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Effect of cotton herbicide programs on weed population trajectories and frequency of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*)

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Abstract

The adoption of dicamba-resistant cotton (*Gossypium hirsutum* L.) cultivars allows using dicamba to reduce weed populations across growing seasons. However, the overuse of this tool risks selecting new herbicide-resistant biotypes. The objectives of this research were to determine the population trajectories of several weed species and track the frequency of glyphosate-resistant (GR) Palmer amaranth (*Amaranthus palmeri* S. Watson) over 8 yr in dicamba-resistant cotton. An experiment was established in North Carolina in 2011, and during the first 4 yr, different herbicide programs were applied. These programs included postemergence applications of glyphosate, alone or with dicamba, with or without residual herbicides. During the last 4 yr, all programs received glyphosate plus dicamba. Biennial rotations of postemergence applications of glyphosate only and glyphosate plus dicamba postemergence with and without preemergence herbicides were also included. Sequential applications of glyphosate plus dicamba were applied to the entire test area for the final 4 yr of the study. No herbicide program was entirely successful in controlling the weed community. Weed population trajectories were different according to species and herbicide program, creating all possible outcomes; some increased, others decreased, and others remained stable. Density of resistant *A. palmeri* increased during the first 4 yr with glyphosate-only programs (up to 11,739 plants m^{-2}) and decreased a 96% during the final 4 yr, when glyphosate plus dicamba was implemented. This species had a strong influence on population levels of other weed species in the community. Goosegrass [*Eleusine indica* (L.) Gaertn.] was not affected by *A. palmeri* population levels and even increased its density in some herbicide programs, indicating that not only herbicide resistance but also reproductive rates and competitive dynamics are critical for determining weed population trajectories under intensive herbicide-based control programs. Frequency of glyphosate resistance reached a maximum of 62% after 4 yr, and those levels were maintained until the end of the experiment.

Introduction

Dicamba-resistant cotton (*Gossypium hirsutum* L.) cultivars have been commercialized in the United States, and the adoption of varieties with this trait has been widespread in cotton production, because their use enables management of weed species with evolved resistance to glyphosate and acetolactate synthase (ALS) inhibitors, such as Palmer amaranth (*Amaranthus palmeri* S. Watson). The dicamba-resistance trait is being incorporated into most glyphosate- and glufosinate-based herbicide-resistant (HR) cultivars (Cahoon et al. 2015b; Merchant et al. 2013; Meyer et al. 2015). The increased use of dicamba will increase selection pressure on weed communities and potentially lead to weed species shifts over time (Culpepper 2006; Shaner 2000). Diversifying cropping systems, integrating cultural and mechanical weed management practices, and reducing overall herbicide use of the same herbicide are required to reduce selection pressure and slow down the evolutionary process leading to herbicide resistance (Boerboom 1999; Kruger et al. 2009; Neve et al. 2011; Norsworthy et al. 2012; Vencill et al. 2012). Furthermore, to steward dicamba-resistant cropping systems and keep them as a viable and

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effective technology for weed management, weed dynamics must be understood not only within one season but also over multiple seasons.

Introducing new management tools that include both chemical and nonchemical controls can produce weed species shifts (Cordeau et al. 2017; Culpepper 2006; de la Fuente et al. 2021 Tuesca et al. 2001). The repeated use of a few herbicides over time in weed management can reduce weed diversity (Oreja et al. 2022), resulting in a few dominant species that are difficult to manage, frequently exhibit herbicide resistance, and ultimately can cause important yield losses (Storkey and Neve 2018). Herbicide programs play a key role in weed shifts and the pace at which these occur (Menalled et al. 2001). The inclusion of different mechanisms of action (MOAs), rather than a simple herbicide program with a few MOAs applied repeatedly, expands the spectrum of different weed species controlled, and the chance of selecting HR biotypes is reduced (Vencill et al. 2012). However, herbicides are not the only factors acting on community assembly; others, such as competition among weeds (Swanton et al. 1993), can have a strong influence, depending on the species. There are no studies that report the weed density variability in dicamba-resistant cotton crops exposed to different herbicide programs that include the repetitive application of this herbicide.

Long term studies are needed to examine weed density variations in response to weed management decisions and determine overall trends. Such studies are useful tools for understanding both weed community changes over time in response to agronomic practices (Clements et al. 1994; Hobbs and Humphries 1995) and the selection pressure on HR biotypes. Therefore, the objectives of this research were (1) to determine whether herbicide programs with wider control spectra reduce weed species communities over time more so than simple herbicide programs with narrower control spectra; (2) to ascertain whether the effects on the population trajectories are maintained after changing herbicide programs; and (3) to compare *A. palmeri* population frequency of glyphosate resistance over 8 yr in response to herbicide programs that included glyphosate, dicamba, and residual herbicides.

Materials and Methods

Two experiments were established in two separate fields in a randomized complete block design with four replications each in 2011 at the Upper Coastal Plain Research Station in Rocky Mount, NC (35.893°N, 77.681°W). Cotton (BollGard II® XtendFlex®; Monsanto, St. Louis, MO) resistant to dicamba, glufosinate, glyphosate, and Lepidopteran insects (events MON-88701-6, MON-88913-6, MON-15985) was planted in conventionally tilled, raised beds at a seeding of 17 seed m^{-1} of row. Cotton in all years was planted in the second or third week of May. Other than treatments imposed for the experiment, cotton was managed according to North Carolina Cooperative Extension Service recommendations (Edmisten et al. 2018). At the beginning of the study, fields were naturally infested with *A. palmeri*, including both glyphosate-susceptible (GS) and ALS-susceptible as well as glyphosate-resistant (GR) and ALS-resistant *A. palmeri*. The frequency of ALS resistance and glyphosate resistance was approximately 30% and <10%, respectively.

Seven herbicide programs composed of different MOAs were compared (Tables 1 and 2). Herbicides were applied using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles (AIXR 11002, TeeJet® Technologies, Wheaton, IL) calibrated to deliver 140 L ha^{-1} at 152 kPa. Depending on the treatment, preemergence herbicides were applied immediately after planting (G+R, G+D+R, and G+½D+R), and postemergence herbicides were applied 2 (early postemergence), 4 (medium postemergence), and 6 (late postemergence) wk after planting.

Weed Population Density

The germinable seedbank method was used to determine the weed density of each species (Reinhardt and Leon 2018). Ten soil cores (10.2 cm by 7.6 cm for a total volume of 4,630 ml) were collected from each plot before preemergence herbicide applications, in May of each year, except in 2015, when collection was done in January. Soil cores were placed in flats to a depth of 4 cm with a total surface area of 1,550 cm^2, maintained in a climate-controlled greenhouse (25 to 35 C at 80% to 90% relative humidity), and irrigated periodically to promote germination and adequate seedling growth. A seedling count was done 3 wk after establishment, when no new seedlings were observed. Seedlings were identified, and the number of individuals per species was recorded.

Frequencies of HR Biotypes

To determine the frequency of GR *A. palmeri* individuals in flats from the soil cores each year, plants were treated with glyphosate at 946 g ae ha^{-1} after seedling count and identification, and the number of surviving plants was determined at 2 wk after application. In May 2019, at the end of the study, plants surviving glyphosate were treated with dicamba at 560 g ae ha^{-1} to determine whether there were any individuals with tolerance to dicamba after 8 yr of dicamba use. A known dicamba-susceptible *A. palmeri* population was included as a positive control.

Statistical Analysis

While 17 species were registered throughout the experiment (Table 3), those detected just a few times across all plots, treatments, and years were not considered in the analysis. To evaluate the effect of herbicide programs on seedling population density, data from two fields were pooled, maintaining the independence of each replicate. Seedling number per species was log-transformed before analysis and then subjected to regression procedures using GraphPad Prism 6 (GraphPad Software, San Diego, CA) program testing for linear, quadratic functions (Tables 4 and 5). The PROC Mixed procedure in SAS (v. 9.4; SAS Institute, Cary, NC) was used to analyze the log-transformed frequency of GR *A. palmeri* glyphosate resistance over time.

Results and Discussion

Weed Population Trajectories

Population density varied during the 96 mo that the experiment lasted, showing different patterns among weed species and/or herbicide programs (Figure 1). Some species increased, others decreased, and others maintained their population stability. Furthermore, after 48 mo, subjecting all populations to a single, uniform program based on the postemergence herbicides glyphosate and dicamba did not have the same effect on the population trajectories of all weed species studied.

Treatments that included the use of dicamba every year (G+D, G+D+R, and G+D+Ac) were the only treatments that reduced the number of *A. palmeri* seedlings from the beginning of the experiment (Figure 1A; Table 4). The treatment in which only glyphosate postemergence was used during the first 4 yr (G)

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Table 1. Herbicide active ingredient, trade name, formulation, application rate, and manufacturer.

Herbicide active ingredient	Trade name[a]	Formulation concentration	Application rate	Manufacturer
Acetochlor	Warrant®	359 g ai L^{-1}	1,260 g ai ha^{-1}	Monsanto Co., St Louis, MO
Dicamba diglycolamine salt	Clarity®	480 g ae L^{-1}	560 g ae ha^{-1}	BASF Ag Products, Research Triangle Park, NC
Dicamba *N,N*-Bis-(3-aminopropyl) methylamine salt	Engenia®	600 g ae L^{-1}	560 g ae ha^{-1}	BASF Ag Products, Research Triangle Park, NC
Diuron	Direx® 4L	480 g ai L^{-1}	840 g ai ha^{-1}	Makhtesshim Agan of North America, Raleigh, NC
Glyphosate potassium salt	Roundup WeatherMax®	540 g ae L^{-1}	946 g ae ha^{-1}	Monsanto Co.
Pendimethalin	Prowl® H_2O	452 g ai L^{-1}	1,065 g ai ha^{-1}	BASF Ag Products

[a] Clarity® was used during 2011 to 2015. Engenia® was used during 2016 to 2018.

Table 2. Preemergence and early (EPOST), medium (MPOST), and late (LPOST) postemergence herbicide treatments applied in Rocky Mount, NC, in 2011–2014.[a]

	Herbicide treatments				
Treatment	Preemergence	EPOST	MPOST	LPOST	Years
G	None	Glyphosate	Glyphosate	Glyphosate	2011–2014
G+D	None	Glyphosate plus dicamba	Glyphosate plus dicamba	Glyphosate plus dicamba	2011–2014
G+R	Pendimethalin plus diuron	Glyphosate	Glyphosate	Glyphosate	2011–2014
G+D+R	Pendimethalin plus diuron	Glyphosate plus dicamba	Glyphosate plus dicamba	Glyphosate plus dicamba	2011–2014
G+D+Ac	None	Glyphosate plus dicamba plus acetochlor	Glyphosate plus dicamba	Glyphosate plus dicamba	2011–2014
G+½D+R	Pendimethalin plus diuron	Glyphosate	Glyphosate	Glyphosate	2011 and 2013
		Glyphosate plus dicamba	Glyphosate plus dicamba	Glyphosate plus dicamba	2012 and 2014
2G+½D	None	Glyphosate	Glyphosate	Glyphosate	2011 and 2013
		Glyphosate plus dicamba	Glyphosate plus dicamba	Glyphosate plus dicamba	2012 and 2014

[a] All plots received glyphosate plus dicamba POST for the remainder of the study (2015–2018).

Table 3. Weed species detected in the germinable seedbank during the experiment.

Species
Amaranthus palmeri (S.) Watson
Ambrosia artemisiifolia L.
Anoda cristata (L.) Schltdl.
Chenopodium album L.
Cyperus compressus L.
Cyperus esculentus L.
Dactyloctenium aegyptium (L.) Willd.
Digitaria sanguinalis (L.) Scop
Eclipta prostrata (L.) L.
Eleusine indica (L.) Gaertn
Ipomoea hederacea L. Jacq
Ipomoea lacunosa L.
Ipomoea purpurea L. Roth
Mollugo verticillata L.
Portulaca oleracea L.
Spergula arvensis L.
Urochloa platyphylla (Munro ex C. Wright) R.D. Webster

exhibited the highest number of *A. palmeri* seedlings, but this was expected, due to the presence of GR individuals in the field from the beginning of the study (Inman et al. 2016). In the first 4 yr, the addition of preemergence herbicides (G+R) helped maintain lower *A. palmeri* densities when compared with glyphosate-only postemergence (G), but the populations were still higher than in those treatments that included dicamba every year. When preemergence herbicides were used with alternating years of glyphosate and glyphosate plus dicamba (G+½D+R), lower *A. palmeri* densities were observed compared with the program without preemergence herbicides (G+½D) (Figure 1A). After the last 4 yr of glyphosate plus dicamba postemergence applications, a reduction in populations was observed in all treatments. By the end of the experiment, after 96 mo, *A. palmeri* densities did not differ across treatments (Figure 1A), due to the high efficacy of *A. palmeri* control with dicamba (Cahoon et al. 2015b; Tehranchian et al. 2017).

Carpetweed (*Mollugo verticillata* L.) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] populations showed a similar pattern; both were reduced in all treatments in the first 4 yr, with the decline of *D. sanguinalis* being more obvious. The structure of the herbicide program was not the critical factor affecting population density, as all programs exhibited the same trend. *Digitaria sanguinalis* seeds have short persistence in the soil seedbank (Oreja et al. 2020); if control is successful and no new seeds enter the soil seedbank by the end of season, populations should decline as observed at 24 and 36 mo after the beginning of the experiment (Figure 1C). Glyphosate applied alone (G) caused a more marked reduction of *M. verticillata* than other treatments (Figure 1B). This reduction could be due not only to the good control obtained with this herbicide (Culpepper and York 2000; Van Gessel et al. 2001) but also by the absence of the antagonism of dicamba to glyphosate performance when both herbicides are applied together (Meyer et al. 2020). Diuron and pendimethalin controlled *D. sanguinalis* well (Cahoon et al. 2015a); and dicamba, pendimethalin, and acetochlor provided acceptable control of *M. verticillata*. Once all herbicide programs changed to glyphosate plus dicamba, an increase

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Table 4. Equations and R-squared (R^2) of the models for each species and the treatments glyphosate (G), glyphosate plus dicamba (G+D), glyphosate plus residual herbicides (diuron plus pendimethalin) (G+D+R), glyphosate plus dicamba plus acetochlor (G+D+Ac), glyphosate plus alternating dicamba between years plus residual herbicides (diuron plus pendimethalin) (G+½D+R), and glyphosate plus alternating dicamba between years (G+½D).

	Amaranthus palmeri		*Mollugo verticillata*		*Digitaria sanguinalis*		*Cyperus compressus*		*Eclipta prostrata*	
Treatment	Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2
G	$y = 1.39 + 0.40x - 0.00054x^2$	0.72	$y = 1.67 + 0.029x - 0.00021x^2$	0.27	$y = 0.44 - 0.018x + 0.00018x^2$	0.21	$y = 0.11 - 0.0044x + 3.6 \times 10^{-5}x^2$	0.13	$y = 0.064 - 0.0025x + 2.0 \times 10^{-5}x^2$	0.07
G+D	$y = -0.0071x + 1.14$	0.20	$y = 1.27 - 0.018x + 0.00015x^2$	0.16	$y = 0.39 - 0.015x + 0.00015x^2$	0.14	$y = 0.18 - 0.0045x + 2.8 \times 10^{-5}x^2$	0.075	$y = 0.10 + 0.0034x + 2.8 \times 10^{-5}x^2$	0.085
G+R	$y = 1.18 + 0.041x - 0.00053x^2$	0.57	$y = 1.28 - 0.015x + 0.00013x^2$	0.09	$y = 0.54 - 0.02x + 0.00018x^2$	0.25	$y = 0.44 - 0.018x + 0.00018x^2$	0.18	$y = 0.18 - 0.0065x + 6.5 \times 10^{-5}x^2$	0.061
G+D+R	$y = -0.0072x + 1.14$	0.24	$y = 1.41 - 0.013x + 9.9 \times 10^{-5}x^2$	0.12	$y = 0.45 - 0.019x - 0.0002x^2$	0.30	$y\ 0.22 - 0.0071x + 6.0 \times 10^{-5}x^2$	0.12	$y = 0.25 + 0.0084x + 6.9 \times 10^{-5}x^2$	0.11
G+D+Ac	$y = -0.0074x + 1.07$	0.19	$y = 1.54 - 0.017x + 0.00011x^2$	0.16	$y = 0.39 - 0.015x + 0.00015x^2$	0.17	$y = 0.22 - 0.0084x + 6.9 \times 10^{-5}x^2$	0.32	$y = 0.2 - 0.0072x + 5.6 \times 10^{-5}x^2$	0.16
G+½D+R	$y = 1.24 + 0.015x - 0.00026x^2$	0.44	$y = 1.55 - 0.021x + 0.00018x^2$	0.17	$y = 0.43 - 0.016x - 0.00016x^2$	0.16	$y = 0.13 - 0.0042x + 3.5 \times 10^{-5}x^2$	0.10	$y = 0.08 - 0.0019x + 1.4 \times 10^{-5}x^2$	0.017
2G+½D	$y = 1.32 + 0.027x - 0.0004x^2$	0.65	$y = 1.34 - 0.011x + 7.1 \times 10^{-5}x^2$	0.09	$y = 0.4 - 0.016x + 0.00017x^2$	0.19	$y = 0.26 - 0.0097x + 7.7 \times 10^{-5}x^2$	0.27	$y = 0.18 + 0.0064x + 5.0 \times 10^{-5}x^2$	0.18

in seedling number was observed for both *M. verticillata* and *D. sanguinalis* (Figure 1B and C). These patterns seem to be not only the result of the change in the herbicide program but also of interspecific competition. Otherwise, the program with glyphosate plus dicamba during the last 4 yr would have maintained the decreasing trend observed during the first 4 yr. For example, the *A. palmeri* populations increased in most treatments during the first 4 yr (Figure 1A), while *D. sanguinalis* and *M. verticillata* populations decreased, and the opposite was observed during the last 4 yr of the study (Figure 1B and C). Interspecific competition strongly influences community assembly (Aschehoug et al. 2016), so it seems that significant decreases in *A. palmeri* populations during the last 4 yr reduced interference on other weed species allowing those to recover their populations.

In the cases of annual sedge (*Cyperus compressus* L.) and eclipta [*Eclipta prostrata* (L.) L.] (Figure 1D and E), recovery after the unification of herbicide programs was minimal, almost null, and the behavior of the populations was more erratic (Figure 1D and E; see low R^2 values in Table 5), probably due to a smaller seedbank and lower competitive ability than other weed species exhibiting faster population recovery. In contrast, corn spurry (*Spergula arvensis* L.) was practically absent in the first 4 yr, but its populations increased rapidly in the second half of the study (Figure 2D). This weed is usually sufficiently controlled with several of the herbicides used in the experiment, such as glyphosate (Ivany 2004), dicamba (Velloso and Dal'Piaz 1982), and pendimethalin (Haar et al. 2001). *Spergula arvensis* is a summer annual species that can also behave as a winter annual, depending on location and due to seed dimorphism creating variable dormancy levels (Wagner 1988). Therefore, reduced levels of *A. palmeri* could free up resources for this plant to grow and increase its reproductive success, as was observed for *D. sanguinalis* and *M. verticillata*.

No herbicide program reduced *E. indica* populations. Even under the most aggressive programs (G+D+Ac, G+½D+R, and G+½D), populations were more or less stable or tended to increase throughout the 96 mo (Figure 2A). After the first 4 yr, the treatment with glyphosate alone (G) had one of the lowest values of seedling density, but immediately after the herbicide programs were unified, the seedling numbers increased to levels that surpassed the original population (Figure 2A). Glyphosate is an effective herbicide for control of *E. indica* (Chuah et al. 2004), but the antagonism exerted by dicamba (Meyer et al. 2020) could have reduced glyphosate phytotoxicity in this grass. Furthermore, this is the only species that seems to be unaffected by *A. palmeri* populations, while the rest of species exhibited low densities when *A. palmeri* density was high during the first 4 yr (Figure 2A). Despite its low stature, *E. indica* can maintain high reproductive rates (i.e., >300,000 seeds per plant; Ma et al. 2019), even under shaded conditions, by shifting photoassimilate allocation. For example, under 40% to 80% shading, this weed reduced total dry weight and tillers per plant and partitioned more resources to inflorescences and less to roots (Ismail et al. 2003).

For species such as common ragweed (*Ambrosia artemisiifolia* L.) and common lambsquarters (*Chenopodium album* L.), initial densities were low, and all herbicide programs were successful at maintaining the populations at low levels (Figure 2B and C). Also, programs that included residual herbicides favored a faster decline in the populations of these species. It is known that pendimethalin can provide adequate levels of preemergence control of *C. album* (Alebrahim et al. 2012), with higher efficacy than acetochlor (Chomas and Kells 2004; Soltani et al. 2013). Residual herbicides can also complement postemergence broad-spectrum

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Table 5. Equations and R-squared (R^2) of the models for each species and the treatments glyphosate (G), glyphosate plus dicamba (G+D), glyphosate plus residual herbicides (diuron plus pendimethalin) (G+D+R), glyphosate plus dicamba plus acetochlor (G+D+Ac), glyphosate plus alternating dicamba between years plus residual herbicides (diuron plus pendimethalin) (G+½D+R), and glyphosate plus alternating dicamba between years (G+½D).

	Eleusine indica		*Ambrosia artemisiifolia*		*Chenopodium album*		*Spergula arvensis*	
Treatment	Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2
G	$y = -0.004x + 0.54$	0.09	$y = -0.0015x - 0.24$	0.02	$y = -0.001x + 0.13$	0.03	$y = 0.003 - 0.0032x + 0.00011x^2$	0.32
G+D	$y = -0.004x + 0.54$	0.06	$y = -0.0015x - 0.22$	0.03	$y = -0.003x + 0.24$	0.08	$y = -0.071 + 0.0049x + 1.28 \times 10^{-5}x^2$	0.22
G+R	$y = -0.004x + 0.41$	0.08	$y = -0.003x - 0.32$	0.10	$y = -0.003x + 0.22$	0.12	$y = -0.012 + 0.0005x + 1.97 \times 10^{-5}x^2$	0.13
G+D+R	$y = -0.0009x + 0.78$	0.003	$y = -0.0027x - 0.30$	0.08	$y = -0.003x + 0.24$	0.12	$y = 0.01 - 0.0031x + 9.14 \times 10^{-5}x^2$	0.29
G+D+Ac	$y = -0.004x + 0. 57$	0.07	$y = -0.0036x - 0.35$	0.13	$y = -0.001x + 0.13$	0.01	$y = -0.03 + 0.001x + 6.78 \times 10^{-5}x^2$	0.31
G+½D+R	$y = -0.004x + 0.44$	0.08	$y = -0.0009x - 0.13$	0.02	$y = -0.001x + 0.09$	0.01	$y = -0.018 + 0.001x + 5.05 \times 10^{-5}x^2$	0.20
2G+½D	$y = -0.004x + 0.59$	0.08	$y = -0.0016x - 0.20$	0.04	$y = -0.0001x + 0.10$	0.02	$y = -0.028 + 0.001x + 3.81 \times 10^{-5}x^2$	0.20

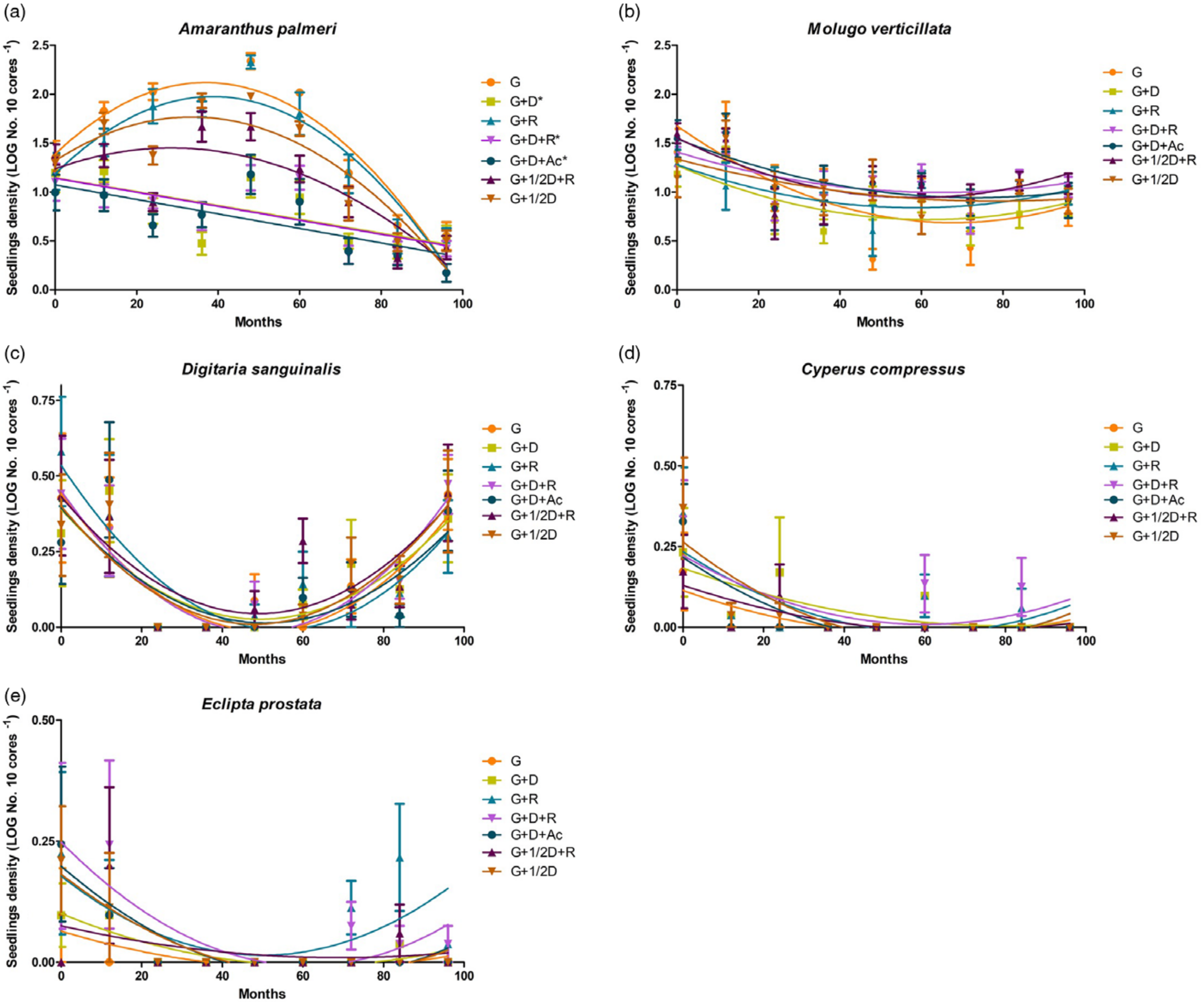

Figure 1. Seedling population density for (A) *Amaranthus palmeri*, (B) *Mollugo verticillata*, (C) *Digitaria sanguinalis*, (D) *Cyperus compressus*, and (E) *Eclipta prostrata* from soil cores in response to herbicide programs: glyphosate (G), glyphosate plus dicamba (G+D), glyphosate plus residual herbicides (diuron plus pendimethalin) (G+D+R), glyphosate plus dicamba plus acetochlor (G+D+Ac), glyphosate plus alternating dicamba between years plus residual herbicides (diuron plus pendimethalin) (G+½D+R) and glyphosate plus alternating dicamba between years (G+½D), throughout the months after experiment initiation. Error bars represent standard error of the mean for each data point; an asterisk (*) indicates regression line slope is different from zero.

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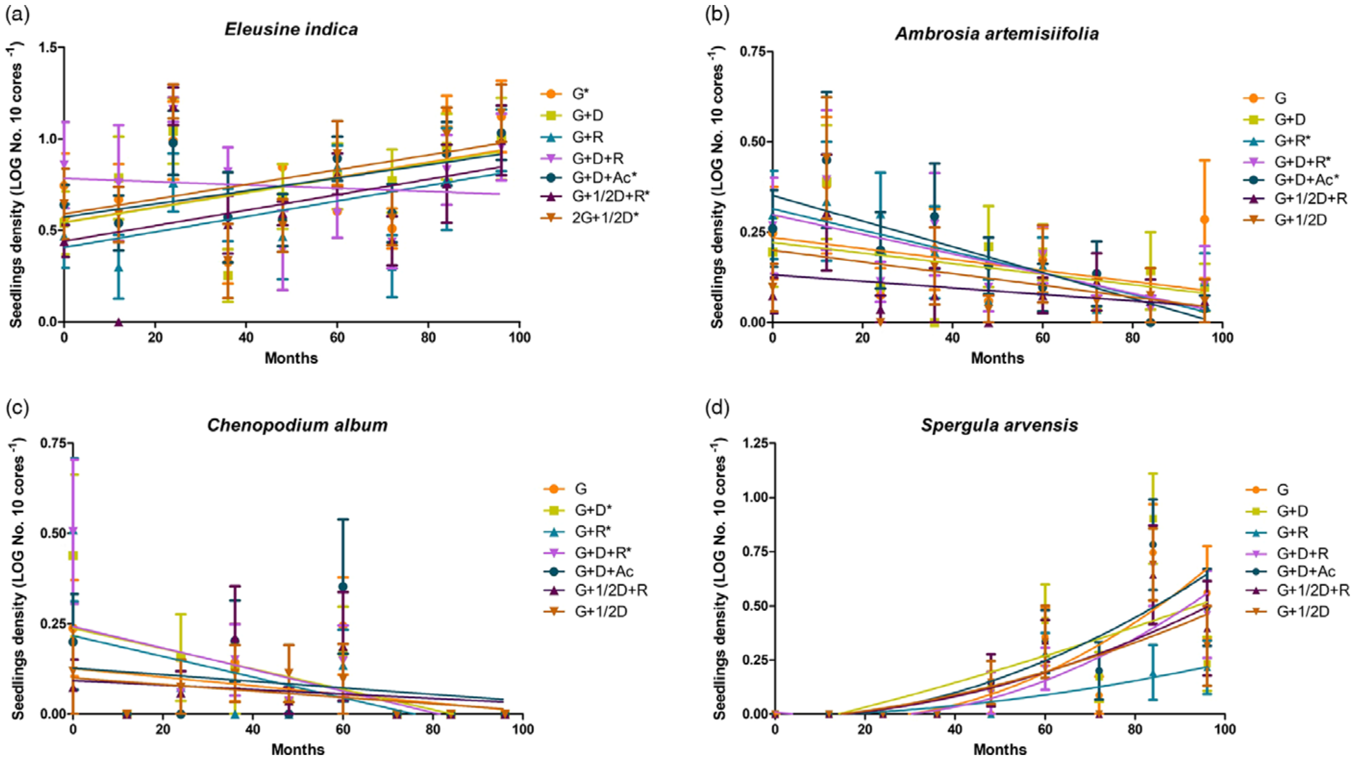

Figure 2. Density of (A) *Eleusine indica*, (B) *Ambrosia artemisiifolia*, (C) *Chenopodium album*, and (D) *Spergula arvensis* from soil cores with their respective regression lines for the different treatments: glyphosate (G), glyphosate plus dicamba (G+D), glyphosate plus residual herbicides (diuron plus pendimethalin) (G+D+R), glyphosate plus dicamba plus acetochlor (G+D+Ac), glyphosate plus alternating dicamba between years plus residual herbicides (diuron plus pendimethalin) (G+½D+R) and glyphosate plus alternating dicamba between years (G+½D), throughout the months after experiment initiation (Months). Error bars represent standard error of the mean for each data point; an asterisk (*) indicates regression line slope is different from zero.

herbicides by improving control of *A. artemisiifolia* (Armel et al. 2003; Barnes et al. 2017).

Frequency of Resistance

At initiation of the experiment, the frequency of GR *A. palmeri* ranged from 1% to 9% (Figure 3). After 1 yr, the frequency of GR *A. palmeri* increased in all programs, being greatest in the glyphosate-only program, but after 4 yr, there were no differences in glyphosate resistance frequency among treatments (Figure 3). Shergill et al. (2018) reported evolution of GR giant ragweed (*Ambrosia trifida* L.) in a susceptible population after 4 yr of continuous use of glyphosate only. In this study, no differences in glyphosate resistance frequency were observed among herbicide programs after 8 yr (Figure 3), despite good control of GR *A. palmeri* plants using continuous glyphosate plus dicamba (Figure 1A). This was likely the result of GR pollen movement from adjacent plots and surrounding fields. It has been documented that GR *A. palmeri* pollen disperses up to 300 m under normal field conditions (Sosnoskie et al. 2012).

This study shows the effectiveness of dicamba in decreasing dense populations of GR *A. palmeri* over the course of several years. Furthermore, these data are consistent with other research that showed a larger increase in the frequency of glyphosate resistance when glyphosate is used alone (Culpepper et al. 2006; Shergill et al. 2018). As observed in the first 4 yr of this study, weed seed production is critical for rapid evolution of GR *A. palmeri*. To date, research has shown a lack of fitness penalties in GR *A. palmeri* compared with GS *A. palmeri*; therefore, there is little delay in the buildup of GR individuals (Jasieniuk et al. 1996), and as the results of the present study suggest, the frequency of glyphosate resistance in a population is not easily reduced.

Although dicamba has been shown to be an effective tool in managing GR *A. palmeri* in cotton over 8 continuous years, diligent stewardship of dicamba should be a priority. Residual herbicides should be incorporated into herbicide programs, and postemergence-only programs must be avoided. Also, mixing two different herbicide MOAs would delay herbicide-resistance evolution more than rotating MOAs (Powles et al. 1997). Dicamba resistance has been reported in *A. palmeri* after three generations of exposure to sublethal rates (Tehranchian et al. 2017). However, at the end of our study, there were no survivors from GR *A. palmeri* populations treated with a field rate (560 g ae ha^{-1}) of dicamba, thus indicating that no tolerance to this herbicide was found among any GR *A. palmeri* populations. The present study differed from the Tehranchian et al. (2017) study in its application of full rather than sublethal rates (560 g ae ha^{-1} vs. 140, 280, and 420 g ae ha^{-1}). In the present research, very few, if any, weed escapes were allowed to grow and contribute to the soil weed seedbank. While we are trying to reduce the incidence of a problematic species (e.g., *A. palmeri*) in the long term we might end up favoring other species (e.g., *E. indica* and *D. sanguinalis*), which ultimately could evolve resistance or increase their weediness. Therefore, special attention must be paid to the rest of the species in the community throughout time. Diverse weed control and production practices are necessary not only to delay the evolution of resistance (Norsworthy et al. 2012; Vencill et al. 2012), but to maintain weed population levels below those that are economically damaging.

Population trajectories can be very different depending on the herbicide program, weed species, and interactions among them, all

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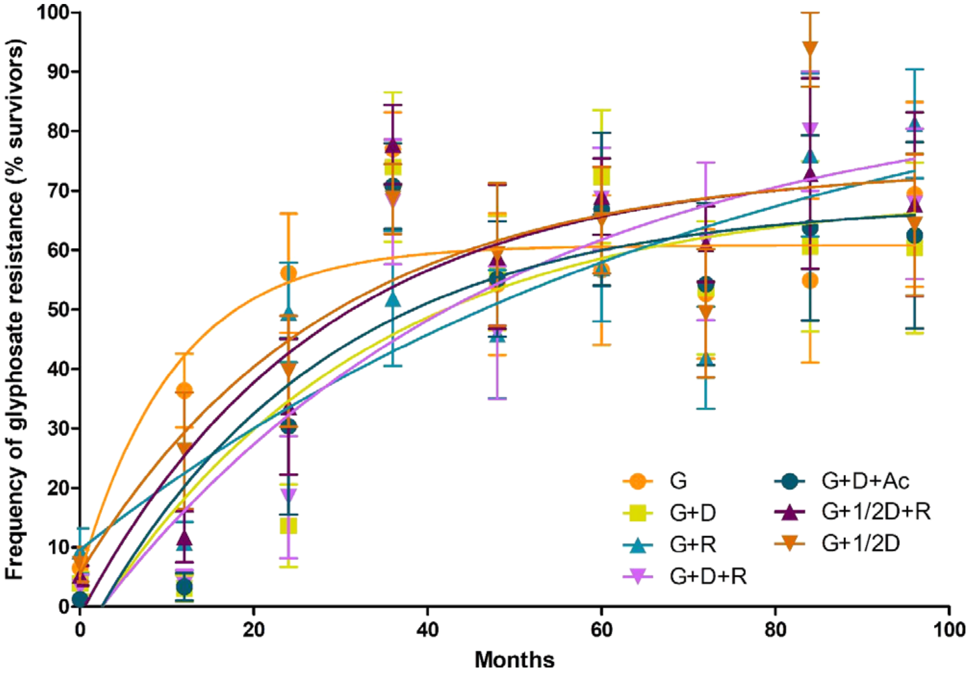

Figure 3. Frequency of glyphosate resistance of *Amaranthus palmeri* from soil cores in response to the herbicide treatments: glyphosate (G), glyphosate plus dicamba (G+D), glyphosate plus residual herbicides (diuron plus pendimethalin) (G+D+R), glyphosate plus dicamba plus acetochlor (G+D+Ac), glyphosate plus alternating dicamba between years plus residual herbicides (diuron plus pendimethalin) (G+½D+R) and glyphosate plus alternating dicamba between years (G+½D), throughout the months after experiment initiation (Months). Error bars represent standard error of the mean for each data point.

of which shape the community over time. The present study illustrates how intensifying herbicide use (number of applications and MOAs) did not result in the reduction of the populations of all weed species present in the field. Thus, species with prolific reproduction can affect weed community assembly and "mask" the overall weed control potential of herbicide programs. The present study shows that intensive herbicide programs targeting dominant weed species can provide excellent control of those species but not necessarily of the entire weed community. In fact, the removal of dominant weed species seemed to have freed resources or reduced competitive interactions, favoring the increase of populations of other weed species. Ignoring those community dynamics can induce researchers to reach erroneous conclusions about the efficacy of weed control strategies and especially of herbicide programs.

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