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Influence of herbicides on germination and quality of Palmer amaranth (*Amaranthus palmeri*) seed

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Research Article

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Nomenclature:
acifluorfen; dicamba; ethephon; flumioxazin; fomesafen; halosulfuron; linuron; metribuzin; oryzalin; pendimethalin; pyroxasulfone; *S*-metolachlor; saflufenacil; trifluralin; 2,4-D; Palmer amaranth, *Amaranthus palmeri* S. Watson; AMAPA

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Abstract

Laboratory and greenhouse studies were conducted to evaluate the effects of chemical treatments applied to Palmer amaranth seeds or gynoecious plants that retain seeds to determine seed germination and quality. Treatments applied to physiologically mature Palmer amaranth seed included acifluorfen, dicamba, ethephon, flumioxazin, fomesafen, halosulfuron, linuron, metribuzin, oryzalin, pendimethalin, pyroxasulfone, *S*-metolachlor, saflufenacil, trifluralin, and 2,4-D plus crop oil concentrate applied at 1× and 2× the suggested use rates from the manufacturer. Germination was reduced by 20% when 2,4-D was used, 15% when dicamba was used, and 13% when halosulfuron and pyroxasulfone were used. Use of dicamba, ethephon, halosulfuron, oryzalin, trifluralin, and 2,4-D resulted in decreased seedling length by an average of at least 50%. Due to the observed effect of dicamba, ethephon, halosulfuron, oryzalin, trifluralin, and 2,4-D, these treatments were applied to gynoecious Palmer amaranth inflorescence at the 2× registered application rates to evaluate their effects on progeny seed. Dicamba use resulted in a 24% decrease in seed germination, whereas all other treatment results were similar to those of the control. Crush tests showed that seed viability was greater than 95%, thus dicamba did not have a strong effect on seed viability. No treatments applied to Palmer amaranth inflorescence affected average seedling length; therefore, chemical treatments did not affect the quality of seeds that germinated.

Introduction

Palmer amaranth is the most troublesome weed in broadleaf, grass, fruit, and vegetable crops in the United States (Van Wychen 2019, 2020). The troublesome nature of this weed is due in part to its large and rapid growth (Horak and Loughin 2000; Keeley et al. 1987; Norsworthy et al. 2008; Sellers et al. 2003), high fecundity (Keeley et al. 1987; Sellers et al. 2003; Sosnoskie et al. 2014), and dioecious reproduction causing obligate outcrossing (Ward et al. 2013). The high fecundity and obligate outcrossing create large genetic diversity. High genetic diversity coupled with intensive herbicidal selection pressure has caused herbicide resistance in Palmer amaranth to become commonplace (Heap 2021), thereby increasing the difficulty of managing it.

Effective long-term Palmer amaranth management requires strategies that limit or eliminate contributions of this weed to the soil seedbank. Continuous control of weeds prior to reproductive maturity can provide minimal seedbank additions, however, weeds often escape management strategies and produce seeds. Flowering Palmer amaranth severed at the soil surface may resprout and produce 22,000 seeds plant^{-1} (Sosnoskie et al. 2012). Harvest weed seed control techniques have proven effective for Palmer amaranth management (Norsworthy et al. 2016; Schwartz-Lazaro et al. 2017a). Palmer amaranth seed retention at soybean [*Glycine max* (L.) Merr.] harvest was 95% to 100% (Schwartz et al. 2016). One month after soybean harvest, corresponding to 19 wk after planting (WAP), Palmer amaranth retained 95% of total seed produced (Schwartz-Lazaro et al. 2017b). Palmer amaranth seeds entering an integrated Harrington Seed Destructor mill were 100% destroyed (Schwartz-Lazaro et al. 2017a). Harvest weed seed control can reduce the amount of viable seeds added into the soil seedbank for crops harvested with a combine, but this technology has few applications in vegetable crops. Thus, additional methods for reducing weed seedbank additions from escaped weeds are needed.

Control of Palmer amaranth with herbicides is well documented (Barkley et al. 2016; Meyers et al. 2013; Moore et al. 2021; Ward et al. 2013; Whitaker et al. 2011). However, because herbicides are most effective in preventing weed interference when applied at early growth stages,

Table 1. Herbicides and growth regulator trade names, use rates, and manufacturers.[a]

Active ingredient	Trade name	Rates	Manufacturer	City, State	Website
		g ai/ae ha^{-1}			
Acifluorfen	Ultra Blazer	420, 840	United Phosphorus, Inc.	King of Prussia, PA	www.upi-usa.com
Dicamba	Xtendimax	1,120, 2,230	Bayer CropScience	St. Louis, MO	www.cropscience.bayer.us
Ethephon	Super Boll	1,090, 2,180	Nufarm Americas Inc.	Alsip, IL	www.nufarm.com
Flumioxazin	Valor SX	110, 210	Valent U.S.A. Corporation	Walnut Creek, CA	www.valent.com
Fomesafen	Reflex	420, 840	Syngenta Crop Protection, LLC	Greensboro, NC	www.syngenta-us.com
Halosulfuron	Sandea	40, 80	Gowan Company	Yuma, AZ	www.gowanco.com
Linuron	Linex 4L	840, 1,680	Tessenderlo Kerley, Inc.	Phoenix, AZ	www.novasource.com
Metribuzin	Tricor 4F	560, 1,120	United Phosphorus, Inc.	King of Prussia, PA	www.upi-usa.com
Oryzalin	Surflan	1,120, 2,240	United Phosphorus, Inc.	King of Prussia, PA	www.upi-usa.com
Pendimethalin	Pendulum AquaCap	1,070, 2,130	BASF Corporation	Research Triangle Park, NC	www.basf.com
Pyroxasulfone	Zidua WG	120, 240	BASF Corporation	Research Triangle Park, NC	www.basf.com
S-metolachlor	Dual Magnum	800, 1,600	Syngenta Crop Protection, LLC	Greensboro, NC	www.syngenta-us.com
Saflufenacil	Detail	50, 100	BASF Corporation	Research Triangle Park, NC	www.basf.com
Trifluralin	Treflan 4L	1,120, 2,240	Loveland Products, Inc.	Greeley, CO	www.lovelandproducts.com
2,4-D	Enlist One	1,070, 2,130	Corteva Agriscience	Wilmington, DE	www.Corteva.com

[a]Crop oil concentrate (1% vol vol^{-1}) was included in all treatments.

little research has evaluated their effect on the viability of seeds present at application. Jha and Norsworthy (2012) reported that Palmer amaranth seed viability was reduced by 36% to 51% when 2,4-D, dicamba, glufosinate, and glyphosate were used and when plants were treated at first signs of female inflorescence. In addition, seed production was reduced by 62% to 95%. Other research evaluating 2,4-D, dicamba, glufosinate, and glyphosate applied at first female Palmer amaranth inflorescence resulted in decreased seed production but not germination or viability (Scruggs et al. 2020). Similarly, registered rates of acifluorfen, dicamba, fluthiacet, fomesafen, glyphosate, and lactofen applied to Palmer amaranth up to 90 cm tall did not affect seed viability but seed production was reduced (de Sanctis et al. 2021). Each of these studies evaluated herbicides that were applied before seed maturity. When Palmer amaranth plants were treated when inflorescences had mature seeds (i.e., brown to black seed coat), 2,4-D, dicamba, glufosinate, MSMA, paraquat, and pyraflufen-ethyl use resulted in a 35% reduction in viable seed production (Sarangi et al. 2020).

Only a few herbicides have been registered for use to control Palmer amaranth among vegetable crops, thus the use of hand removal is often required to prevent additions into the seedbank (SC Smith and LD Moore, unpublished data). Acifluorfen, flumioxazin, fomesafen, saflufenacil (protoporphyrinogen oxidase-inhibitors, G14), halosulfuron (acetolactate synthase (ALS)-inhibitor, G2), linuron, metribuzin (photosynthesis at PSII-inhibitors, G5), oryzalin, pendimethalin, trifluralin (microtubule assembly-inhibitors, G3), pyroxasulfone, and *S*-metolachlor (very long-chain fatty acid synthesis-inhibitors, G15) are herbicides that have activity on susceptible biotypes of Palmer amaranth and are registered for use in certain vegetable crop production. Previous research reported that trifluralin (1.7 g ai kg seeds^{-1}) reduced cheat (*Bromus secalinus* L.) emergence by more than 90% when applied in a sprayer-equipped auger (Stone et al. 2001). Dicamba and 2,4-D are auxin-mimic herbicides (Group 4) that may be used as preplant burndown treatments in some vegetable crops. Ethephon is a plant growth regulator used for turf, apple (*Malus domestica* Borkh.), cotton (*Gossypium hirsutum* L.), and many vegetable crops (Anonymous 2019, 2020). Ethephon promotes fruit ripening, abscission, and flower induction (EPA 1995). In addition, ethephon can break the seed dormancy of several weed species (Goudey et al. 1987). Herbicides can reduce reproductive Palmer amaranth seed production by decreasing photosynthesis; however, the effects of chemicals on the viability of seeds already present at application are unknown. Thus, these studies were conducted to determine the seed germination and quality of Palmer amaranth seeds and gynoecious plants that retain seeds after chemical treatments used in vegetable crops.

Materials and Methods

Chemical Seed Treatment

Seeds were collected from mature Palmer amaranth at the Horticultural Crops Research Station near Clinton, NC (35.022° N, 78.280°W) in 2016 and stored at 4 C and 25% relative humidity (RH) for at least 2 yr before their use in evaluations. Resistance to glyphosate and ALS is common in this population, and susceptibility to other herbicides at labeled rates is still present. Laboratory studies were initiated on November 3 and November 13, 2018, in Raleigh, NC. The experimental design was completely randomized with four replications in each study. Treatments (Table 1) included herbicides, or a growth regulator (ethephon) applied at 1× and 2× registered application rates plus crop oil concentrate (1% vol vol^{-1}). Additionally, a control, treated with 1% vol vol^{-1} crop oil concentrate, was included for comparison. For each treatment, 20 mature (black) Palmer amaranth seeds per experimental unit were placed into a 14 by 14 by 2.5 cm-deep plastic weigh boat and misted with water for imbibition. After approximately 1 h, once imbibition was visually confirmed and excess water had evaporated from the weigh boats, treatments were applied to one experimental unit at a time in a spray chamber equipped with an 8002EVS nozzle (TeeJet Technologies, Springfield, IL) pressurized with 210 kPa CO_2 and calibrated to administer a 375 L ha^{-1} spray solution. The application pressure was confirmed to be low enough to prevent seeds from moving off of the weigh boats.

Approximately 2 h after treatment, once weigh boats containing seeds were dry, treated seeds from each experimental unit were transferred to a 10-cm-diam petri dish containing filter paper moistened with 10 ml of water. Dishes were placed on a laboratory bench with fluorescent lighting provided at an 8-h photoperiod. Temperature was set at 21 C, and additional water was added equally to all dishes daily. Germination was counted, then seedlings (radicle and hypocotyl) were imaged 3 d after treatment (DAT)

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using a flatbed scanner (Expression 10000 XL; Epson America, Long Beach, CA). Seedlings were arranged to avoid overlapping during scanning. Root measurement image analysis software (WinRHIZO 2019a; Regent Instruments, Quebec, QC, Canada) was used to measure total seedling length. Average seedling length was calculated by dividing the total length of seedlings by the number of germinated seeds in the experimental unit.

Residual plots were assessed for normality and homogeneity of variance. Arcsine square root transformations were applied for germination data. Data were subjected to ANOVA using the GLM procedure in SAS software (version 9.4; SAS Institute, Cary, NC). Dunnett's test was used to compare treatments to the control ($\alpha = 0.05$). Back-transformed germination means were presented.

Chemical Inflorescence Treatment

Treatments from the *Chemical Seed Treatment* studies that limited the average seedling length to <1 cm were included in a further examination to determine their effects on Palmer amaranth progeny from treatments applied to reproductively mature plants. Studies were initiated at the North Carolina State University Method Road Greenhouses (35.788°N, 78.694°W) on October 30 and December 17, 2018. The experimental design was a randomized complete block with eight replications in each study. Palmer amaranth seeds, from the same lot as the *Chemical Seed Treatment* studies, were surface seeded into 10 by 10 by 9 cm-deep pots (Square Injection Molded Pots; Greenhouse Megastore, Danville, IL) containing moistened peat-perlite substrate (SunGro Fafard 4P Mix; Agawam, MA) and thinned to one plant per pot. Pots were overhead irrigated, temperature in the greenhouse was 30/25 ± 5 C d/night, and supplemental lighting was provided at 150 μmol m^{-2} s^{-1} for a 16-h photoperiod. Initially, contiguous arrangement of pots encouraged vertical growth with a single primary inflorescence. Once reproductive structures were visible, pots were spaced 10 cm apart and arranged so that each sex was aside the opposite. Additionally, androecious plants were shaken daily over gynoecious plants to ensure adequate pollination. On January 26, 2019, and March 16, 2019, gynoecious plants were grouped into blocks by plant height and treatments were applied at a 2× rate using a CO_2-pressurized backpack sprayer equipped with 8003VS nozzles pressurized with 170 kPa. Treatments were applied vertically to both sides of the plant directed at the inflorescence with a total output of 470 L ha^{-1}.

After treatment, plants were placed back into the greenhouse, without androecious plants, for 2 wk. Then, inflorescences were harvested and threshed, and seeds were separated from floral material using a vertical air column seed cleaner and stored at 4 C and 25% RH. Six months after being placed in cold storage, 30 mature (black) seeds per experimental unit were placed into 10-cm-diam petri dishes with filter paper moistened with 10 ml water, sealed with parafilm, and placed back into cold storage for 4 wk to overcome dormancy. Then, parafilm was removed, additional water was added equally to all dishes, and they were placed into a germination chamber at a 16-h photoperiod set to 35/25 C d/night and 100% RH for 3 d. Germination was determined, and seedling length was measured as previously described. Seed viability was assessed on 30 seeds per plot using a crush test (Sawma and Mohler 2002).

Residual plots were assessed for normality and homogeneity of variance. Data were subjected to ANOVA using the MIXED procedure in SAS software. Fixed effects included experimental run, treatment, and their interaction, and the random effect included replication nested within experimental run. Dunnett's test was used to compare treatments to the control ($\alpha = 0.05$).

Table 2. Influence of chemicals applied to Palmer amaranth seeds on germination and seedling length.[a,b,c]

	Germination		Average seedling length		Total seedling length	
	%		cm			
Crop oil control	95	–	1.8	–	34	–
Acifluorfen	85		1.7		29	
Dicamba	80	***	0.9	***	15	***
Ethephon	96		0.9	***	16	***
Flumioxazin	88		1.5		26	
Fomesafen	88		1.7		31	
Halosulfuron	82	***	0.9	***	14	***
Linuron	87		1.9		32	
Metribuzin	92		1.7		32	
Oryzalin	91		0.6	***	12	***
Pendimethalin	88		1.1	***	20	***
Pyroxasulfone	82	***	1.4		23	***
S-metolachlor	88		1.4		24	
Saflufenacil	89		1.5		26	
Trifluralin	91		0.8	***	15	***
2,4-D	75	***	0.8	***	11	***

[a]Crop oil concentrate (1% vol vol^{-1}) was included in all treatments.
[b]Data were pooled across 1× and 2× registered use rates.
[c]Means followed by *** are statistically different from the control according to Dunnett's test ($\alpha = 0.05$).

Results and Discussion

Chemical Seed Treatment

Interactions with experimental run were not significant for germination ($P > 0.07$) or average ($P > 0.06$) and total ($P > 0.06$) seedling length data; therefore, data were pooled over experimental runs. For germination and total and average seedling length data, the treatment effect was significant ($P < 0.0001$), and the rate effect and treatment by rate interaction were not significant ($P > 0.5$); therefore, only treatment main effects are presented. Germination was reduced by 20% from 2,4-D, 15% from dicamba, and 13% from halosulfuron and pyroxasulfone (Table 2). Dicamba, ethephon, halosulfuron, oryzalin, trifluralin, and 2,4-D use resulted in a seedling length that was reduced by at least 50%; thus, these treatments were evaluated further in the *Chemical Inflorescence Treatment* studies.

Chemical Inflorescence Treatment

Interactions with experimental run were not significant for germination ($P = 0.09$) or average ($P = 0.2$) and total ($P = 0.5$) seedling length data; therefore, data were pooled over experimental runs. Treatment had a significant effect on seed germination ($P < 0.0001$). Germination was reduced by 24% when dicamba was used compared to crop oil alone (Table 3). Germination among all other treatments were similar to those of the control. Crush tests results showed that seeds from plants treated with dicamba had 95% viability compared with 98% viability in the crop oil control ($P = 0.01$; data not shown). Thus, treatments did not have a strong effect on seed viability. Total seedling length was reduced ($P = 0.01$) when dicamba and 2,4-D were used, but no treatments affected average seedling length ($P = 0.17$). Total seedling length is the compound effects of the number of germinated

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Table 3. Influence of chemicals applied to Palmer amaranth inflorescence on progeny seed germination and seedling length.[a,b,c]

	Germination		Average seedling length		Total seedling length	
	%		cm			
Crop oil control	82	–	4.1	–	99	–
Dicamba	58	***	3.8		69	***
Ethephon	83		3.8		93	
Halosulfuron	83		3.9		99	
Oryzalin	81		4.1		101	
Trifluralin	80		4.1		97	
2,4-D	78		3.6		83	***

[a]Crop oil concentrate (1% vol vol^{-1}) was included in all treatments.
[b]Treatments were applied at 2× registered use rates.
[c]Means followed by *** are statistically different from the control according to Dunnett's test ($\alpha = 0.05$).

seeds and the seedling lengths, whereas average seedling length is the length of the seedlings regardless of the total number of germinated seeds. Therefore, because the average seedling lengths were not affected, treatments did not have a strong effect on the quality of seeds that germinated. The differences between results from the seed treatment and the inflorescence treatment studies were likely due to the inflorescences protecting seeds from direct contact with the full application rate of the chemicals.

Late-season herbicide applications can reduce seed production (de Sanctis et al. 2021; Jha and Norsworthy 2012; Sarangi et al. 2020; Scruggs et al. 2020). However, no treatments applied to Palmer amaranth after seed development caused a great decrease in seed viability in the present study, although previous research reported that trifluralin applied to cheat seed can reduce emergence by more than 90% (Stone et al. 2001). Based on the results of this study, late-season chemical application will likely not replace weed removal or seed destruction as a form of seedbank management.

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