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Multiple herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) in Connecticut: confirmation and response to POST herbicides

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Abstract

Palmer amaranth is the latest pigweed species documented in Connecticut; it was identified there in 2019. In a single-dose experiment, the Connecticut Palmer amaranth biotype survived the field-use rates of glyphosate (840 g ae ha^{-1}) and imazaquin (137 g ai ha^{-1}) herbicides applied separately. Additional experiments were conducted to (1) determine the level of resistance to glyphosate and acetolactate synthase (ALS) inhibitors in the Connecticut-resistant (CT-Res) biotype using whole-plant dose-response bioassays, and (2) evaluate the response of the CT-Res biotype to POST herbicides commonly used in Connecticut cropping systems. Based on the effective dose required for 90% control (ED₉₀), the CT-Res biotype was 10-fold resistant to glyphosate when compared with the Kansas-susceptible (KS-Sus) biotype. Furthermore, the CT-Res biotype was highly resistant to ALS-inhibitor herbicides; only 18% control was achieved with 2,196 g ai ha⁻¹ imazaquin. The CT-Res biotype was also cross-resistant to other ALS-inhibitor herbicides, including chlorimuron-ethyl (13.1 g ai ha⁻¹), halosulfuron-methyl (70 g ai ha⁻¹), and sulfometuron-methyl (392 g ai ha⁻¹). The CT-Res Palmer amaranth was controlled 75% to 100% at 21 d after treatment (DAT) with POST applications of 2,4-D (386 g ae ha⁻¹), carfentrazone-ethyl (34 g ai ha⁻¹), clopyralid (280 g ae ha⁻¹), dicamba (280 g ae ha⁻¹), glufosinate (595 g ai ha⁻¹), lactofen (220 g ai ha⁻¹), oxyfluorfen (1,121g ai ha⁻¹), and mesotrione (105 g ai ha⁻¹) herbicides. Atrazine (2,240 g ai ha⁻¹) controlled the CT-Res biotype only 52%, suggesting the biotype is resistant to this herbicide as well. Here we report the first case of Palmer amaranth from Connecticut with multiple resistance to glyphosate and ALS inhibitors. Growers should proactively use all available weed control tactics, including the use of effective PRE and alternative POST herbicides (tested in this study), for effective control of the CT-Res biotype.

Introduction

Palmer amaranth, a member of the Amaranthaceae, is native to northwestern Mexico, southern California, New Mexico, and Texas (Sauer 1957). It has been ranked the most troublesome and difficult-to-control weed in several agricultural and horticultural crops in the United States (Van Wychen 2017). Palmer amaranth interference and resultant yield losses have been documented in several crops, such as bell pepper (*Capsicum annuum* L.), cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), grain sorghum (*Sorghum bicolor* L. Moench), muskmelon (*Cucumis melo* L.), peanut (*Arachis hypogaea* L.), pecan [*Carya illinoinensis* (Wangenh) K. Koch], soybean (*Glycine max* L.), sweet potato (*Ipomoea batatas* L. Lam.), tomato (*Solanum lycopersicum* L.), and watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] (Aulakh et al. 2011, 2012, 2013; Bertucci et al. 2019; Burke et al. 2007; Chahal et al. 2017; Garvey et al. 2013; Grichar 1997; Mayers et al. 2010; Mohseni-Moghadam et al. 2013; Moore et al. 2004; Nerson 1989; Norsworthy et al. 2008; Price et al. 2018).

Palmer amaranth is extremely competitive and invasive because of C4 photosynthesis, high water use efficiency, rapid growth (0.10–0.21 cm growing degree-day⁻¹), tall stature (≥ 2 m), and prolific seed production (>600,000 seeds plant⁻¹) (Bensch et al. 2003; Horak and Loughin 2000; Keeley et al. 1987; Massinga et al. 2001; Smith et al. 2000; Sosnoskie et al. 2014; Steckel 2007; Ward et al. 2013). Compared to waterhemp [*Amaranthus rudis* (L.) Sauer], redroot pigweed (*A. retroflexus* L.), and tumble pigweed (*A. albus* L.), Palmer amaranth produced the greatest plant dry weight, leaf area, and height, and had the fastest growth rate (Horak and Loughin 2000). Palmer amaranth and waterhemp are dioecious, whereas redroot pigweed, Powell amaranth, spiny amaranth (*A. spinosis* L.), and smooth pigweed (*A. hybridus* L.) are monoecious (Bryson and DeFelice 2010).

The most concerning characteristic of Palmer amaranth is its tendency to rapidly evolve herbicide resistance. The repeated use of a single herbicide with a specific site of action has led to the evolution of herbicide resistance in many weeds, including Palmer amaranth (Heap 2020). Currently in the United States, Palmer amaranth is resistant to eight herbicide sites of action, including acetolactate-synthase inhibitors, enolpyruvyl shikimate-3-phosphate synthase (EPSPS) inhibitors, 4-hydroxyphenylpyruvate dioxygenase inhibitors, long-chain fatty acid inhibitors, microtubule inhibitors, photosystem II (PSII) inhibitors, protoporphyrinogen oxidase inhibitors, and synthetic auxins (Chahal et al. 2017; Culpepper et al. 2006; Gossett et al. 1992; Jhala et al. 2014; Salas et al. 2016; Sprague et al. 1997; Thompson et al. 2012). In addition, many Palmer amaranth populations are resistant to herbicides from more than one site of action (Heap 2020). In the United States, Palmer amaranth resistant to acetolactate synthase (ALS)-inhibiting herbicides and glyphosate (an EPSPS inhibitor) is widespread (Heap 2020). The first case of ALS-inhibitor resistant Palmer amaranth was reported in Kansas in 1993 (Horak and Peterson 1995). Burgos et al. (2001) reported that Palmer amaranth biotypes resistant to imazaquin, an ALS-inhibiting herbicide, were cross-resistant to other ALS-inhibiting herbicides such as chlorimuron-ethyl, diclosulam, and pyrithiobac. Likewise, glyphosate-resistant Palmer amaranth was first discovered in Macon County, GA, in 2006 (Culpepper et al. 2006). As of 2020, glyphosate-resistant Palmer amaranth populations have been confirmed in 28 U.S. states (Heap 2020). Some of these glyphosate-resistant Palmer amaranth biotypes required 115 times higher glyphosate application than the susceptible plants to achieve 50% control (Norsworthy et al. 2008; Steckel et al. 2008).

Palmer amaranth is the most recently identified pigweed species in Connecticut. It was first discovered in a pumpkin (Cucurbita pepo L.) field in Hartford County, CT, in the fall of 2019 (Aulakh 2019). Palmer amaranth has also been confirmed in the neighboring northeastern states of Massachusetts and New York (USDA-APHIS 2020). Common pigweeds in the Amaranthaceae reported to occur in Connecticut are livid amaranth (A. blitum L.), Powell amaranth (A. powellii S. Watson), prostrate pigweed (A. blitoides S. Watson), redroot pigweed, smooth pigweed, spiny amaranth, waterhemp, and tumble pigweed. The Palmer amaranth biotype in Connecticut is believed to have been introduced from Massachusetts via contaminated farm equipment. As of 2019, Palmer amaranth infestation in Connecticut was reported on approximately 80 ha under pumpkin production. Because of widespread resistance in Palmer amaranth to ALS-inhibiting herbicides and glyphosate in the United States, it was necessary to understand the response of the newly discovered Palmer amaranth population in Connecticut to ALS-inhibitor and glyphosate herbicides. Therefore, Palmer amaranth seeds were collected randomly from multiple plants to conduct whole-plant dose-response bioassays with the objectives to (1) determine if the Connecticut Palmer amaranth was resistant to ALS-inhibitor and glyphosate herbicides and (2) evaluate the response of the Connecticut Palmer amaranth to POST herbicides from alternate sites of action commonly used in various cropland and noncropland areas in Connecticut.

Materials and Methods

Seed Collection and Preparation

In the fall of 2019, approximately 50 Palmer amaranth seed heads were collected randomly from a pumpkin field in Hartford County, CT (41.93°N, 72.53°W). The seed heads were manually threshed and seeds were cleaned thoroughly using a vertical air column blower and stored separately in airtight polyethylene bags at 5 C until used. The Connecticut biotype was designated "CT-Res." In addition, seeds of a Palmer amaranth biotype from Kansas State University Agricultural Research Center near Hays, KS, with a known history of effective control with the field-use rate of glyphosate (designated KS-Sus) were included for comparison.

Single-Dose Experiments for Resistance Confirmation

Greenhouse experiments were conducted at the Connecticut Agricultural Experiment Station, Windsor, CT, to determine the response of the CT-Res biotype to field-use rates of glyphosate (840 g ae ha⁻¹) and imazaquin (137 g ai ha⁻¹) herbicides. Seeds of the CT-Res biotype were sown on the surface of flat trays (52 \times 26 \times 6 cm) containing a 2:1 composted pine bark to peat moss mixture. After emergence, Palmer amaranth seedlings were thinned to 50 seedlings tray⁻¹. The plants were supplied with water and nutrients and kept in a greenhouse maintained at a 32/27 C day/night temperature regimen with a 16-h photoperiod supplemented by overhead sodium halide lamps. The study was conducted in a completely randomized design with two flat tray (50 seedlings tray⁻¹) for each tested herbicide. Seedlings were treated with glyphosate (MADDOG*; Loveland Products, Inc., Loveland, CO) or imazaquin (Scepter®; BASF Corp., Research Triangle Park, NC) at the 5- to 6-leaf stage (8–10 cm tall). Each herbicide treatment was prepared in distilled water and mixed with a nonionic surfactant (Induce; Helena Chemical Co., Collierville, TN) at 0.25% vol/vol. Herbicide treatments were applied with a compressed CO₂ backpack sprayer through a single flat-fan spray nozzle AIXR 8002 (TeeJet*; Spraying Systems Co., Wheaton, IL) calibrated to deliver 190 L ha⁻¹ spray volume at 207 kPa and 3.5 kph. Palmer amaranth control was assessed visually at 7 and 14 d after treatment (DAT) on a scale ranging from 0% (no control) to 100% (complete control or death of plants). Control ratings were recorded on the basis of symptoms such as chlorosis, necrosis, and stunting of treated plants compared with nontreated control plants.

Dose-Response Bioassay

Whole-plant dose-response bioassays were conducted in a greenhouse at the Connecticut Agricultural Experiment Station, Windsor, CT, to determine the level of ALS-inhibitor and glyphosate resistance in the CT-Res biotype. Because Palmer amaranth was not known to exist in Connecticut before 2019, the known glyphosate susceptible biotype from Kansas (KS-Sus) was included for comparison. Plants from both CT-Res and KS-Sus biotypes were separately grown in plastic pots (10-cm diam) containing the same potting mixture as previously described. The study was laid out in a randomized complete block design with a 9×2 factorial arrangement of treatments and 21 replications (each replication was 1 plant pot⁻¹). The two factors were (1) nine glyphosate or imazaquin rates (0, 0.125×, 0.25×, $0.5\times$, $1\times$, $2\times$, $4\times$, $8\times$, and $16\times$), where $1\times$ is the field-use rate of glyphosate (840 g ae ha^{-1}) or imazaquin (137 g ai ha^{-1}) and (2) two Palmer amaranth biotypes (CT-Res and KS-Sus). Greenhouse conditions were maintained as described previously and the experiment was conducted five times under similar growing conditions.

Palmer amaranth seedlings were separately treated with different glyphosate or imazaquin rates at the 5- to 6-leaf stage (8–10 cm tall) as described in the single-dose experiments. Palmer amaranth control was assessed visually at 7 and 14 DAT using the same injury scale as described for the single-dose experiments. Control ratings were recorded on the basis of symptoms such as chlorosis, necrosis, and stunting of treated plants compared with nontreated control plants. Plants were harvested at 14 DAT, oven dried for 4 d at 65 C, and aboveground dry weight was determined. The biomass data were converted into percent biomass reduction compared to the nontreated control (Wortman 2014) as shown in Equation 1:

Biomass reduction (%) =
$$\frac{(\overline{C} - B)}{\overline{C}} \times 100$$
 [1]

where C is the mean biomass of the nontreated control and B is the biomass of an individual treated plant.

Response to POST Herbicides

The responses of the CT-Res Palmer amaranth biotype to the selected POST herbicides were evaluated (Table 1). Plants were grown in greenhouse conditions at the Windsor Valley Laboratory of the Connecticut Agricultural Experiment Station using the same procedures as described previously. Two experimental runs were conducted in a completely randomized design with 21 replications. Palmer amaranth seedlings were treated with different herbicides, as described for the single-dose experiments. Visual control estimates of Palmer amaranth were recorded at 7 and 21 DAT on a scale of 0% to 100% as described for the dose-response study. Plants were cut at the soil surface at 21 DAT and oven dried for 4 d at 65 C, and dry biomass was recorded. Percent biomass reduction of treated plants was calculated using Equation 1.

Statistical Analyses

Palmer amaranth visual control and biomass reduction data collected at 14 DAT only were used for estimating the regression parameters. A three-parameter log-logistic model (Equation 2) was used to estimate the effective dose of glyphosate or imazaquin needed to control each Palmer amaranth biotype by 50% (ED_{50}) and 90% (ED_{90}) using the *drc* package (*drc* 2.3 in R 3.1.0 (R statistical software; R Foundation for Statistical Computing, Vienna, Austria) (Knezevic et al. 2007):

$$Y = \frac{d}{1 + \exp[b \ (logx - loge)]}$$
[2]

where *Y* is the percent visual control or percent aboveground biomass reduction, *x* is the herbicide rate, *d* is the upper limit, *e* is the ED_{50} or ED_{90} values, and *b* represents the relative slope around the parameter *e*. The level of resistance was calculated by dividing the ED_{90} value of the resistant biotype (CT-Res) by that of the susceptible biotype (KS-Sus).

Data from POST herbicides study were subjected to ANOVA using the PROC GLIMMIX procedure in SAS, version 9.3 (SAS Institute Inc., Cary, NC). Percent control and biomass reduction data were analyzed excluding the nontreated control as well as the treatments containing ALS-inhibitor herbicides. Herbicide treatments, experimental run, and their interactions were considered fixed effects, whereas replication was considered a random effect in the model. Before analysis, data were tested for normality using PROC UNIVARIATE and homogeneity of variance with the modified Levene test. The ANOVA requirements of normality and homogeneity of variance assumptions were met; therefore, no data transformation was needed. When the F test was significant ($P \le 0.05$), percent control and biomass reduction means were separated using the Fisher LSD test at P = 0.05.

Results and Discussion

Single-Dose Experiments to Determine Resistance Concern

The preliminary single-dose bioassay revealed complete control failure for the CT-Res biotype with glyphosate applied at 840 g ae ha⁻¹ and imazaquin applied at 137 g ai ha⁻¹ (data not shown). The treated plants exhibited no chlorotic, necrotic, or stunting injury compared to the nontreated control plants. Response of the CT-Res biotype to glyphosate and imazaquin dose-response bioassays are discussed separately in the following paragraphs.

Glyphosate Dose-Response Bioassay

Experiment run-by-treatment interactions for Palmer amaranth control (P = 0.121) and biomass reduction (P = 0.193) were not significant; therefore, data were pooled over experimental runs. Glyphosate applied at the labeled field-use rate (840 g ae ha⁻¹) controlled the KS-Sus biotype by 96%, whereas the CT-Res biotype was controlled only 10%. To achieve 50% and 90% control, the CT-Res biotype required respective glyphosate rates of 1,593 g ae ha⁻¹ and 4,204 g ae ha⁻¹, almost 2- and 5-fold higher than the labelled fielduse rate (Table 2). Almost similar ED_{50} (1,778 g ae ha⁻¹) and ED_{90} (4,713 g ae ha⁻⁻¹) values were observed for reduction in aboveground biomass of the CT-Res biotype. In contrast, the KS-Sus biotype required 69 g ae ha^{-1} and 460 g ae ha^{-1} for 50% and 90% reduction in biomass, respectively (Figure 1A; Table 2). On the basis of percent visual control or biomass reduction (ED₉₀ values), the CT-Res biotype manifested a 10-fold glyphosate resistance compared with the KS-Sus biotype. Similar levels of glyphosate resistance have been reported with respect to Palmer amaranth biomass reduction from Kansas, Mississippi, and Nebraska (Chahal et al. 2017; Kumar et al. 2019; Kumar et al. 2020; Nandula et al. 2012). In contrast, Mohseni-Moghadam et al. (2013) reported a lower resistance level (7-fold) in glyphosateresistant Palmer amaranth biotype from New Mexico. On the basis of ED₉₀ values for biomass reduction, the level of glyphosate resistance in CT-Res biotype is 2.6- and 3.6-fold lower than reported in the Arkansas (ED₉₀ =12,500 g ae ha⁻¹) and Nebraska (ED₉₀ =16,797 g ae ha⁻¹) biotypes, respectively (Chahal et al. 2017; Norsworthy et al. 2008).

Imazaquin Dose-Response Bioassay

Experiment run-by-treatment interactions for Palmer amaranth control (P = 0.096) and biomass reduction (P = 0.133) were not significant; therefore, data were pooled over the experimental runs. A known ALS-inhibitor susceptible biotype was not available for comparison. This lack of a susceptible biotype is not surprising, given that ALS-inhibitor–resistant Palmer amaranth has become wide-spread in the United States due to the continuous use of ALS-inhibiting herbicides in corn, cotton, and soybean. Therefore, the KS-Sus biotype, which was suspected to be ALS-inhibitor resistant, was used for comparison in this study.

The CT-Res biotype demonstrated a very high level of resistance to imazaquin. Imazaquin applied at $16 \times (i.e., 2,196 \text{ g ai } ha^{-1})$ the effective field-use rate controlled the CT-Res biotype only 18%. Comparatively, the KS-Sus biotype was controlled 50% and 90% with imazaquin rates of 194 g ai ha^{-1} and 727 g ai ha^{-1} , respectively (Table 2). Biomass reduction data indicated similar levels of

Table 1. Details of POST herbicides used in the greenhouse study conducted at the Valley Laboratory in Windsor, CT, to determine response of glyphosate-resistant Palmer amaranth

Herbicide	Trade name	Rate	Manufacturer
		g ae or g ai ha ⁻¹	
2,4-D ester	Weedone [®] LV6	386	Nufarm Inc., Alsip, IL 60803
Atrazine	Atrazine 90 DF	2,240	Drexel Chemical Co., Memphis, TN 38113
Carfentrazone-ethyl	Aim [®] EC	34	FMC Corp., Philadelphia, PA 19103
Chlorimuron-ethyl	Classic [®]	13.1	Corteva Agriscience, Indianapolis, IN 46268
Clopyralid	Stinger [®]	280	Corteva Agriscience
Dicamba	Clarity®	280	BASF Corp., Research Triangle Park, NC 27709
Glufosinate	Rely [®] 280	595	BASF Corp.
Halosulfuron-methyl	Sandea®	70	Gowan Co., Yuma, AR 85364
Imazaguin	Scepter [®] 70	137	BASF Corp.
Lactofen	Cobra®	220	Valent USA Corp., Walnut Creek, CA 94596
Mesotrione	Callisto®	105	Syngenta Crop Protection, Basel, Switzerland
Oxyflurfen	Goal [®] 2XL	1,121	Corteva Agriscience
Sulfometuron-methyl	Oust [®] XP	392	Bayer Crop Science

 Table 2.
 Estimates of Regression Parameters and Herbicide Dose Required for 50% and 90% Visual Control and Biomass Reduction of Palmer Amaranth Biotypes at 14 d After Treatment in a Greenhouse Whole-Plant Dose Response Study Conducted at the Valley Laboratory, Windsor, CT

Variable	Herbicide	Biotype ^a	Regression par	ameter (±SE) ^b	ED ₅₀ (±SE)	ED ₉₀ (±SE)	Resistance level
			b	d	g ae	/ai ha ^{_1_}	
Visual control	Glyphosate	CT-Res	-1.9 (±0.96)	102 (±1.5)	1,593 (±36)	, 4,204 (±253)	10×
		KS-Sus	-1.2 (±0.09)	100 (±1.4)	67 (±4)	424 (±70)	
	Imazaquin	CT-Res	-0.3 (±NA)	48 (±NA)	NA	NA	NA
		KS-Sus	-1.7 (±0.09)	99 (±2.7)	194 (±7)	727 (±55)	
Biomass reduction	Glyphosate	CT-Res	2.2 (±0.17)	99 (±1.9)	1,778 (±69)	4,713 (±390)	10×
		KS-Sus	1.1 (±0.07)	99 (±2.5)	69 (±5.4)	460 (±50)	
	Imazaguin	CT-Res	3.5 (±0.73)	100 (±2.3)	NA	NA	NA
	·	KS-Sus	2.0 (±0.17)	99 (±2.7)	211 (±9)	632 (±59)	

^aAbbreviations: DAT, days after treatment; CT-Res, resistant Palmer amaranth biotype found in Hartfield Co., Connecticut; ED₅₀, effective herbicide dose required for 50% biomass reduction or visual control at 14 DAT; ED₉₀, effective herbicide dose required for 90% biomass reduction or visual control at 14 DAT; KS-Sus, Palmer amaranth biotype collected from Kansas State University Agricultural Research Center near Hays, KS; NA, not applicable (ED₅₀, ED₉₀, or resistance level could not be determined).

Pregression parameters b and d of a three-parameter log-logistic model were obtained using the nonlinear least-square function of R statistical software.

^cResistance level was calculated by dividing the ED₉₀ value of the CT-Res biotype by that of the KS-Sus biotype.

imazquin resistance in the CT-Res and KS-Sus biotypes as observed with visual control estimates (Figure 1B; Table 2). Previously, Gossett and Toler (1999) reported 80% control of the ALS-susceptible Palmer amaranth with imazaquin applied at 140 g ai ha⁻¹. The imazaquin herbicide label also indicates control of 15-cm tall ALSsusceptible Palmer amaranth at 137 g ai ha⁻¹. This confirms that both the CT-Res and KS-Sus biotypes were ALS-inhibitor herbicide resistant. Furthermore, Burgos et al. (2001) observed that Palmer amaranth biotypes resistant to imazaquin were also cross-resistant to chlorimuron-ethyl, dichosulam, and pyrithiobac herbicides.

Our results from the response to POST herbicides (Table 3) also indicated that the CT-Res biotype was cross-resistant to other ALS-inhibiting herbicides. Chlorimuron-ethyl (13.1 g ai ha⁻¹), halosulfuron-methyl (70 g ai ha⁻¹), and sulfometuron-methyl (392 g ai ha⁻¹) had no effect on visual control and biomass reduction of the CT-Res biotype compared to the nontreated control. Currently, multiple resistance to glyphosate and ALS-inhibitors has been confirmed in several Palmer amaranth biotypes in the United States (Heap 2020; Kumar et al. 2019, 2020).

Response to POST Herbicides

Experimental run-by-treatment interactions for Palmer amaranth control (P = 0.271) and biomass reduction (P = 0.183) were not significant; therefore, data from both experiment runs were combined. Because the DAT main effect was significant, data were analyzed by DAT. The glutamine synthetase–inhibitor (glufosinate) and protoporphyrinogen oxidase (PPO)-inhibitors (carfentrazone-ethyl, lactofen, and oxyfluorfen) provided the quickest control of the CT-Res biotype compared with all other POST herbicides tested in this study (Table 3). At 7 DAT, the CT-Res biotype was controlled 90% or more with carfentrazone-ethyl (34 g ai ha⁻¹), glufosinate (595 g ai ha⁻¹), lactofen (220 g ai ha⁻¹), and oxyfluorfen (1,121 g ai ha⁻¹) herbicides. By 21 DAT, the CT-Res biotype was completely controlled (100%) with glufosinate and all PPO-inhibitors tested in this study. A similar trend was observed in biomass reduction of the CT-Res biotype at 21 DAT (Table 3).

These results are consistent with the findings of Kumar et al. (2019), who reported complete control (100% at 21 DAT) of a five-way resistant (2,4-D, atrazine, chlorsulfuron, glyphosate, and mesotrione) Palmer amaranth from Kansas with glufosinate in a greenhouse study. Similarly, glufosinate applied at a concentration of 409 g ai ha⁻¹ or greater provided 93% or better control of Palmer amaranth up to 10 cm tall in studies conducted by Aulakh et al. (2011) and Corbett et al. (2004). Mohseni-Moghadam et al. (2013) in New Mexico obtained more than 94% biomass reduction of two Palmer amaranth biotypes with carfentrazone-ethyl, glufosinate, or oxyfluorfen at rates similar to those used in the present study. Likewise, Palmer amaranth was controlled greater than 85% with lactofen applied at 220 g ai ha⁻¹ (Jhala et al. 2014; Sweat et al. 1998). Aulakh et al. (2016) observed complete control of waterhemp with lactofen applied at 220 g ai ha⁻¹.

Auxinic herbicides such as 2,4-D and dicamba provided greater control than clopyralid at both 7 and 21 DAT. In this study, control of the CT-Res biotype ranged from 60% to 75% with clopyralid, 73%

Table 3. Glyphosate-resistant Palmer amaranth control with POST herbicides at 7 and 21 DAT and biomass reduction at 21	DA
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		Control ^{b,c}		Biomass reduction ^{b,c}	
Herbicide ^a	Rate	7 DAT	21 DAT	21 DAT	
	g ae or g ai ha ⁻¹		%		
2,4-D	386	73 b	88 ab	90 ab	
Atrazine	2,240	40 d	52 d	48 d	
Carfentrazone-ethyl	34	96 a	100 a	100 a	
Chlorimuron-ethyl	13.1	0 e	0 e	0 e	
Clopyralid	280	60 c	75 c	70 c	
Dicamba	280	78 b	92 ab	95 a	
Glufosinate	595	93 a	100 a	100 a	
Halosulfuron-methyl	70	0 e	0 e	0 e	
Lactofen	220	100 a	100 a	100 a	
Mesotrione	105	70 bc	82 bc	80 bc	
Oxyflurfen	1,121	90 a	100 a	100 a	
Sulfometuron-methyl	392	0 e	0 e	0 e	

^aNonionic surfactant (Induce; Helena Chemical Co., Collierville, TN) at 0.25% vol/vol was added to 2,4-D, dicamba, chlorimuron-ethyl, clopyralid, glufosinate, halosulfuron-methyl, lactofen, mesotrione, oxyfluorfen, and sulfometuron-methyl. Crop oil concentrate (Agridex; Helena Chemical Co.) at 1% vol/vol was added to atrazine and carfentrazone-ethyl herbicides. ^bMeans within columns with no common letter(s) are significantly different according to Fisher protected LSD test where P < 0.05.

^cPercent visual control and biomass reduction data from the nontreated control, and treatments with zero visual control or biomass reduction were not included in analysis. Biomass reduction was calculated on the basis of comparison of the average biomass of the nontreated control, using Equation 1 in the text.



Figure 1. Dose-response curves for the CT-Res and KS-Sus biotypes. (A) Percent biomass reduction at 14 d after glyphosate application, and (B) percent biomass reduction at 14 d after imazaquin application, in greenhouse whole-plant dose-response studies conducted at the Windsor Valley Laboratory. Percent biomass reduction was calculated using Equation 1 in the text. CT-Res, resistant Palmer amaranth biotype found in Hartfield Co., Connecticut; KS-Sus, Palmer amaranth biotype collected from Kansas State University Agricultural Research Center near Hays, KS.

to 88% with 2,4-D, and 78% to 92% with dicamba at 7 and 21 DAT, respectively. Similar visual control and biomass reduction with 2,4-D and dicamba have been reported in Palmer amaranth biotypes from Nebraska and New Mexico (Chahal et al. 2017; Jhala et al. 2014; Mohseni-Moghadam et al. 2013). Furthermore,

mesotrione, a 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor, controlled the CT-Res biotype 70% and 82% at 7 and 21 DAT, respectively. Biomass reduction at 21 DAT was similar to the corresponding percent visual control. Mesotrione is widely used in seed corn and sweet corn because of high tolerance of corn varieties to these herbicides, a wide weed-control spectrum, flexibility for application timings, and compatibility for tank mixes with other herbicides (Bollman et al. 2008; McMullan and Green 2011). Atrazine, a PSII-inhibitor, controlled the CT-Res biotype less than 55% in this study. Previous studies have shown high variation in Palmer amaranth control with atrazine. Chahal et al. (2017) reported less than 25% control with atrazine applied at the same rate tested in this study (2,240 g ai ha^{-1}). In contrast, Jhala et al. (2014) observed 45% to 73% visual control and 44% to 68% biomass reduction 21 DAT in two Palmer amaranth biotypes with atrazine applied POST at 560 g ai ha⁻¹. However, Palmer amaranth was completely controlled with atrazine at concentrations of at least 2,150 g at ha^{-1} in studies conducted by Mohseni-Moghadam et al. (2013), Norsworthy et al. (2008), and Salas et al. (2016).

ALS inhibitors (i.e., chlorimuron-ethyl, halosulfuron-methyl, imazaquin, and sulfometuron-methyl) did not affect visual control and biomass reduction in the CT-Res biotype compared to the nontreated control. Burgos et al. (2001) reported Palmer amaranth biotypes resistant to imazaquin were also cross-resistant to chlor-imuron-ethyl, dichosulam, and pyrithiobac herbicides. Chahal et al. (2017) observed less than 40% control with chlorimuron-ethyl and halosulfuron-methyl. Alternatively, some researchers reported 69% or higher control Palmer amaranth with similar rates of chlorimuron-ethyl, halosulfuron-methyl, imazaquin, and sulfometuron-methyl (Gossett and Toler 1999; Jhala et al. 2014).

Practical Implications

The newly reported Palmer amaranth biotype from Hartford County, CT, is resistant to both ALS-inhibitor herbicides and glyphosate. The CT-Res biotype was controlled only 18% with imazaquin applied at 2,196 g ai ha⁻¹, which is 16-fold higher than the labelled imazaquin rate (137 g ai ha⁻¹) for Palmer amaranth control. Furthermore, the CT-Res biotype required five times more glyphosate (4,204 g ae ha⁻¹) for 90% visual control compared to the KS-Sus biotype. Presence of ALS-and glyphosate-resistant

Palmer amaranth in Connecticut is a serious management concern for specialty crops producers because of limited POST herbicide options. The response of the CT-Res biotype to POST herbicides suggests the array of control options will vary with the crop. For instance, the CT-Res biotype was controlled 75% or better with an HPPD inhibitor (mesotrione), PPO inhibitors (carfentrazone-ethyl, lactofen, oxyfluorfen), glutamine synthetase inhibitor (glufosinate), and synthetic auxins (2,4-D, clopyralid, and dicamba) in this study. This suggests that adequate effective POST herbicides exist for use in corn grown for forage, seed, popcorn (Z. mays L var. Everta), and sweet corn (Z. mays L. var. rugosa). However, in most vegetables, such as bell peppers, cucurbits (Cucurbita spp.), and tomatoes (Solanum lycopersicum L.), and small fruits, such as blackberry (Rubus fructicosus L.), blueberry (Vaccinium corymbosum L.), and raspberry (R. idaeus L.), cultivation, hand weeding, or directed applications of POST herbicides such as carfentrazone-ethyl, glufosinate, or paraquat are the only viable alternatives for control of Palmer amaranth resistant to ALS inhibitors and glyphosate. Moreover, mesotrione is labelled for use as a prebloom POST directed spray in blackberry, blueberry, and raspberry and can still be used to control herbicide-resistant Palmer amaranth biotypes. The CT-Res biotype was not completely controlled with recommended field-use rates of some of the POST herbicides tested in this study. Therefore, additional control tactics, including cultural and physical methods, should be integrated to improve Palmer amaranth control and prevent replenishment of the soil seedbank.

The CT-Res biotype also demonstrated reduced sensitivity to a PSII-inhibitor herbicide (atrazine). Furthermore, Palmer amaranth resistant to long-chain fatty acid–inhibitor and microtubule inhibitor herbicides is also present in the United States (Heap 2020). These concerns warrant the need for additional dose-response bioassays to determine the response of the CT-Res bio-type to herbicides from these sites-of-action groups.

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References

- Aulakh JS (2019) Weed alert: first report of Palmer amaranth in Connecticut-watch out for this pigweed! https://portal.ct.gov/-/media/CAES/DOCUMENTS/ Publications/Fact_Sheets/Palmer-Amaranth.pdf?la=en. Accessed: August 10, 2020
- Aulakh JS, Chahal PS, Jhala AJ (2016) Glyphosate-resistant weed control and soybean injury in response to different PPO-inhibiting herbicides. J Agr Sci 8:1–9
- Aulakh JS, Price AJ, Balkcom KS (2011) Weed management and cotton yield under two row spacings in conventional and conservation tillage systems utilizing conventional, glufosinate-, and glyphosate-based weed management systems. Weed Technol 25:542–547
- Aulakh JS, Price AJ, Enloe SF, van Santen E, Wehtje G, Patterson MG (2012) Integrated Palmer amaranth management in glufosinate-resistant cotton, I: soil-inversion, high residue cover crops and herbicide regimes. Agronomy 2:295–311
- Aulakh JS, Price AJ, Enloe SF, Wehtje G, Patterson MG (2013) Integrated Palmer amaranth management in glufosinate resistant cotton, II: primary, secondary and conservation tillage. Agronomy 3:28–42
- Bensch CN, Horak MJ, Peterson D (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Sci 51:37–43

- Bertucci MB, Jennings KM, Monks DW, Schultheis JR, Louws FJ, Jordan DL, Brownie C (2019) Critical period for weed control in grafted and nongrafted watermelon grown in plasticulture. Weed Sci 76:1–8
- Bollman JD, Boerboom CM, Becker RL, Fritz VA (2008) Efficacy and tolerance to HPPD-inhibiting herbicides in sweet corn. Weed Technol 22:666-674
- Bryson CT, DeFelice MS (2010) Weeds of the Midwestern United States & Central Canada. Athens, GA: University of Georgia Press. 427 p
- Burgos NR, Kuk YI, Talbert RE (2001) Amaranthus palmeri resistance and differential tolerance of Amaranthus palmeri and Amaranthus hybridus to ALSinhibitor herbicides. Pest Manag Sci 57:449–457
- Burke IC, Schroeder M, Thomas WE, Wilcut JW (2007) Palmer amaranth interference and seed production in peanut. Weed Technol 21:367–371
- Chahal PS, Varanasi VK, Jugulum M, Jhala AJ (2017) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Nebraska: confirmation, EPSPS gene amplification, and response to POST corn and soybean herbicides. Weed Technol 31:80–93
- Corbett JL, Askew SD, Thomas WE, Wilcut JW (2004) Weed efficacy evaluations for bromoxynil, glufosinate, glyphosate, pyrithiobac, and sulfosate. Weed Technol 18:443–453
- Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci 54:620–626
- Garvey PV Jr, Meyers SL, Monks DW, Coble HD (2013) Influence of Palmer amaranth (*Amaranthus palmeri*) on the critical period for weed control in plasticulture-grown tomato. Weed Technol 27:165–170
- Gossett BJ, Murdock EC, Toler JE (1992) Resistance of Palmer amaranth (*Amaranthus palmeri*) to the dinitroaniline herbicides. Weed Technol 6:587-591
- Gossett BJ, Toler JE (1999) Differential control of Palmer amaranth (*Amaranthus palmeri*) and smooth pigweed (*Amaranthus hybridus*) by postemergence herbicides in soybean (*Glycine max*). Weed Technol 13:165-168
- Grichar WJ (1997) Control of Palmer amaranth (*Amaranthus palmeri*) in peanut (*Arachis hypogaea*) with postemergence herbicides. Weed Technol 11:739–743
- Heap I (2020) International survey of herbicide-resistant weeds. http://www. weedscience.org/Summary/Species.aspx. Accessed: July 5, 2020
- Horak MJ, Loughin TM (2000) Growth analysis of four Amaranthus species. Weed Sci 48:347–355
- Horak MJ, Peterson DE (1995) Biotypes of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. Weed Technol 9:192–195
- Jhala AJ, Sandell LD, Rana N, Kruger GR, Knezevic SZ (2014) Confirmation and control of triazine and 4-hydroxyphenylpyruvate dioxygenase-inhibiting herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) in Nebraska. Weed Technol 28:28–38
- Keeley PE, Carter CH, Thullen RJ (1987) Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci 35:199–204
- Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R software package for dose-response studies: the concept and data analysis. Weed Technol 21:840–848
- Kumar V, Liu R, Boyer G, Stahlman PW (2019) Confirmation of 2,4–D resistance and identification of multiple resistance in a Kansas Palmer amaranth (*Amaranthus palmeri*) population. Pest Manag Sci 75:2925–2933
- Kumar V, Liu R, Stahlman PW (2020) Differential sensitivity of Kansas Palmer amaranth populations to multiple herbicides. Agron J 112:2152–2163
- Massinga RA, Currie RS, Horak MJ, Boyer J Jr (2001) Interference of Palmer amaranth in corn. Weed Sci 49:202–208
- McMullan PM, Green JM (2011) Identification of a tall waterhemp (*Amaranthus tuberculatus*) biotype resistant to HPPD-inhibiting herbicides, atrazine, and thifensulfuron in Iowa. Weed Technol 25:514–548
- Meyers SL, Jennings KM, Schultheis JR, Monks DW (2010) Interference of Palmer amaranth (*Amaranthus palmeri*) in sweetpotato. Weed Sci 58:199–203
- Mohseni-Moghadam M, Schroeder J, Heerema R, Ashigh J (2013) Resistance to glyphosate in Palmer amaranth (*Amaranthus palmeri*) populations from New Mexico pecan orchards. Weed Technol 27:85–91

- Moore JW, Murray DS, Westerman RB (2004) Palmer amaranth (*Amaranthus palmeri*) effects on the harvest and yield of grain sorghum (*Sorghum bicolor*). Weed Technol 18:23–29
- Nandula VK, Reddy KN, Kroger CH, Poston DH, Rimando AM, Duke SO, Bond JA, Ribeiro DN (2012) Multiple resistance to glyphosate and pyrithiobac in Palmer amaranth (*Amaranthus palmeri*) from Mississippi and response to flumiclorac. Weed Sci 60:179–188
- Nerson H (1989) Weed competition in muskmelon and its effects on yield and fruit quality. Crop Prot 8:439-442
- Norsworthy JK, Griffith GM, Scott RC, Smith KL, Oliver LR (2008) Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technol 22:108–113
- Price AJ, Korres N, Norsworthy JS, Li S (2018) Influence of a cereal rye cover crop and conservation tillage on the critical weed-free period in cotton. Weed Technol 32:683–690
- Salas RA, Burgos NR, Tranel PJ, Singh S, Glasgow L, Scott RC, Nichols RL (2016) Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas. Pest Manag Sci 72:864–869
- Sauer JD (1957) Recent migration and evolution of the dioecious amaranths. Evolution 11:11-31
- Smith DT, Baker RV, Steele GL (2000) Palmer amaranth (Amaranthus palmeri) impacts on yield, harvesting, and ginning in dryland cotton (Gossypium hirsutum). Weed Technol 14:122–126
- Sosnoskie LM, Webster TM, Grey TL, Culpepper AS (2014) Severed stems of *Amaranthus palmeri* are capable of regrowth and seed production in *Gossypium hirsutum*. Ann Appl Biol 165:147–154

- Sprague CL, Stoller EW, Wax LM, Horak MJ (1997) Palmer amaranth (Amaranthus palmeri) and common waterhemp (Amaranthus rudis) resistance to selected ALS-inhibiting herbicides. Weed Sci 45:192–197
- Steckel LE (2007) The dioecious *Amaranthus* spp.: here to stay. Weed Technol 21:567–570
- Steckel LE, Main CL, Ellis AT, Mueller TC (2008) Palmer amaranth (Amaranthus palmeri) in Tennessee has low level glyphosate resistance. Weed Technol 22:119–123
- Sweat JK, Horak MJ, Peterson DE, Lloyd RW, Boyer JE (1998) Herbicide efficacy on four Amaranthus species in soybean (Glycine max). Weed Technol 12:315–321
- Thompson CR, Peterson D, Lally NG (2012) Characterization of HPPD-resistant Palmer amaranth. http://wssaabstracts.com/public/9/ abstract-413.html. Accessed: July 14, 2020
- [USDA APHIS]U.S. Department of Agriculture, Animal and Plant Health Service (2020) Weed risk assessment for Amaranthus palmeri (Amaranthaceae)– Palmer's amaranth. https://www.aphis.usda.gov/plant_health/plant_pest_ info/weeds/downloads/wra/amaranthus-palmeri.pdf. Accessed: July 15, 2020
- Van Wychen L (2017) 2017 Survey of the most common and troublesome weeds in grass crops, pasture and turf in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. http://wssa.net/wpcontent/uploads/2017-Weed-Survey_Grass-crops.xlsx. Accessed: July 19, 2020
- Ward SM, Webster TM, Steckel LE (2013) Palmer amaranth (Amaranthus palmeri): a review. Weed Technol 27:12–27
- Wortman SE (2014) Integrating weed and vegetable crop management with multifunctional air-propelled abrasive grits. Weed Technol 28:243–252