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

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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. Coetzter 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. (Coetzter 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 (Coetzter et al. 2001).

Herbicide application time of day (TOD) is a 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; Muller 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
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 diflufenopyr (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 herbaecae (Mill.) McVaugh], and sicklepod [Senna obtusifolia (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 et al. 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 PPO2 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 CO2-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
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 Goldust, 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 Goldust 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 environments (Blouin et al. 2011; Carmer et al. 1989). TOD was selected randomly from a population of years (Steckel et al. 2011). Data were analyzed over the 2 years because each year was considered to be a No. of Palmer amaranth plants m$^{-2}$.

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
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 was 99% and 100%, respectively. Control of PPO-R and PPO-S Palmer amaranth was similar after glufosinate alone or glufosinate plus fomesafen was applied. 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).

### 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 | Mortality |
|-------------------|---------|---------|-----------|
| Glufosinate       | PPO-R   | 95%     | 74%       |
|                   | PPO-S   | 99%     | 100%      |
| P value           |         | 0.0756  | 0.0756    |
| Acifluorfen       | PPO-R   | 98%     | 83%       |
|                   | PPO-S   | 99%     | 100%      |
| P value           |         | 0.3632  | 0.3632    |

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

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 was similar after glufosinate alone or glufosinate plus fomesafen 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 was 99% and 100%, respectively. Control of PPO-R and PPO-S Palmer amaranth was similar after glufosinate alone or glufosinate plus fomesafen was 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).
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; Stopp et al. 2013); however, this does not apply to all herbicides (Lee and Oliver 1982; Montgomery et al. 2017). 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 (Norsworthy et al. 2012). In midday applications (Montgomery et al. 2017). TOD of paraquat plus metribuzin applications did not affect control of PPO-R Palmer amaranth 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).

TOD of paraquat plus fomesafen applications did not affect control of PPO-R or PPO-S Palmer amaranth control 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 fomesafen,

### 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.

| Biotype | TODa | Control 7 DAA | Living 7 DAA | Control 21 DAA | Living 21 DAA | Paraquatb | Fomesafenb | Acifluorfenb |
|---------|------|--------------|-------------|---------------|-------------|---------|-----------|------------|
| 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.

### 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.

| Biotype | TODa | Control 7 DAA | Living 7 DAA | Control 21 DAA | Living 21 DAA | Paraquatb | Paraquat plus metribuzinb | Paraquat plus fomesafenb |
|---------|------|--------------|-------------|---------------|-------------|---------|-------------------------|-------------------------|
| PPO-R   | Sunrise | 92 | 88 | 4 | 99 | 96* | 1 | 95 | 86 | 8 |
|         | Midday  | 91 | 80 | 11* | 97 | 91 | 92 | 92 | 85 | 97 | 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.

Analysis of variance with LSD was used to determine the significance of differences among the six variables. Values reported in the table indicate significant differences at P = 0.05 (indicated by *).
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 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 Gold Dust 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.

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**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.

| Biotype | TOD | Glufosinate<sup>a</sup> | Glufosinate plus fomesafen<sup>b</sup> |
|---------|-----|----------------------|-----------------------------------|
|         |     | Control | Living | Control | Living |
|         |     | 7 DAA | 21 DAA | plants<sup>c</sup> | 7 DAA | 21 DAA | plants<sup>c</sup> |
| PPO-R   | Sunrise | 63 | 41 | 73* | 69 | 49 | 44* |
|         | Midday | 97* | 85* | 6 | 97* | 85* | 13 |
| PPO-S   | Sunrise | 61 | 26 | 76* | 99 | 99* | 0 |
|         | Midday | 97* | 91* | 10 | 0.0591 | <0.0001 | 0.0337 |

<sup>a</sup> Abbreviations: DAA, days after application; PPO-R, protoporphyrinogen IX oxidase resistant; PPO-S, protoporphyrinogen IX oxidase susceptible; TOD, time of day.

<sup>b</sup> Asterisk (*) indicates significance according to Fisher protected LSD (P = 0.05).

<sup>c</sup> Surviving plants counted at 21 DAA in 1 m², expressed as a percentage of the nontreated check.
Cover crops, row spacing, crop rotation, and tillage practices should all be included in an integrated management plan to combat Palmer amaranth.

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