, USA; [2]Technology Development Representative, Bayer Crop Sciences, St. Louis, MO, USA; and [3]Professor, Department of Plant Sciences, University of Tennessee, Jackson, TN, USA

Abstract

Studies to evaluate the effect of application time of day (TOD) and protoporphyrinogen IX oxidase (PPO)-inhibiting herbicide–resistant Palmer amaranth on the efficacy of commonly used herbicides was conducted in Tennessee in 2017 and 2018. Treatments of fomesafen, lactofen, acifluorfen, paraquat, glufosinate, glufosinate plus fomesafen, paraquat plus fomesafen, and paraquat plus metribuzin were applied to PPO-resistant (PPO-R) and PPO-susceptible (PPO-S) Palmer amaranth at sunrise and midday. Control of Palmer amaranth with acifluorfen, glufosinate, and glufosinate plus fomesafen was greater with the midday application. However, control of Palmer amaranth with paraquat-based treatments was greater with the sunrise application. TOD effects on PPO-inhibiting herbicides and paraquat-based treatments were more prominent for the PPO-R Palmer amaranth biotype. The TOD effect observed when applying glufosinate in early morning hours on PPO-S Palmer amaranth can be minimized by adding fomesafen to the tank mix. However, this strategy did not provide consistent performance on PPO-R Palmer amaranth. The percentages of living Palmer amaranth plants and control were greater when paraquat plus metribuzin was applied to both biotypes. These results highlight the necessity of at least two effective herbicide sites of action for POST applications intended for controlling PPO-R Palmer amaranth. In addition, the timing of herbicide applications can affect their activity in both PPO-R and PPO-S Palmer amaranth populations.

Introduction

Palmer amaranth resistance to protoporphyrinogen IX oxidase (PPO)-inhibiting herbicides (WSSA Group 14) has complicated chemical control tactics in the mid-South (Giacomini et al. 2017; Heap 2018; Johnston et al. 2018; Schwartz-Lazaro et al. 2017). Over the last decade, management of glyphosate and acetolactate synthase inhibitor–resistant Palmer amaranth has relied on PPO-inhibiting herbicides applied PRE and POST for control. Coupled with Palmer amaranth's prolific growth and ability to spread, the aforementioned reliance has selected for PPO-resistant (PPO-R) Palmer amaranth biotypes throughout Arkansas, Illinois, and Tennessee (Copeland et al. 2018a; Heap 2018; Varanasi et al. 2017; Ward et al. 2013). Therefore, POST herbicide applications that include multiple, effective sites of action for control of PPO-R Palmer amaranth are a valuable resistance-management strategy.

Efficacy of herbicides applied POST on *Amaranthus* spp. is greatly affected by environmental factors. Coetzer et al. (2001) reported increasing relative humidity from 35% to 90% increased glufosinate efficacy on control of Palmer amaranth, redroot pigweed (*Amaranthus retroflexus* L.), and common waterhemp [*A. tuberculatus* (Moq.) J.D. Sauer]. Increases in temperature also increased glufosinate injury to *Amaranthus* spp. (Coetzer et al. 2001). Temperature regimens increasing from 26/21 C (day/night) to 31/26 C increased visual injury from 51% to 71%, respectively, 14 d after treatment with glufosinate at 410 g ha^{-1} (Coetzer et al. 2001).

Herbicide application time of day (TOD) is an parameter applicators have control over and can affect the efficacy of many herbicides (Doran and Andersen 1976; Martinson et al. 2002; Sellers et al. 2004; Stopps et al. 2013). The TOD effect is weed-species specific (Fausey and Renner 2001; Lee and Oliver 1982) and herbicide specific (Doran and Andersen 1976; Miller et al. 2003; Stewart et al. 2009). Culpepper et al. (2013) found that glufosinate (WSSA Group 10) efficacy on Palmer amaranth was significantly reduced with applications made near sunrise or sunset. Glufosinate applications made near sunrise or sunset ultimately reduced lint

yield of cotton (*Gossypium hirsutum* L.) compared with cotton treated with glufosinate during midday hours, because of crop competition from surviving Palmer amaranth.

Application timing can also affect the efficacy of paraquat (WSSA Group 22), a photosystem I–inhibiting herbicide. For example, horseweed [*Conyza canadensis* (L.) Cronq.] control after applications of paraquat at sunrise and sunset was greater than control reported from midday applications (Montgomery et al. 2017). Conversely, photosystem II–inhibiting herbicides have greater control of common ragweed (*Ambrosia artemisiifolia* L.) and common lambsquarters (*Chenopodium album* L.) between 0900 and 1800 hours as opposed to applications made at 0600 or 2100 hours (Stewart et al. 2009). However, dicamba (WSSA Group 4) and diflufenzopyr (WSSA Group 19) applications provided greater than 95% control of common ragweed, common lambsquarters, and redroot pigweed regardless of TOD (Stewart et al. 2009). These findings suggest the efficacy of an herbicide applied a various times of the day is influenced by the herbicide's site of action.

Morphological and physiological factors of specific weed species can play a role in species-specific TOD effects for POST herbicides (Hess and Falk 1990; Stopps et al. 2013). Diurnal changes in leaf angle of velvetleaf (*Abutilon theophrasti* Medik.), prickly sida (*Sida spinosa* L.), hemp sesbania [*Sesbania herbacea* (Mill.) McVaugh], and sicklepod [*Senna obtusiifolia* (L.) H.S. Irwin & Barneby] have been reported to negatively affect herbicide activity in low-light environments (Andersen and Koukkari 1978; Norsworthy et al. 1999; Sellers et al. 2004). POST herbicide coverage and subsequent absorption and translocation are also affected by factors such as exposed leaf surface area and orientation (Andersen and Koukkari 1978; Coetzer et al. 2001; Mohr et al. 2007; Norsworthy et al. 1999). Herbicide physiology factors, such as site of action, can influence the TOD effects on efficacy. Miller et al. (2003) reported glyphosate (WSSA Group 9), glufosinate (WSSA Group 10), fomesafen (WSSA Group 14), and chlorimuron ethyl (WSSA Group 2) have different optimal peaks in efficacy on broadleaf weeds throughout the day.

In Arkansas and Tennessee populations, marginal control of PPO-R Palmer amaranth was reported after applications of various POST herbicides, which had no previous reports of resistance, in greenhouse studies (Schwartz-Lazaro et al. 2017; Umphres 2017). Researchers found that control of PPO-R biotypes after applications of dicamba or mesotrione (WSSA Group 27) was approximately 25% less than control reported with PPO-susceptible (PPO-S) biotypes (Schwartz-Lazaro et al. 2017; Umphres 2017). After applications of paraquat (WSSA Group 22) and glufosinate, more consistent control of PPO-R biotypes was reported, both 95% or better (Schwartz-Lazaro et al. 2017; Umphres 2017). Furthermore, in an effort to delay selection for resistance and sustain effective modes of action, combining herbicides with multiple modes of action, such as paraquat and glufosinate, for control of herbicide-resistant Palmer amaranth is highly recommended (Kupper et al. 2018). However, previous work shows both glufosinate and paraquat are affected by the TOD the application is made.

Based on previous research (Montgomery et al. 2017), herbicides not applied at the most effective time are unlikely to control PPO-R Palmer amaranth. To date, to our knowledge, research has not been conducted on the TOD effect of combining multiple sites of action for control of PPO-R Palmer amaranth. The implications of tank mixing herbicides that have multiple sites of action and the time of application for control of PPO-R Palmer amaranth are not well understood. Therefore, the objectives of this study were to (1) evaluate the response of PPO-R compared with PPO-S Palmer amaranth with common tank mixes of PPO-inhibiting herbicides, paraquat- and glufosinate-based treatments applied POST in the greenhouse, and (2) determine the effect of application TOD of the aforementioned herbicides on control of PPO-R and PPO-S Palmer amaranth in separate field studies.

Materials and Methods

Greenhouse Study

Greenhouse experiments were conducted at West Tennessee Research and Education Center, Jackson, Tennessee. In the fall of 2017, Palmer amaranth seed was collected from the Jackson (PPO-S) and Golddust, Tennessee (PPO-R), locations. To determine the presence of PPO resistance, before conducting these experiments, fomesafen (265 g ai ha^{-1}) was applied to Palmer amaranth at each location when weeds were 6- to 10-cm tall. Palmer amaranth at the Jackson location was completely controlled (100%), whereas less than 10% control was observed at the Golddust location. Molecular procedures were conducted to determine the presence of *PPX2* mutations in Palmer amaranth, as described by Copeland et al. (2018a), using genomic DNA from plant tissue from each location to screen for mutations that confer PPO resistance. The Palmer amaranth biotype at Golddust harbored both the ΔG210 and R128G mutations that confer PPO resistance and is referred to as the PPO-R biotype in this study (Giacomini et al. 2017; Varanasi et al. 2017). The Palmer amaranth biotype at Jackson did not contain any of the mutations that confer PPO resistance and is referred to hereafter as the PPO-S biotype.

Seed from each location was scattered in separate flats and watered to stimulate germination. Plants at the cotyledon stage were transplanted into pots of 10-cm diameter, one plant per pot, containing commercial potting soil (Sun-Gro Redi-Earth Plug and Seedling Mix; Sun-Gro Horticulture, Bellevue, WA). Day/night temperatures ranged from 20 to 30 C. Natural light was supplemented by lamps (NXT2 lamp; P.L. Light Systems, Beamsville, ON, Canada) on a 13-h photoperiod (0600 to 1900 hours) delivering 400 μmol m^{-2} s^{-1} photosynthetic photon flux density. Plants received a dilute nutrient solution weekly and were watered daily (Miracle Gro: All Purpose Plant Food 24-8-16; The Scotts Company LLC, Marysville, OH).

The experiment was replicated six times within a randomized complete block design and repeated once. The first factor was herbicide treatment and consisted of fomesafen, lactofen, acifluorfen, paraquat, glufosinate, glufosinate plus fomesafen, paraquat plus fomesafen, and paraquat plus metribuzin. With the exception of glufosinate applied alone, all treatments contained 1% vol/vol of methylated seed oil (Fire-Zone; Helena Chemical Co., Collierville, TN). Herbicide common names, trade names, rates, and manufacturers are listed in Table 1. The second factor was biotype and consisted of the PPO-R and PPO-S biotypes. A nontreated check was included for comparison purposes. Treatments were applied to 12-cm Palmer amaranth using a CO_2-pressurized backpack sprayer equipped with AIXR 11003 nozzles (TeeJet Technologies, Glendale Heights, IL) delivering 140 L ha^{-1} at pressure to generate medium droplet sizes for optimal coverage.

Visual estimates of Palmer amaranth control were conducted 10 d after application (DAA). Mortality was also determined by the number of dead plants in each treatment 10 DAA, and expressed as a percentage. Plants were considered dead if zero green material or regrowth was present. Data were subjected to

https://doi.org/10.1017/wet.2019.43 Published online by Cambridge University Press

Table 1. Herbicide common and trade names, application rates, and registrant information for treatments evaluating the time-of-day effect on protoporphyrinogen IX oxidase–resistant and –susceptible Palmer amaranth in 2017 and 2018.

Common name	Trade name	Rate	Manufacturer
		g ai ha^{-1}	
Fomesafen	Flexstar®	265	Syngenta Crop Protection, Greensboro, NC
Lactofen	Cobra®	175	Valent USA Corp., Walnut Creek, CA
Acifluorfen	Ultra Blazer®	280	UPI, King of Prussia, PA
Paraquat	Gramoxone® SL 2.0	700	Syngenta Crop Protection, Greensboro, NC
Glufosinate	Liberty® 280 SL	655	Bayer Crop Science, Rhein, Germany
Metribuzin	Tricor® DF	210	UPI, King of Prussia, PA

Table 2. Application dates, Palmer amaranth density, and environmental conditions in field studies conducted in Golddust, TN (protoporphyrinogen IX oxidase [PPO]-resistant Palmer amaranth) and Jackson, TN (PPO-susceptible Palmer amaranth), in 2017 and 2018.

Application	Jackson, TN		Golddust, TN	
	2017	2018	2017	2018
Date	May 25	May 18	May 24	May 15
Palmer amaranth density[a]	170	205	195	210
Sunrise				
Time	0520	0500	0500	0515
Air temperature, C	13	20	11	23
Soil temperature, C	15	25	15	25
Relative humidity, %	97	99	96	67
Dew presence	Yes	Yes	Yes	Yes
Soil moisture	Moderate	High	Moderate	Moderate
Cloud cover, %	50	50	0	90
Midday				
Time	1300	1100	1100	1115
Air temperature, C	23	29	21	34
Soil temperature, C	20	27	20	32
Relative humidity, %	45	63	53	54
Dew presence	No	No	No	No
Soil moisture	Moderate	High	Moderate	Moderate
Cloud cover, %	20	60	15	20

[a] No. of Palmer amaranth plants m^{-2}.

an ANOVA using PROC Glimmix procedure in SAS, version 9.4 (SAS Institute, Cary. NC). Replication served as a random effect. The impact of Palmer amaranth biotype on individual herbicide treatments was determined by analyzing data for each treatment separately and making no comparisons among herbicides. Type III statistics were used to test the fixed effects, and least square means were separated using Fisher protected LSD at $P = 0.05$.

Field Study

Studies were conducted at the West Tennessee Research and Education Center in Jackson, Tennessee, and on farmland in Golddust, Tennessee, in 2017 and 2018 to evaluate the TOD effects of herbicides on PPO-R and PPO-S Palmer amaranth. Plants at the Jackson location were PPO-S; they were susceptible to POST-applied fomesafen and had no history of lack-of-performance issues with fomesafen. Plants at the Golddust location were PPO-R; they were resistant to POST-applied fomesafen and had a history of Palmer amaranth control failure with fomesafen. Over the last 5 yr, both locations have been in continuous soybean production and are located 93-km apart. Each location had similarly dense populations of Palmer amaranth (Table 2), ranging from a low of 170 m^{-2} in Jackson in 2017 (PPO-S) to a high of 210 m^{-2} in Golddust in 2018 (PPO-R). Rainfall each year at both locations was adequate for rapid weed growth and was not limiting during the time experiments were conducted.

Plot areas at each site were weed free before germination of Palmer amaranth. At both locations, glyphosate at 1,260 g ae ha^{-1} and dicamba at 560 g ae ha^{-1} were applied in the early spring to allow Palmer amaranth to germinate without the competition of winter annuals or early summer annual weeds. Each experiment was conducted as a randomized complete block design with 17 treatments. Individual plot sizes were 1.5 m by 9.1 m. The first factor was herbicide treatment and consisted of fomesafen, lactofen, acifluorfen, paraquat, glufosinate, glufosinate plus fomesafen, paraquat plus fomesafen, and paraquat plus metribuzin. With the exception of glufosinate applied alone, all treatments contained 1% vol/vol of methylated seed oil (Fire-Zone). Herbicide common names, trade names, rates, and manufacturers are listed in Table 1. The second factor was application TOD and consisted of an application 0.5 h before sunrise and at 1200 hours. Environmental data and application times are listed in Table 2. A nontreated control was included for comparison purposes.

Herbicide treatments were applied POST when Palmer amaranth averaged 7.5-cm tall at each location. Herbicide treatments were applied with a CO_2-pressurized backpack sprayer calibrated to 140 L ha^{-1} at 220 kPa at each location, using AIXR 11003 nozzles spaced 50-cm apart (AIXR). Visual estimates of control for Palmer amaranth were assessed 7 and 21 DAA on a scale of 0% (no weed control) to 100% (complete control). Surviving Palmer amaranth plants from 1-m^2 quadrants were counted 21 DAA, and the number of living plants in each treatment plot was compared with the number in the nontreated check in each replication and calculated as a percentage. This study contained four replications and was repeated at each location.

All data were subjected to ANOVA using the PROC Glimmix procedure in SAS, version 9.4. The DANDA.sas design and analysis macro collection (Saxton 2013) was used to create all PROC Glimmix (MMAOV) procedures. Random effects were year and replication nested within year (Blouin et al. 2011). Data were analyzed over the 2 years because each year was considered to be selected randomly from a population of years (Steckel et al. 2003). Considering year an environmental or random effect permits inferences about treatments to be made over a range of environments (Blouin et al. 2011; Carmer et al. 1989). TOD was considered a fixed effect. The impact of application TOD on individual herbicide treatments was determined by analyzing data for each treatment separately and making no comparisons among herbicides. Type III statistics were used to test the fixed effects, and least square means were separated using Fisher protected LSD at $P = 0.05$.

Results and Discussion

PPO-R and PPO-S Palmer Amaranth Response to Herbicides Applied POST Under Greenhouse Conditions

After PPO-inhibiting herbicides were applied, control of PPO-R and PPO-S biotypes differed (Table 3). Palmer amaranth control ranged from 91% to 99% when lactofen, fomesafen, or acifluorfen was applied to PPO-S plants and was greater than when these

https://doi.org/10.1017/wet.2019.43 Published online by Cambridge University Press

Table 3. Control and mortality of Palmer amaranth plants 10 d after application of protoporphyrinogen IX oxidase–inhibiting herbicides, paraquat-based tank mixes, and glufosinate-based tank mixes as affected by biotype in greenhouse experiments conducted at Jackson, TN.

Treatment	Biotype	Control[a,b]	Mortality
		%	
Lactofen	PPO-R	11b	0b
	PPO-S	91a	83a
P value		0.0002	0.0041
Fomesafen	PPO-R	19b	0b
	PPO-S	99a	100a
P value		<.0001	<.0001
Acifluorfen	PPO-R	39b	16b
	PPO-S	99a	100a
P value		0.0114	0.0041
Paraquat	PPO-R	99	100
	PPO-S	99	100
P value		N/A[c]	N/A[c]
Paraquat + metribuzin	PPO-R	99	100
	PPO-S	99	100
P value		N/A[c]	N/A[c]
Paraquat + fomesafen	PPO-R	99	100
	PPO-S	99	100
P value	N/A[c]	N/A[c]	
Glufosinate	PPO-R	95	74
	PPO-S	99	100
P value	0.0756	0.0756	
Glufosinate + fomesafen	PPO-R	98	83
	PPO-S	99	100
P value		0.3632	0.3632

[a] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.
[b] Abbreviations: N/A, not applicable; PPO-R, protoporphyrinogen IX oxidase resistant; PPO-S, protoporphyrinogen IX oxidase susceptible.
[c] No factorial was run, because of the lack of variance.

herbicides were applied to PPO-R plants; control of PPO-R plants ranged from 11% to 39%. Plant death after application of lactofen, fomesafen, and acifluorfen followed the same trend as visual estimates of control. The mortality rate of PPO-S plants (83% to 100%) was greater than that of PPO-R plants (0% to 16%) (Table 3).

With paraquat-based treatments, complete control of both PPO-R and PPO-S biotypes was observed with or without a tank-mix partner. For instance, control and percent mortality 10 DAA of paraquat, paraquat plus metribuzin, and paraquat plus fomesafen were 99% and 100%, respectively. Control of PPO-R and PPO-S Palmer amaranth was similar after glufosinate alone or glufosinate plus fomesafen were applied. Control 10 DAA of glufosinate alone and glufosinate plus fomesafen applied to PPO-R plants ranged from 95% to 98%. Similarly, 99% control was observed 10 DAA of both glufosinate alone and glufosinate plus fomesafen (Table 3). Percent mortality after application of glufosinate and glufosinate plus fomesafen on PPO-S plants was 100% (Table 3).

Although significant differences were not observed, percent mortality of the PPO-R and PPO-S biotypes after application of glufosinate and glufosinate plus fomesafen was 74% and 83%, respectively (Table 3). Confounding factors, such as relative humidity, sunlight, and temperature, affect glufosinate activity on Palmer amaranth (Montgomery et al. 2017). However, this experiment was conducted in a controlled greenhouse environment ideal for glufosinate activity. These data suggest differential tolerances to glufosinate may exist when comparing the PPO-R and PPO-S biotypes from Tennessee populations (Copeland et al. 2017).

PPO-R and PPO-S Palmer Amaranth Control Affected by TOD of PPO-Inhibiting Herbicide Applications

Control of PPO-R Palmer amaranth 7 DAA of lactofen and acifluorfen was affected by time of application (Table 4). Lactofen and acifluorfen applied midday resulted in 8% greater control than when applied at sunrise. With lactofen, PPO-R Palmer amaranth control was poor (26% with sunrise application; 34% with midday application). Greater control with lactofen was observed for the PPO-S biotype (92% with sunrise application; 84% with midday application) 7 DAA. Control of PPO-S Palmer amaranth was affected by the TOD of acifluorfen application 7 DAA. Control of PPO-S Palmer amaranth was 18% greater when acifluorfen was applied midday. Interestingly, the TOD of fomesafen applications did not affect PPO-R or PPO-S Palmer amaranth control 7 DAA. PPO-S Palmer amaranth control was greater (88% to 95%) than control observed on PPO-R Palmer amaranth (32% to 36%).

TOD of lactofen application did not affect Palmer amaranth control of either biotype 21 DAA. Similar to 7 DAA, PPO-R Palmer amaranth control ranged from 9% to 13%, whereas 69% to 71% control was observed for PPO-S Palmer amaranth (Table 4). In addition, the percentage of living Palmer amaranth plants was greater 21 DAA of lactofen among the PPO-R Palmer amaranth (97% to 100%), whereas the mortality rate of PPO-S Palmer amaranth ranged from 14% to 26%, which was expected because of PPO resistance documented at the PPO-R biotype location (Table 4).

Control of PPO-R Palmer amaranth with fomesafen was poor. However, control and the number of living PPO-R Palmer amaranth plants 21 DAA of fomesafen was affected by application TOD. Control with fomesafen applied at midday (16%) was greater than that achieved with sunrise applications (10%) and, likewise, 22% more living plants were in plots treated with fomesafen at sunrise. No TOD effect was observed on PPO-S Palmer amaranth, and control 21 DAA of fomesafen ranged from 68% to 80% and the percentage of living plants was not greater than 11% (Table 4).

Regardless of time of acifluorfen application, PPO-R Palmer amaranth control and the percentage of living plants 21 DAA were similar. Control of PPO-R Palmer amaranth was 13% and the percentage of living plants ranged from 96% to 98% (Table 4). Similar to control after application of lactofen and fomesafen, acifluorfen poorly controlled PPO-R Palmer amaranth regardless of application timing. Control of PPO-S Palmer amaranth was affected by application TOD of acifluorfen; however, the percentage of living plants was not affected. Greater control was achieved when acifluorfen was applied midday (82%) compared with application at sunrise (56%). Acifluorfen applied in the dark provides more effective control than sunrise or midday applications (Lee and Oliver 1982). Previous TOD research with acifluorfen was conducted on hemp sesbania, pitted morningglory (*Ipomoea lacunosa* L.) and smooth pigweed (*Amaranthus hybridus* L.). The morphological and physiological factors of specific weeds can result in species-specific TOD effects (Stopps et al. 2013).

PPO-R and PPO-S Palmer Amaranth Control Affected by TOD of Paraquat-Based Applications

Control at 7 and 21 DAA of PPO-R and PPO-S Palmer amaranth was not affected by application TOD of paraquat (Table 5). At the PPO-R and PPO-S biotype locations, Palmer amaranth control rates were similar for applications made at sunrise (92% and 99%, respectively, 7 DAA; 88% and 99%, respectively, 21 DAA) and at midday (91% and 97%, respectively, 7 DAA; 80% and

https://doi.org/10.1017/wet.2019.43 Published online by Cambridge University Press

Table 4. Control of PPO-R and PPO-S Palmer amaranth biotypes in field studies 7 and 21 DAA, and percentage of living plants 21 DAA of lactofen, fomesafen, or acifluorfen, as affected by application TOD.

		Lactofen[b]			Fomesafen[b]			Acifluorfen[b]		
		Control		Living	Control		Living	Control		Living
Biotype	TOD[a]	7 DAA	21 DAA	plants[c]	7 DAA	21 DAA	plants[c]	7 DAA	21 DAA	plants[c]
		%			%			%		
PPO-R	Sunrise	26	9	100	32	10	100*	34	13	96
	Midday	34*	13	97	36	16*	78	42*	13	98
	P value	0.0490	0.1111	0.4221	0.3311	0.0248	0.0258	0.0137	1.000	0.8879
PPO-S	Sunrise	92	69	14	88	68	11	75	56	33
	Midday	84	71	26	95	80	10	93*	82*	14
	P value	0.1321	0.8627	0.1443	0.2008	0.1685	0.8476	0.0165	0.0036	0.0807

[a] Abbreviations: DAA, days after application; PPO-R, protoporphyrinogen IX oxidase resistant; PPO-S, protoporphyrinogen IX oxidase susceptible; TOD, time of day.
[b] Asterisk (*) indicates significance according to Fisher protected LSD (P = 0.05).
[c] Surviving plants counted at 21 DAA in 1 m^2, expressed as a percentage of the nontreated check.

Table 5. Control of PPO-R and PPO-S Palmer amaranth biotypes in field studies 7 and 21 DAA and percentage of living plants 21 DAA of paraquat, paraquat plus metribuzin, or paraquat plus fomesafen, as affected by application TOD.

		Paraquat[b]			Paraquat plus metribuzin[b]			Paraquat plus fomesafen		
		Control		Living	Control		Living	Control		Living
Biotype	TOD[a]	7 DAA	21 DAA	plants[c]	7 DAA	21 DAA	plants[c]	7 DAA	21 DAA	plants[c]
		%			%			%		
PPO-R	Sunrie	92	88	4	99	96*	1	95	86	8
	Middy	91	80	11*	97	91	2	92	85	13
	P value	0.2507	0.0537	0.0065	0.1927	0.0255	0.3555	0.3035	0.7317	0.0887
PPO-S	Sunrise	99	99	0	99	99	0	99	98	0
	Midday	97	90	5	99	98	2	99	94	2
	P value	0.0702	0.1565	0.3507	0.6576	0.3506	0.3506	1.000	0.1816	0.3507

[a] Abbreviations: DAA, days after application; PPO-R, protoporphyrinogen IX oxidase resistant; PPO-S, protoporphyrinogen IX oxidase susceptible; TOD, time of day.
[b] Asterisk (*) indicates significance according to Fisher protected LSD (P = 0.05).
[c] Surviving plants counted at 21 DAA in 1 m^2, expressed as a percentage of the nontreated check.

90%, respectively, 21 DAA). When making no comparison between the two biotypes in this study, overall control with paraquat was greater at the PPO-S location. Interestingly, TOD of paraquat applications affected the percentage of living PPO-R plants but did not affect the percentage of living PPO-S plants. More living PPO-R plants were observed in plots treated at midday (11%) compared with plots treated at sunrise (4%) (Table 5). Paraquat applied at sunrise provided more effective control than midday applications.

Most application TOD research has reported greater efficacy when a herbicide is applied in the middle of the day (Miller et al. 2003; Sellers et al. 2004; Stewart et al. 2009; Stopps et al. 2013); however, this does not apply to all herbicides (Lee and Oliver 1982; Montgomery et al. 2017; Putnam and Ries 1968). Putnam and Ries (1968) found that a 6-hr dark period after an application further enhanced ^{14}C-paraquat movement from the treated quackgrass [*Elymus repens* (L.) Gould] leaf. The increased translocation observed in the dark resulted in enhanced growth inhibition of rhizome segments (Putnam and Ries 1968). A similar trend was noted in glyphosate-resistant horseweed, with sunrise and sunset applications of paraquat providing better control than the midday applications (Montgomery et al. 2017).

TOD of paraquat plus metribuzin applications did not affect PPO-R or PPO-S Palmer amaranth control 7 DAA. Regardless of TOD, Palmer amaranth control ranged from 97% to 99% (Table 5). PPO-R Palmer amaranth control was affected by application TOD of paraquat plus metribuzin 21 DAA; however, the PPO-S biotype was not affected. Significantly greater control of PPO-R Palmer amaranth was observed after paraquat plus metribuzin was applied at sunrise (96%) compared with midday applications (91%). However, differences were not observed for either biotype in regard to percentage of living plants. The percentage of living plants observed in plots that received an application of paraquat plus metribuzin ranged from 0% to 2%, compared with the nontreated plots, regardless of time of application or biotype (Table 5). These data provide evidence that the more troublesome Palmer amaranth biotype (i.e., the PPO-R biotype) should be targeted with multiple, effective modes of action (Norsworthy et al. 2012). In burndown scenarios, the addition of metribuzin (WSSA Group 5) to paraquat can improve control of Palmer amaranth, thus delaying the selection for resistant biotypes by minimizing selection pressure of using a single site of action (Eubank et al. 2012; Norsworthy et al. 2012).

TOD of paraquat plus fomesafen applications did not affect control of PPO-R or PPO-S Palmer amaranth 7 and 21 DAA. Palmer amaranth control at the PPO-R and PPO-S Palmer amaranth locations, respectively, at sunrise (95% and 99% 7 DAA; 86% and 98% 21 DAA) and at midday (92% and 99% 7 DAA; 85% and 94% 21 DAA) was similar for each application timing (Table 5). Similar to paraquat alone, PPO-S Palmer amaranth control was numerically greater than control reported at the PPO-R location. The percentage of living plants after application of paraquat plus

https://doi.org/10.1017/wet.2019.43 Published online by Cambridge University Press

Table 6. Control of PPO-resistant and -susceptible Palmer amaranth biotypes in field studies 7 and 21 DAA and percentage of living plants 21 DAA of glufosinate or glufosinate plus fomesafen as affected by application TOD.

		Glufosinate[b]			Glufosinate plus fomesafen[b]		
		Control		Living	Control		Living
Biotype	TOD[a]	7 DAA	21 DAA	plants[c]	7 DAA	21 DAA	plants[c]
		%			%		
PPO-R	Sunrise	63	41	73*	69	49	44*
	Midday	97*	85*	6	97*	85*	13
	P value	0.0012	0.0057	0.0012	0.0036	0.0113	0.0449
PPO-S	Sunrise	61	26	76*	92	90	7
	Midday	97*	91*	10	99	99*	0
	P value	0.0017	<0.0001	0.0337	0.0591	0.0187	0.4017

[a] Abbreviations: DAA, days after application; PPO-R, protoporphyrinogen IX oxidase resistant; PPO-S, protoporphyrinogen IX oxidase susceptible; TOD, time of day.
[b] Asterisk (*) indicates significance according to Fisher protected LSD (P = 0.05).
[c] Surviving plants counted at 21 DAA in 1 m^2, expressed as a percentage of the nontreated check.

fomesafen was similar, regardless of time of application, for both biotypes. The percentage of living plants ranged from 8% to 13% and 0% to 2% at the PPO-R and PPO-S location, respectively. These data would suggest that unlike paraquat plus metribuzin, the addition of fomesafen is not additive when paired with paraquat on PPO-R Palmer amaranth.

PPO-R and PPO-S Palmer Amaranth Control Affected by TOD of Glufosinate-Based Applications

Glufosinate applications at sunrise adversely affected control and percentage of living of PPO-R and PPO-S Palmer amaranth. Control of Palmer amaranth was greatest when glufosinate was applied midday (97% at both the PPO-R and PPO-S locations) compared with glufosinate applied at sunrise (63% at the PPO-R location and 61% at the PPO-S location) (Table 6). The percentage of living plants mirrored the control observed with glufosinate. Plots that received an application of glufosinate at sunrise had at least a 7-fold increase in the percentage of living plants when compared with glufosinate applied midday (Table 6). Greater Palmer amaranth control rates have been reported when glufosinate was applied in the middle portion of the day (Culpepper et al. 2013; Martinson et al. 2002; Stewart et al. 2009). Furthermore, it has been reported that light is an essential requirement for glufosinate activity (Kocher 1983). Sellers et al. (2004) reported that significant glutamine synthetase inhibition only occurred during applications made in the light period. Therefore, ammonium accumulation levels were lower in plants treated with glufosinate at 2200 hours (dark) than plants treated at 1400 hours (light); thus, better glufosinate activity is observed from applications made in the light (Sellers et al. 2004).

In our study, control of PPO-S Palmer amaranth 7 DAA was not affected by the time of glufosinate plus fomesafen applications and ranged from 92% to 99% (Table 6). However, control of the PPO-S biotype 21 DAA was affected by the time of application. Although PPO-S control was 90% for the sunrise application of glufosinate plus fomesafen, greater control was observed after the midday application (99%). TOD of glufosinate plus fomesafen applications affected PPO-R Palmer amaranth control 7 and 21 DAA and the number of living plants. PPO-R Palmer amaranth control after glufosinate plus fomesafen applied midday (97% 7 DAA; 85% 21 DAA) was greater than control in plots treated at sunrise (69% 7 DAA; 49% 21 DAA). These differences in PPO-R Palmer amaranth control produced similar trends in observations of the percentage of living plants. More living Palmer amaranth plants were in plots treated with glufosinate plus fomesafen at sunrise (44%) compared with midday applications (13%). These data suggest the glufosinate plus fomesafen tank mix no longer is appropriate for a PPO-R Palmer amaranth biotype on which fomesafen is no longer effective. Glufosinate plus fomesafen on the PPO-S biotype reduces the selection pressure on both herbicides; however, this is no longer an option on the PPO-R biotype (Norsworthy et al. 2012).

PPO-R Palmer amaranth responded differently than PPO-S populations to selected herbicides and application timings used in this study. When comparing the two biotypes in the greenhouse, PPO-inhibiting herbicides did not control PPO-R Palmer amaranth. Biotype differences in this study were generally obvious because Palmer amaranth at the Golddust location was highly resistant compared with the sensitive populations in Jackson. Palmer amaranth biotypes with similar resistance mechanisms have exhibited a greater tolerance to herbicidal control, regardless of site of action (Copeland et al. 2018b; Schwartz-Lazaro et al. 2017). Marginal tolerance to herbicides, such as paraquat alone and glufosinate alone, was observed with the PPO-R biotype in this study. However, no differences in living PPO-R and PPO-S Palmer amaranth plants in plots treated with paraquat plus metribuzin were observed in this study. Applying herbicides with two effective sites of action was critical for management of PPO-R Palmer amaranth. Moreover, previous research has indicated that sequential applications of POST herbicides provide more consistent control of PPO-R Palmer amaranth (Steckel et al. 2018). For example, treatments that included 2,4-D plus glyphosate, 2,4-D or dicamba plus or followed by glufosinate provided greater than 92% control (Steckel et al. 2018). Moreover, herbicides within the current Enlist® or Xtend® systems effectively control PPO-resistant Palmer amaranth (Steckel et al. 2018).

The TOD effect on PPO-S Palmer amaranth with glufosinate was overcome by adding fomesafen, a PPO-inhibiting herbicide. However, the addition of fomesafen to glufosinate added no value for the control of PPO-R Palmer amaranth. Ineffective tank mixes are costly when trying to control Palmer amaranth and will likely result in a yield loss (Copeland et al. 2018b).

Understanding the implications of the application TOD effect on PPO-R Palmer amaranth will assist farmers in developing control strategies. Moreover, PRE herbicide applications should include two or more effective sites of action followed by overlapping WSSA Group 15 herbicides applied POST. However, nonherbicidal control measures should be seriously considered as well.

https://doi.org/10.1017/wet.2019.43 Published online by Cambridge University Press

Cover crops, row spacing, crop rotation, and tillage practices should all be included in an integrated management plan to combat Palmer amaranth.

Author ORCIDs. Lawrence E. Steckel 0000-0002-4004-5102

Acknowledgements. Partial funding was provided by the Tennessee Soybean Promotion Board and Cotton Inc. and is greatly appreciated by the authors. The authors also thank R. Hayes, J. Buck, C. Kemp, C. Little, and H. Love for their assistance in these studies.

Conflicts of interest. J.D.C. is employed by FMC and G.B.M. is employed by Bayer Crop Sciences.

References

Andersen RN, Koukkari WL (1978) Response of velvetleaf (*Abutilon theophrasti*) to bentazon as affected by leaf orientation. Weed Sci 26:393–395

Blouin DC, Webster EP, Bond JA (2011) On the analysis of combined experiments. Weed Technol 25:165–169

Carmer SG, Nyquist WE, Walker WM (1989) Least significant differences in combined analyses of experiments with two- or three-factor treatment designs. Agron J 81:655–672

Coetzer E, Al-Khatiband K, Loughin TM (2001) Glufosinate efficacy, absorption, and translocation in amaranth as affected by relative humidity and temperature. Weed Sci 49:8–13

Copeland JD, Everman WJ, York AC (2017) Investigating Palmer amaranth response to glufosinate in a North Carolina population. Page 124 *in* 70th Proceedings of the Southern Weed Science Society. Westminster, CO: Southern Weed Science Society

Copeland JD, Giacomini DA, Tranel PJ, Montgomery GB, Steckel LE (2018a) Distribution of *PPX2* mutations conferring PPO-inhibitor resistance in Palmer amaranth populations of Tennessee. Weed Technol 32:592–596

Copeland JD, Wiggins M, Steckel LE (2018b) Influence of residual herbicide application rate on PPO-resistant and -susceptible Palmer amaranth in Tennessee. *58th Proceedings of the Weed Science Society*. http://www.wssaabstracts.com/public/54/proceedings.html. Accessed: July 9, 2018

Culpepper AS, York AC, Steckel L, Bond JA, Stephenson D (2013) Palmer amaranth control significantly influenced by the time of day in which Liberty is applied. Page 168 *in* 66th Proceedings of the Southern Weed Science Society. Westminster, CO: Southern Weed Science Society

Doran DL, Andersen RN (1976) Effectiveness of bentazon applied at various times of the day. Weed Sci 24:567–570

Eubank TW, Nandula VK, Poston DH, Shaw DR (2012) Multiple resistance of horseweed to glyphosate and paraquat and its control with paraquat and metribuzin combinations. Agronomy 2:358–370

Fausey JC, Renner KA (2001) Environmental effects on CGA-248757 and flumiclorac efficacy/soybean tolerance. Weed Sci 49:668–674

Giacomini DA, Umphres AM, Nie H, Mueller TC, Steckel LE, Young BG, Scott RC, Tranel PJ (2017) Two new *PPX2* mutations associated with resistance to PPO-inhibiting herbicides in *Amaranthus palmeri*. Pest Manag Sci 73:1559–1563

Heap I (2018) International Survey of Herbicide Resistant Weeds. http://www.weedscience.org Accessed: July 5, 2018

Hess FD, Falk RH (1990) Herbicide deposition on leaf surfaces. Weed Sci 38:280–288

Johnston CR, Eure PM, Grey TL, Culpepper AS, Vencil WK (2018) Time of application influences translocation of auxinic herbicides in Palmer amaranth (*Amaranthus palmeri*). Weed Sci 66:4–14

Kocher H (1983) Influence of the light factor on physiological effects of the herbicide Hoe 39866. Aspects Appl Biol 4:227–234

Kupper A, Manmathan HK, Giacomini D, Patterson EL, McCloskey WB, Gaines TA (2018) Population genetic structure in glyphosate-resistant and -susceptible Palmer amaranth (*Amaranthus palmeri*) populations using genotyping-by-sequencing (GBS). Front Plant Sci 9:29

Lee SD, Oliver LR (1982) Efficacy of acifluorfen on broadleaf weeds. Times and methods for application. Weed Sci 30:520–526

Martinson KB, Sothern RB, Koukkari WL, Durgan BR, Gunsolus JL (2002) Circadian response of annual weeds to glyphosate and glufosinate. Chronobiol Int 19:405–422

Miller R, Martinson KB, Sothern RB, Durgan BR, Gunsolus JL (2003) Circadian response of annual weeds in a natural setting to high and low application rates of four herbicides with different modes of actions. Chronobiol Int 20:299–324

Mohr KB, Sellers BA, Smeda RJ (2007) Application time of day influences glyphosate efficacy. Weed Technol 21:7–13

Montgomery GB, Treadway JA, Reeves JL, Steckel LE (2017) Effect of time of day of application of 2, 4-D, dicamba, glufosinate, paraquat, and saflufenacil on horseweed (*Conyza canadensis*) control. Weed Technol 31:550–556

Norsworthy JK, Oliver LR, Purcell LC (1999) Diurnal leaf movement effects on spray interception and glyphosate efficacy. Weed Technol 13:466–470

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci: 60:31–62

Putnam AR, Ries SK (1968) Factors influencing the phytotoxicity and movement of paraquat in quackgrass. Weed Sci 16:80–83

Saxton AM (2013) DandA.sas. Design and Analysis Macro Collection, Version 2.11. Knoxville, TN: University of Tennessee. Publication 17

Schwartz-Lazaro LM, Norsworthy JK, Scott RC, Barber LT (2017) Resistance of two Arkansas Palmer amaranth populations to multiple herbicide sites of action. Crop Prot 96:158–163

Sellers BA, Smeda RJ, Li J (2004) Glutamine synthetase activity and ammonium accumulation is influenced by time of glufosinate application. Pestic Biochem Physiol 78:9–20

Steckel LE, Simmons FW, Sprague CL (2003) Soil factor effects on tolerance of two corn (*Zea mays*) hybrids to isoxaflutole plus flufenacet. Weed Technol 17:599–604

Steckel SS, Copeland JD, Steckel LE (2018) Postemergence options for control of multiple-resistant Palmer amaranth in Tennessee. *58th Proceedings of the Weed Science Society*. http://www.wssaabstracts.com/public/54/proceedings.html. Accessed: October 24, 2018

Stewart CL, Nurse RE, Sikkema PH (2009) Time of day impacts postemergence weed control in corn. Weed Technol 23:346–355

Stopps GJ, Nurse RE, Sikkema PH (2013) The effect of time of day on the activity of postemergence soybean herbicides. Weed Technol 27:690–695

Umphres AM (2017) Response of glyphosate resistant Palmer amaranth (*Amaranthus palmeri*) to protoporphyrinogen oxidase inhibiting herbicides in Tennessee. PhD dissertation. Knoxville, TN: University of Tennessee. http://trace.tennessee.edu/utk_graddiss/4663 Accessed: October 21, 2018

Varanasi VK, Brabham C, Norsworthy JK, Nie H, Young BG, Houston M, Barber T, Scott RC (2017) A statewide survey of PPO-inhibitor resistance and the prevalent target-site mechanisms in Palmer amaranth (*Amaranthus palmeri*) accessions from Arkansas. Weed Sci 68:1–10

Ward SM, Webster TM, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*): a review. Weed Technol 27:12–27

https://doi.org/10.1017/wet.2019.43 Published online by Cambridge University Press