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Research Report

The silver bullet that wasn't: Rapid agronomic weed adaptations to glyphosate in North America

Christopher Landau[a,*], Kevin Bradley[b], Erin Burns[c], Michael Flessner[d], Karla Gage[e], Aaron Hager[f], Joseph Ikley[g], Prashant Jha[h], Amit Jhala[i], Paul O. Johnson[j], William Johnson[k], Sarah Lancaster[l], Travis Legleiter[m], Dwight Lingenfelter[n], Mark Loux[o], Eric Miller[p], Jason Norsworthy[q], Micheal Owen[h], Scott Nolte[r], Debalin Sarangi[s], Peter Sikkema[t], Christy Sprague[c], Mark VanGessel[u], Rodrigo Werle[v], Bryan Young[k] and Martin M. WilliamsII[a]

[a]Global Change and Photosynthesis Research Unit, USDA-ARS, Urbana, IL 61801, USA
[b]Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA
[c]Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA
[d]School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 24061, USA
[e]School of Agricultural Sciences/School of Biological Sciences, Southern Illinois University Carbondale, Carbondale, IL 62901, USA
[f]Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA
[g]Department of Plant Sciences, North Dakota State University, Fargo, ND 58108, USA
[h]Department of Agronomy, Iowa State University, Ames, IA 50201, USA
[i]Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[j]Agronomy, Horticulture, & Plant Science, South Dakota State University, Brookings, SD 57007, USA
[k]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA
[l]Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA
[m]Department of Plant and Soil Sciences, University of Kentucky, Princeton, KY 42445, USA
[n]Department of Plant Science, Penn State University, University Park, PA 16802, USA
[o]Department of Horticulture and Crop Science, Ohio State University, Columbus, OH 43210, USA
[p]School of Agricultural Sciences, Southern Illinois University Carbondale, Carbondale, IL 62901, USA
[q]Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701, USA
[r]Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77840, USA
[s]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA
[t]Department of Plant Agriculture, University of Guelph Ridgetown Campus, Ridgetown, ON N0P 2C0, Canada
[u]Department of Plant and Soil Sciences, University of Delaware, Georgetown, DE 19947, USA
[v]Department of Agronomy, University of Wisconsin-Madison, Madison, WI 53705, USA
*To whom correspondence should be addressed: Email: Christopher.landau@usda.gov
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Abstract

The rapid adoption of glyphosate-resistant crops at the end of the 20th century caused a simplification of weed management that relied heavily on glyphosate for weed control. However, the effectiveness of glyphosate has diminished. A greater understanding of trends related to glyphosate use will shed new light on weed adaptation to a product that transformed global agriculture. Objectives were to (1) quantify the change in weed control efficacy from postemergence (POST) glyphosate use on troublesome weeds in corn and soybean and (2) determine the extent to which glyphosate preceded by a preemergence (PRE) improved the efficacy and consistency of weed control compared to glyphosate alone. Herbicide evaluation trials from 24 institutions across the United States of America and Canada from 1996 to 2021 were compiled into a single database. Two subsets were created; one with glyphosate applied POST, and the other with a PRE herbicide followed by glyphosate applied POST. Within each subset, mean and variance of control ratings for seven problem weed species were regressed over time for nine US states and one Canadian province. Mean control with POST glyphosate alone decreased over time while variability in control increased. Glyphosate preceded by a labeled PRE herbicide showed little change in mean control or variability in control over time. These results illustrate the rapid adaptation of agronomically important weed species to the paradigm-shifting product glyphosate. Including more diversity in weed management systems is essential to slowing weed adaptation and prolonging the usefulness of existing and future technologies.

Keywords: weed adaptation, herbicide resistance, corn (*Zea mays*), soybean (*Glycine max*), glyphosate

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Significance Statement

Glyphosate is the most commonly used herbicide in corn and soybean production systems. Due to its continued use and evolution of glyphosate-resistant weeds, the efficacy of glyphosate has decreased. Quantifying changes in glyphosate efficacy and variability of control with glyphosate would improve the development of future weed management systems. Using a database of 25 years of glyphosate from locations across the United States of America and Canada, we showed a decrease in weed control and an increase in variability of control with glyphosate alone, but little to no change when a preemergence herbicide is used prior to glyphosate application. This highlights the need for diversity in weed management programs to provide high and consistent weed control.

Introduction

A central dogma of weed science is that weeds will adapt to changes in their environments, including weed management practices. Widely accepted evidence supporting this dogma is the widespread evolution of herbicide resistance. A noteworthy example is weed resistance to glyphosate, a herbicide once heralded as a "silver bullet" of weed control, and now the most widely used herbicide in the world with 8.6 billion kilograms of product applied annually (1).

The commercialization of glyphosate-resistant (GR) soybean and corn in 1996 and 1998, respectively, allowed glyphosate to be used as a selective in-crop postemergence (POST) herbicide. The adoption of GR corn and soybean, as well as in-crop glyphosate use, was rapid and unprecedented. By 2014, >90% of corn and soybean hectares was GR (2–4). Rapid adoption of the technology along with a reduced cost of glyphosate caused a shift in weed management practices away from a more diverse system using a combination of chemical, biological, cultural, and mechanical techniques to a system that often consisted of glyphosate alone (5). Within the first four years after the commercialization of GR crops, a majority of US cropland experienced upwards of a 10-fold increase in the amount of glyphosate applied (Fig. 1).

The shift in weed management caused intense selection pressure from glyphosate that facilitated the evolution of GR weeds. Since the first confirmed case of glyphosate resistance in 1996 in *Lolium rigidum* Gaud., there have been 354 confirmed cases in 57 weed species globally (7), primarily confined to GR crop systems (8). There are several mechanisms by which weeds have evolved glyphosate resistance. Several species, including *Amaranthus palmeri* S. *Watson* and *Amaranthus tuberculatus* (Moq.) *J. D. Sauer*, evolved resistance via mutations to the herbicide target site enzyme which increased quantities of target site proteins (9, 10). Other species, including *Erigeron canadensis* L. and *Sorghum halepense* (L.) Pers., evolved non-target site resistance mechanisms, such as reduced translocation (11), vacuolar sequestration (12), reduced glyphosate uptake (13), or rapid necrosis of treated tissue followed by regrowth (14, 15). Most recently, enhanced glyphosate metabolism was discovered in *Echinochloa colona* L. (16). While evolved resistance has reduced glyphosate efficacy on certain weed populations, a quantitative understanding of the extent to which weeds adapted to repeated glyphosate use is lacking.

Aside from herbicide resistance, comparatively less is known about evolutionary adaptations of more complex life-history traits to crop/weed management practices. Earlier crop sowing dates shifted *Chenopodium ficifolium* Sm. germination temperatures from a range of 30–40 °C to 0–30 °C and favored earlier flowering in *Chenopodium album* L. (17, 18). Additionally, weed adaptation to management practices may oscillate over time. Ethridge et al. (19) reported decreased competitiveness of *Setaria faberi* Herm. between 1983 and 1991, while the same population increased in competitiveness from 1996 to 2017. A challenge to quantifying such life-history adaptations is that, experimentally, a large sample size from a broad temporal range is required (19).

Life-history trait adaptations, while not necessarily providing herbicide resistance, can reduce the effectiveness of herbicides in other manners. Weed adaptations that shift to earlier emergence and greater seedling growth lead to larger plants at the time of glyphosate application, resulting in insufficient glyphosate concentrations to kill the plant (20–22). Conversely, delayed emergence allows the weed to avoid contact with glyphosate altogether (23). Both resistance and competitive weed adaptations can increase the variability of control with glyphosate within a field; however, the extent to which variability in weed control changes across generations is poorly understood. Few empirical studies include variability in weed response and those that do often represent a small number of years or observations. A better understanding of weed adaptation to management may impact the development of future weed management systems.

Reliance on glyphosate contributed to stagnation of herbicide discovery. During the first two decades after the introduction of GR crops, the use and value of herbicides other than glyphosate plummeted (2, 24, 25). This decline led to many companies discontinuing or greatly reducing their herbicide discovery research (2). Recent consolidations of agricultural pesticide companies further reduced herbicide discovery research (26).

Glyphosate is used across the major US and Canadian corn and soybean growing regions with more than 130 million kilograms applied annually (6, 27). Loss of glyphosate as an effective weed management tool could result in up to $4.17 billion in crop losses per year in North America (28). A better understanding of how glyphosate efficacy has changed over time will reveal the extent to which weeds adapt to a technology that was adopted at a rate not experienced previously in modern crop production. As such, this research was developed with the objectives to (1) quantify the risk of poor weed control from repeated glyphosate use over time on troublesome weed species in corn and soybean production systems and (2) determine the extent to which glyphosate preceded by a preemergence applied soil-residual herbicide (PRE) reduced the risk of poor and inconsistent weed control compared to glyphosate applied alone. Two hypotheses were tested: (1) efficacy of glyphosate has declined over time and increased in variability and (2) addition of a labeled PRE herbicide prior to a POST glyphosate application has provided improved and less variable weed control compared to a single POST application of glyphosate.

Materials and methods

Most North American land-grant universities have an herbicide evaluation program (HEP) that conducts herbicide efficacy trials on various weed species. These programs have been active for decades, with several programs collecting data as far back as

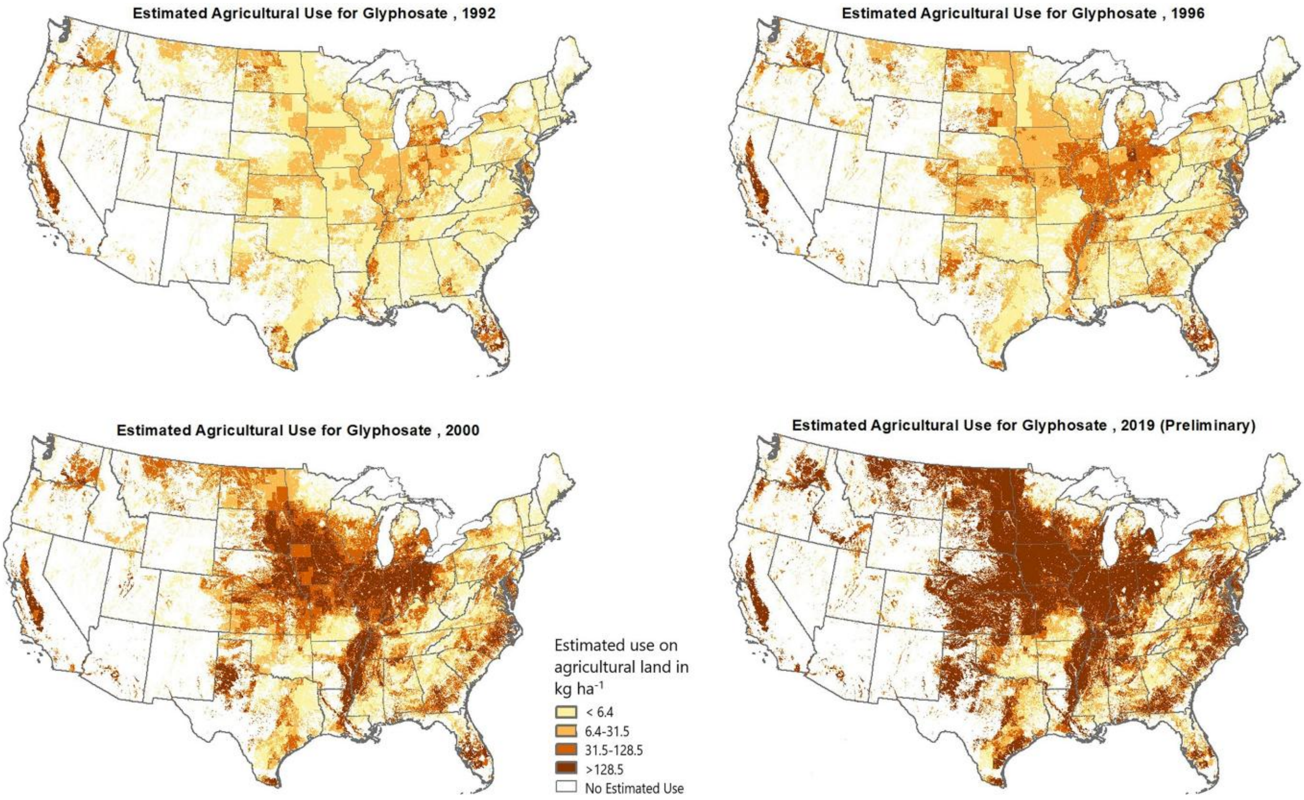

Fig. 1. Estimated glyphosate use in the United States of America from 1992 to 2019. Constructed from figures from USGS-NAWQA (6).

the 1970s. Within each program, upwards of 50 field trials per year were established. Individual trials were conducted in a randomized complete block design with 3–4 replications. Weeds from untreated plots and weeds that survived herbicide application were allowed to produce seed to keep weed populations relatively consistent for data collection. Additionally, trials and plots within trials were rotated throughout the field over time. As such, a given plot may not have always received the same herbicide or herbicide combination in consecutive years. Results from these trials were summarized in yearly field guides by each institution and were then archived in individual databases. Previous research showed how the University of Illinois database is useful in modeling trends in herbicide efficacy, crop yield loss due to weeds, and weather impacts on weed management (29, 30). However, a single institution's database is geographically constrained. In 2021, an effort was made to combine herbicide evaluation data from institutions across North America. Data from 24 institutions were collected and standardized into a single common database (hereafter referred to as the HEP database).

The HEP database was filtered to include only glyphosate treatments to address the objectives of this study. Data typically included visual estimations of mean percent weed control (0% being no control and 100% being complete control) ratings following various treatments containing at least one POST glyphosate application in corn and soybean field trials. Mean control for each treatment was calculated from the 3 to 4 replications within each trial. Mean control ratings were filtered to include only those recorded 14–28 days after the in-crop glyphosate application. Most institutions had more than one research location where data were collected; however, many locations contained a small number of trials from <4 years. As such, only data from the location with a majority of each institution's field trials were used. Eleven institutions representing nine US states and one Canadian province had sufficient data from a single location for one or more weed species; as such, only data from these locations were used for analyses (Fig. S1).

The HEP database contains ratings on more than 50 weed species; however, in order to more accurately model spatiotemporal changes in glyphosate efficacy, only species with more than 50 observations each from three or more locations were used for analysis. The following seven species met these criteria: *Abutilon theophrasti* Medik., *A. palmeri*, *A. tuberculatus*, *Ambrosia artemisiifolia* L., *Ambrosia trifida* L., *C. album*, and *E. canadensis*.

Prior to analysis, the filtered HEP database was split into two subsets; one consisting of treatments with a single, in-crop (i.e. POST) glyphosate application as the only herbicide component, and the other containing treatments consisting of a single in-crop glyphosate application following a labeled soil-residual (i.e. PRE) herbicide application. Treatments would often consist of multiple POST applications following a PRE herbicide, which could confound results. As such, treatments containing a POST application following the initial glyphosate application were included in the subsets only if there was a weed control rating recorded prior to the second POST application. Both subsets were further filtered to include only glyphosate rates between 0.75 and 1.20 kg a.e. ha^{-1} to capture the range in labeled rates between 1996 and 2021.

Statistical analyses

When simple linear regression models were fit through the data for each location, there was an increase in the variability of weed control over time. Heteroscedasticity was determined using the studentized Breusch-Pagan test with the *bptest* function from the *lmtest* package in R (31). As such, within each subset, weighted

Table 1. Number of observations by location with control rating data for the seven evaluated weed species.

Location	University	Years of data	*Abutilon theophrasti*		*Amaranthus palmeri*		*Amaranthus tuberculatus*		*Ambrosia artemisiifolia*		*Ambrosia trifida*		*Chenopodium album*		*Erigeron canadensis*	
			Treatment													
			gly	PRE fb gly	gly	PRE fb gly	gly	PRE fb gly	gly	PRE fb gly	gly	PRE fb gly	gly	PRE fb gly	gly	PRE fb gly
			Number of observations													
Illinois	University of Illinois	1996–2020	675	1,377	–	–	1,299	2,638	144	304	–	–	644	1,525	–	–
	Southern Illinois University	1999–2019	457	498	–	–	908	888	366	266	438	755	–	–	91	214
Indiana	Purdue University	2005–2020	57	261	–	–	–	–	–	–	116	662	107	489	65	152
Kansas	Kansas State University	1998–2020	67	259	304	681	51	65	–	–	–	–	–	–	–	–
Michigan	Michigan State University	2003–2021	53	296	53	82	–	–	104	459	–	–	355	1,081	53	125
North Dakota	North Dakota State University	1996–2018	–	–	–	–	–	–	142	394	–	–	523	610	–	–
Ohio	The Ohio State University	1996–2021	117	616	–	–	–	–	137	378	552	1,335	304	1,041	101	380
Ontario	University of Guelph	1997–2021	237	136	–	–	108	104	428	468	–	–	428	554	60	136
Pennsylvania	The Pennsylvania State University	2002–2021	50	302	–	–	–	–	56	311	–	–	87	376	–	–
South Dakota	South Dakota State University	2012–2021	54	151	–	–	56	232	–	–	–	–	52	199	–	–
Virginia	Virginia Tech	2015–2021	–	–	66	147	–	–	–	–	–	–	–	–	–	–
Total			1,713	3,745	423	763	2,366	3,695	1,377	2,580	1,106	2,752	2,448	5,676	370	1,007

Treatments were separated into those consisting of a foliar-applied glyphosate application as the only component and those consisting of foliar-applied glyphosate following a soil-residual herbicide application. Observations were means of 3–4 replicates from randomized complete block design trials. gly, glyphosate; fb, followed by; PRE, preemergence.

regression models were created by regressing weed control over time for each location-by-species combination with more than 50 observations using the *lm* function in R (32). Additionally, within each subset, a combined weighted regression model was created for each weed species by combining data from all locations with 50 or more observations for a given weed species. Weighted regression is used to remove heteroscedasticity by giving more importance (i.e. weight) to observations with lower variability (33). Several potential weight parameters were tested; however, the reciprocal of the squared residuals from the ordinary least squares regression model was chosen, as it produced the smallest percent residual error (34, 35).

The coefficient of variation (CV) of weed responses was used to quantify variability in glyphosate efficacy. For each year, within both subsets and for each location-by-species combination, CVs were calculated using the following equation:

$$CV_i = \frac{\sigma_i}{\mu_i} * 100 \quad (1)$$

where σ_i is the standard deviation of the weed control of year *i* and μ_i is the mean of the weed control for a year *i*. The CV values were then regressed over time using the *lm* function in R to quantify the changes in the variability of weed control over time. Additionally, within each subset, a combined model regressing CV values over time was created for each weed species by combining data from all locations with 50 or more observations.

Results and discussion

This analysis depicts weed adaptation to glyphosate in North American corn and soybean production systems over the last quarter century (Table 1). The timeframe encompasses an important transition in North American crop production, including the introduction and near-universal adoption of GR corn and soybean (2–4), the simplification of weed management systems to rely almost exclusively on glyphosate (5), the evolution of GR weeds (7), and the current reality of resistant weeds becoming more common and representing an increasing problem. The seven weed species included in this study are major pests in North America and are ranked among the most common and/or troublesome weed species in corn and soybean according to the Weed Science Society of America (36–38). Additionally, five of the seven species (*A. palmeri*, *A. tuberculatus*, *A. artemisiifolia*, *A. trifida*, and *E. canadensis*) have confirmed populations that are not only resistant to glyphosate but are also listed among the most common herbicide-resistant weeds (3).

The United States of America and Canada combined account for 31.6 and 31.7% of the global corn and soybean production, respectively (39, 40). The 10 states/provinces in the database represent a majority of corn and soybean growing regions in the United States of America and Canada, accounting for 13.6 and 15.6 million hectares of corn and soybean, respectively (41, 42).

Insufficient length of time and small sample size are common hurdles to studying the evolutionary adaptation in weeds, particularly for traits other than herbicide resistance (19). Most previous studies on weed response to glyphosate were evaluated in one or two populations over two to three years, often resulting in <20 observations. While the number of observations varied by species, the present study utilized 370–5,676 observations per species over a time period spanning up to 25 years (Table 1). All species were represented by multiple populations. While this research was unable to account for the factors driving weed responses to

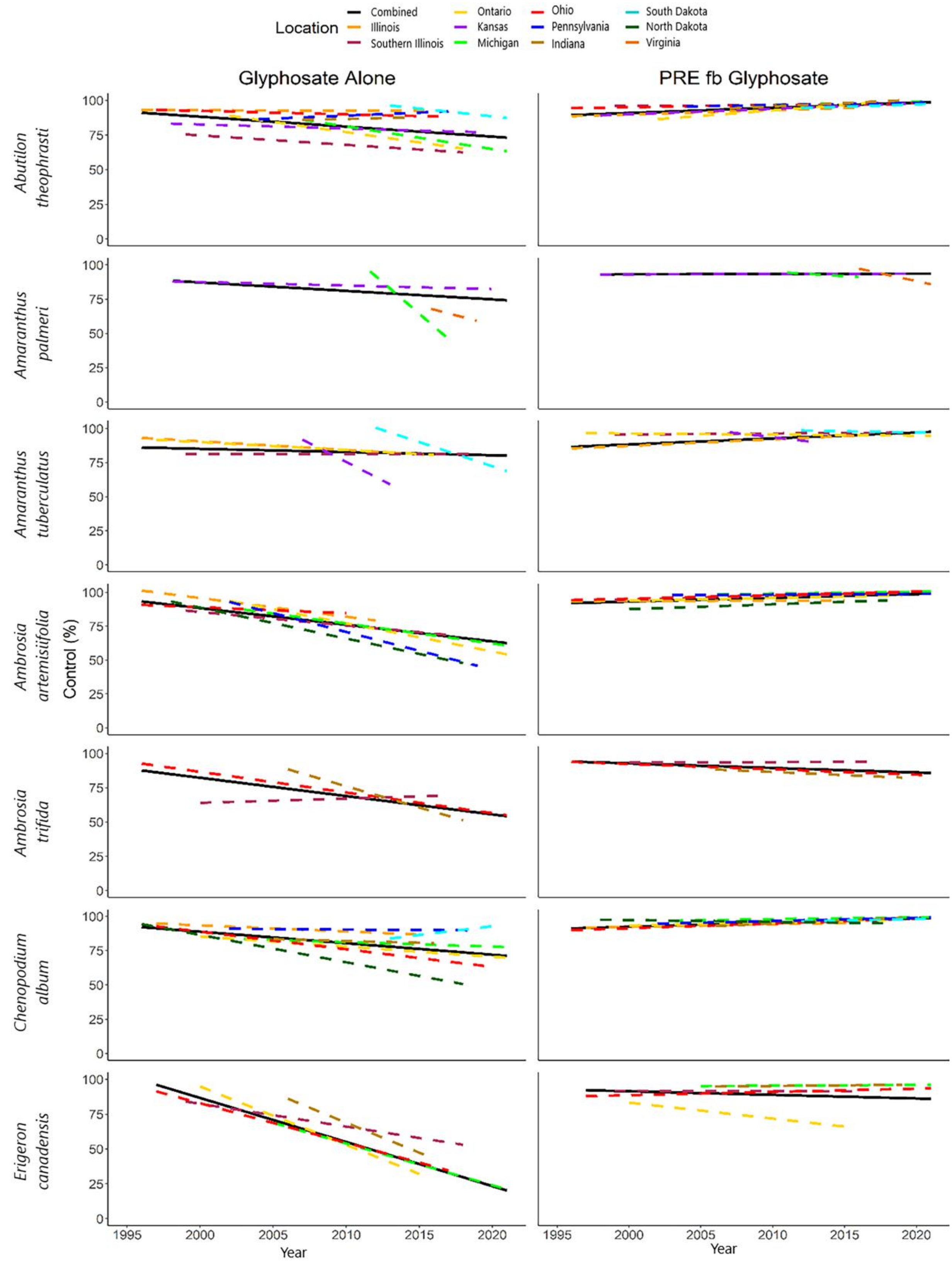

Fig. 2. Weighted regression models for percent weed control of seven weed species treated with POST glyphosate alone and POST glyphosate following a labeled PRE herbicide over time. Separate regression models were constructed for up to 11 sites. A combined weighted regression model was created for each weed species by combining data from all locations with 50 or more observations for a given weed species. gly, glyphosate; fb, followed by; PRE, preemergence.

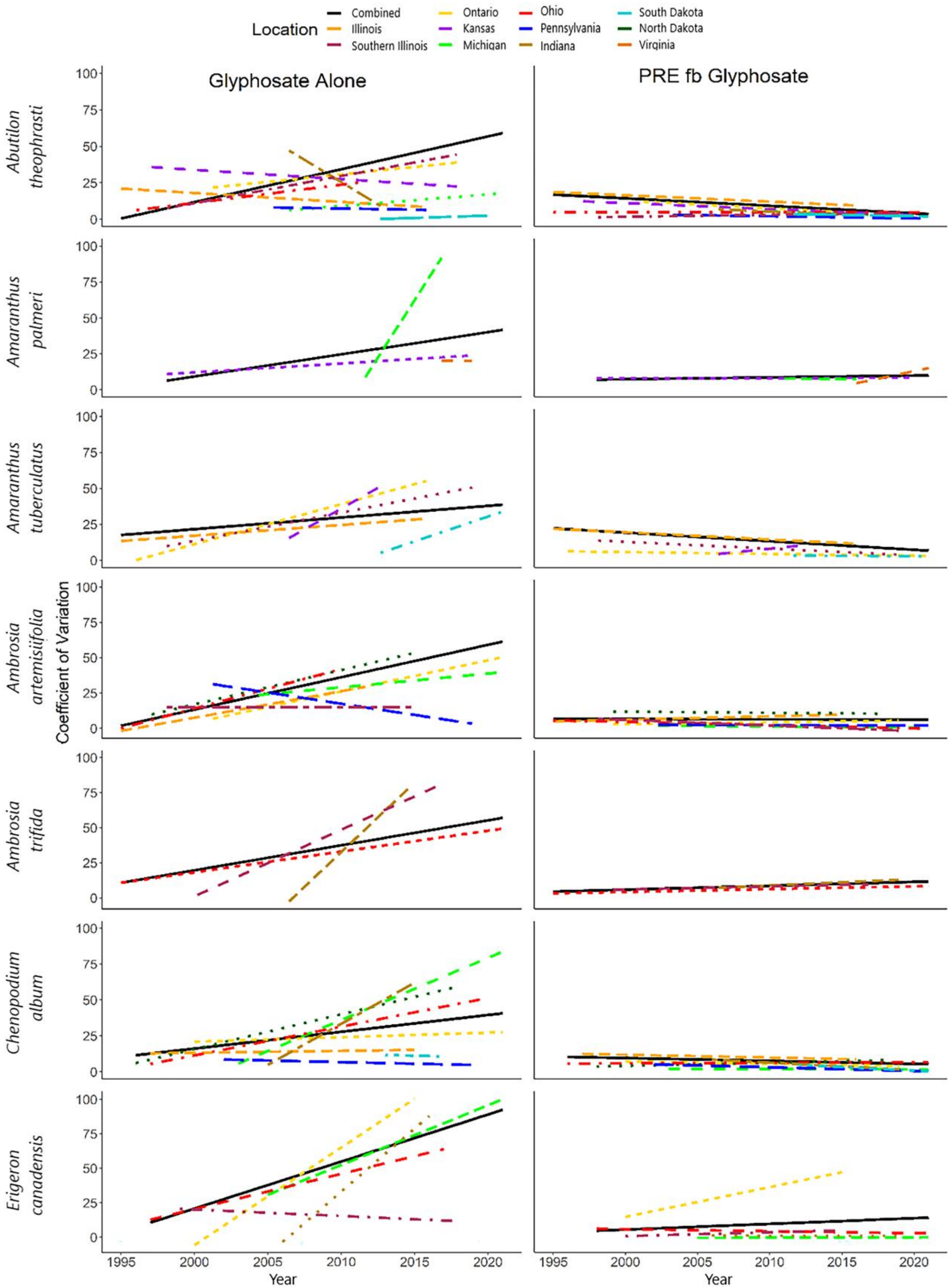

Fig. 3. Regression models for coefficients of variation (standard deviation of control within a given year/mean control in a given year * 100) of percent weed control of seven weed species treated with POST glyphosate alone and POST glyphosate following a labeled PRE herbicide over time. Separate regression models were constructed for up to 11 locations. A combined regression model was created for each weed species by combining data from all locations with 50 or more observations for a given weed species. gly, glyphosate; fb, followed by; PRE, preemergence.

Table 2. Weighted regression slope estimates for annual percent change in weed control on seven weed species treated with POST glyphosate alone or following a labeled PRE herbicide over time.

Location	University	*Abutilon theophrasti*[a] gly	*Abutilon theophrasti*[a] PRE fb gly	*Amaranthus palmeri*[a] gly	*Amaranthus palmeri*[a] PRE fb gly	*Amaranthus tuberculatus*[a] gly	*Amaranthus tuberculatus*[a] PRE fb gly	*Ambrosia artemisiifolia*[a] gly	*Ambrosia artemisiifolia*[a] PRE fb gly	*Ambrosia trifida*[a] gly	*Ambrosia trifida*[a] PRE fb gly	*Chenopodium album*[a] gly	*Chenopodium album*[a] PRE fb gly	*Erigeron canadensis*[a] gly	*Erigeron canadensis*[a] PRE fb gly
		Slope estimates													
Combined	Combined	**−0.72B**[b]	**0.37ab**	**−0.64B**	0.02c	**−0.24A**	**0.44a**	**−1.28C**	**0.27b**	**−1.33C**	**−0.33d**	**−0.84B**	**0.31ab**	**−3.16D**	**−0.26d**
Illinois	University of Illinois	−0.03	**0.31**	-	-	**−0.63**	**0.48**	**−1.38**	0.01	-	-	**−0.45**	**0.19**	-	-
	Southern Illinois University	**−0.68**	0.05	-	-	−0.02	0.08	**−1.01**	**0.24**	0.31	0.04	-	-	**−1.63**	−0.03
Indiana	Purdue University	0.04	**0.49**	-	-	-	-	-	-	**−3.10**	−0.45	−0.27	**0.46**	**−4.30**	0.09
Kansas	Kansas State University	−0.29	**0.83**	**−0.46**	0.03	**−5.57**	**−1.21**	-	-	-	-	-	-	-	-
Michigan	Michigan State University	−0.32	**0.53**	**−9.56**	−0.63	-	-	**−1.47**	**0.15**	-	-	−0.32	**0.14**	**−3.00**	0.05
North Dakota	North Dakota State University	-	-	-	-	-	-	**−2.28**	0.35	-	-	0.35	−0.12	-	-
Ohio	The Ohio State University	−0.25	**0.17**	-	-	-	-	**−0.44**	**0.26**	**−1.51**	−0.39	**−1.28**	**0.34**	**−2.85**	**0.24**
Ontario	University of Guelph	**−1.48**	**0.85**	-	-	**−0.58**	**−0.08**	**−2.08**	**0.20**	-	-	**−0.73**	**0.23**	**−4.18**	−1.14
Pennsylvania	The Pennsylvania State University	0.44	**0.23**	-	-	-	-	**−2.78**	0.05	-	-	−0.06	**0.24**	-	-
South Dakota	South Dakota State University	**−1.12**	**0.54**	-	-	**−3.53**	**−0.22**	-	-	-	-	1.27	0.19	-	-
Virginia	Virginia Tech	-	-	**−2.87**	**−2.27**	-	-	-	-	-	-	-	-	-	-

Separate models were created for each of up to 11 sites and a combined regression model was created for each weed species by combining data from all locations with 50 or more observations. Model slope estimates in boldface were significantly different from zero at $\alpha = 0.05$. gly, glyphosate; fb, followed by; PRE, preemergence.

[a] A significant difference between the glyphosate alone and PRE fb glyphosate combined models for the given species at $\alpha = 0.05$.

[b] Slope estimates for the combined models within a treatment (gly or PRE fb gly) with no common letters are significantly different at $\alpha = 0.05$. Upper case letters are used for comparisons across the gly treatment while lower case letters are used for comparisons across the PRE fb gly treatment.

Table 3. Annual slope estimates for coefficients of variation (standard deviation of control within a given year/mean control in a given year * 100) of percent weed control on seven weed species treated with POST glyphosate alone and POST glyphosate following a labeled PRE herbicide over time.

Location	University	*Abutilon theophrasti*[a]		*Amaranthus palmeri*[a]		*Amaranthus tuberculatus*[a]		*Ambrosia artemisiifolia*[a]		*Ambrosia trifida*[a]		*Chenopodium album*[a]		*Erigeron canadensis*[a]	
		Treatment													
		gly	PRE fb gly	gly	PRE fb gly	gly	PRE fb gly	gly	PRE fb gly	gly	PRE fb gly	gly	PRE fb gly	gly	PRE fb gly
		Slope estimates													
Combined	Combined	**2.34AB**[b]	**−0.53a**	**1.60AB**	0.13c	**0.84A**	**−0.62a**	**2.38AB**	−0.01b	**1.85AB**	**0.28d**	**1.17AB**	**−0.20ab**	**3.41B**	0.39d
Illinois	University of Illinois	−0.63	−0.46	–	–	**0.78**	**−0.52**	**1.95**	0.25	–	–	0.13	−0.20	–	–
	Southern Illinois University	**1.94**	0.14	–	–	**2.04**	−0.49	0.01	**−0.41**	**4.94**	0.22	–	–	−0.47	0.26
Indiana	Purdue University	−6.34	−0.45	–	–	–	–	–	–	**10.30**	**0.46**	**5.71**	**−0.53**	**9.15**	−0.01
Kansas	Kansas State University	−0.66	**−0.43**	**0.65**	0.02	**5.99**	1.08	–	–	–	–	–	–	–	–
Michigan	Michigan State University	0.82	−0.34	**16.65**	−0.08	–	–	1.01	−0.07	–	–	**4.33**	−0.02	**4.33**	0.03
North Dakota	North Dakota State University	–	–	–	–	–	–	**2.53**	−0.08	–	–	−0.08	−0.08	–	–
Ohio	The Ohio State University	**1.30**	−0.01	–	–	–	–	**2.90**	**−0.23**	**1.54**	**0.20**	**2.00**	0.04	**2.56**	−0.14
Ontario	University of Guelph	**1.07**	−0.54	–	–	**2.89**	−0.13	**2.29**	0.13	–	–	0.32	−0.17	**7.08**	2.03
Pennsylvania	The Pennsylvania State University	−0.17	**−0.15**	–	–	–	–	−1.65	−0.01	–	–	−0.22	**−0.26**	–	–
South Dakota	South Dakota State University	0.33	−0.17	–	–	3.58	−0.03	–	–	–	–	−0.26	−0.48	–	–
Virginia	Virginia Tech	–	–	−0.14	2.10	–	–	–	–	–	–	–	–	–	–

Separate models were created for each of up to 11 sites and a combined regression model was created for each weed species by combining data from all locations with 50 or more observations. Model slope estimates in boldface were significantly different from zero at $\alpha = 0.05$. gly, glyphosate; fb, followed by; PRE, preemergence.

[a]A significant difference between the glyphosate alone and PRE fb glyphosate combined models for the given species at $\alpha = 0.05$.

[b]Slope estimates for the combined models within a treatment (gly or PRE fb gly) with no common letters are significantly different at $\alpha = 0.05$. Upper case letters are used for comparisons across the gly treatment while lower case letters are used for comparisons across the PRE fb gly treatment.

glyphosate, it is the largest cumulative measure of how weed communities have adapted to a simplified weed management tactic which became the norm throughout North America.

Weed control over time

Shortly after commercialization of GR crops, glyphosate provided superior weed control in corn and soybean. At the time of first occurrence in the database (1996–1998), average control of *A. theophrasti*, *A. artemisiifolia*, *C. album*, and *E. canadensis* following a single POST glyphosate application was >90%, while control of *A. palmeri*, *A. tuberculatus*, and *A. trifida* was >80% (Fig. 2). The literature is replete with studies showing excellent control of most weed species within the first few years following the introduction of GR crops (43–50). High levels of control for multiple weed species in the early years of in-crop glyphosate use was the major driver of the rapid adoption of glyphosate and GR technology (2).

Weed control with one POST application of glyphosate deteriorated over time for most species at nearly every location. Averaged across 11 locations, weed control decreased by 2.4–31.6% per decade (Table 2). Several factors likely contributed to the decrease in weed control over time. Unconfirmed evolution of glyphosate resistance in these populations from repeated use of glyphosate may have contributed to the decline. Evans et al. (51) reported that fields that received frequent glyphosate applications and little herbicide diversity had the highest incidence of GR *A. tuberculatus*. Furthermore, previous research using a simulation model showed that overuse of glyphosate can lead to glyphosate resistance within 8–12 years of initial use (52).

Additionally, weed populations in this study may have adapted their life-history strategies. Ethridge et al. (19) showed an increase in *S. faberi* competitiveness in populations from 2017 compared with populations from 1996 resulting from increased leaf area and biomass. Increased biomass has been shown to prevent lethal doses of glyphosate from accumulating in the plant, resulting in reduced levels of control (20–22). Although the origin of the declining efficacy cannot be determined in the present study, it does provide the most extensive view to date of the spatiotemporal changes in glyphosate efficacy.

When combined across locations, the decreased control of *E. canadensis* with glyphosate alone (31.6% per decade) was significantly greater than that of the other six species (Table 2). This sharp decrease in control may be due to the ability of *E. canadensis* to move pollen and seed over large distances. Huang et al. (53) showed that, while *E. canadensis* is primarily self-pollinated, under certain environmental conditions pollen can move up to 100 m in height and 480 m downwind from the source plant. Additionally, Wang et al. (54) reported that one GR plant may be sufficient to spread resistant alleles throughout a field. Dauer et al. (55) showed that, while most *E. canadensis* seed remains within 100 m of the source plant, some seeds can be dispersed >500 m from the source plant.

A PRE herbicide applied prior to POST glyphosate significantly improved weed control over time compared to glyphosate applied once POST (Fig. 2). Averaged across sites, the change in weed control ranged from −3.3 to 4.4% per decade (Table 2). Control of *A. theophrasti*, *A. palmeri*, *A. tuberculatus*, *A. artemisiifolia*, and *C. album* with the two-pass herbicide programs showed minor increases over time at most of the locations. Control of *A. trifida* and *E. canadensis* decreased by 3.3 and 2.6% per decade, respectively, with a PRE herbicide followed by POST glyphosate; however, the decrease was significantly smaller compared with glyphosate used alone. The authors attribute the decrease in *E. canadensis* control to the increase in GR populations at the sites. These results are consistent with previous research, which showed increased weed control when a residual PRE herbicide was used prior to POST glyphosate compared to glyphosate applied once POST (56–59).

The addition of a labeled PRE herbicide prior to POST glyphosate application in this study mitigated some risk of reduced weed control with glyphosate alone, perhaps by slowing weed adaptation. Legleiter et al. (60) showed no control of a GR population of *A. tuberculatus* when POST glyphosate was used alone; however, when glyphosate followed a PRE herbicide, control increased by 66 to 91%. Neve et al. (61) showed that PRE fomesafen prior to POST glyphosate application reduced the risk of the evolution of glyphosate resistance in *A. palmeri* by >50%, although it did not completely eliminate the risk of resistance. Herbicide application technology has recently changed to incorporate artificial intelligence for detecting and spot spraying weeds postemergence (62, 63). This technology allows for reduced herbicide volumes and cost per hectare. However, even as a spot spray, overuse of a single selective POST herbicide used alone would lead to reduced efficacy over time. As such, diversifying weed management strategies to include a labeled PRE herbicide, as well as using cultural, mechanical, or biological tactics, will be essential to reducing the risk of weed adaption to POST herbicides, regardless of application strategy.

Annual weeds can rapidly adapt to stresses from their environment. Franks et al. (64) showed that to avoid drought, *Brassica rapa* L. adapted to flower earlier within three generations. Weed species evaluated in the present study, producing a single generation per year, showed signs of adaptation to glyphosate within 2–3 years after the commercialization of GR crops, as evidenced by declining weed control.

By the dawn of the 21st century, glyphosate had become a staple in most corn and soybean production systems and was used widely throughout North America (Fig. 1). The continuous use of glyphosate exerted intense selection pressure on weed communities to adapt to glyphosate. Due to the randomized nature of the treatments within the individual trials in this study, it is unlikely that glyphosate was applied to the exact same area within a field each year resulting in less selection pressure on the weed population compared to what would be observed in a production field subjected to repeated glyphosate applications. As such, this research likely underestimates the consequences of repeated use of the same management strategy. The actual magnitude of weed adaptation is likely higher in production fields. Nonetheless, this research provides the most robust analysis to date on weed adaptation to glyphosate.

Changes in variability over time

Initially, variability of control with POST glyphosate used alone was low but rapidly increased over time for most locations (Fig. 3). When averaged across locations, the CV for the seven evaluated species increased with time, ranging from an increase of 8.4 to 34.1% per decade (Table 3). Except for *A. theophrasti* and *C. album*, weed species showed increased CV over time at most locations. Both *A. theophrasti* and *C. album* had decreased CV at several locations. Further exploration into the causes of this observed variability is necessary to differentiate the drivers of variability.

As hypothesized, the use of a labeled PRE herbicide prior to a POST glyphosate application resulted in significantly less variable control over time compared to glyphosate applied POST alone.

The CVs for *A. theophrasti*, *A. tuberculatus*, *A. artemisiifolia*, and *C. album* remained constant over time when a PRE application was included in the treatment (Table 3). The CVs for *A. palmeri*, *A. trifida*, and *E. canadensis* increased across locations by 1.3–3.9 per decade; however, when compared to the CVs of POST glyphosate alone on these species, the increase was negligible. Rather than exclusively seeking the next silver bullet product to solve weed control issues, these results illustrate the need for additional weed management strategies in order to protect the efficacy of current herbicides and provide higher and more consistent weed control.

Modeling the changes in CVs using historical data provided an accurate means of tracking variability in glyphosate efficacy over time. Most previous research either did not report changes in weed control variability or insufficient data were available to accurately model changes in variability. The observations in this study are the means of 3–4 replications; as such, this study underestimates the temporal changes in variability. However, this study provides the most comprehensive analysis to date of the variability of weed control with glyphosate by quantifying the variability over a time frame that encompasses the initial adoption of GR crops to the present day, as farmers now encounter more weeds poorly controlled by glyphosate.

Conclusions

Research tracking changes in weed management efficacy over time are rare. Nonetheless, mapping such trends, especially to widely adopted herbicide tactics, is essential to building more resilient weed management systems in the future. By analyzing herbicide efficacy data from thousands of trials conducted over a broad spatiotemporal domain, this study shows that the adaptation of seven key weed species to a single, widely adopted management practice in North America was rapid. Results from this study showed that when used alone, glyphosate efficacy decreased and became more variable. Even for a tactic that was initially highly effective, the pace of weed adaptation should not be underestimated. Fortunately, even a simple approach to diversifying a weed management system, such as including a labeled PRE herbicide prior to glyphosate application, slows weed adaptation. While this manuscript focuses on herbicide diversification, additional biological, cultural, and mechanical tactics should be incorporated into weed management programs in order to further improve weed control and slow weed adaptation to POST herbicides. Through evolved resistance and/or life-history traits, the short-lived success of glyphosate for weed control in North American corn and soybean production systems is a testament to the importance of incorporating diversity into current and future weed management systems.

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Supplementary Material

Supplementary material is available at *PNAS Nexus* online.

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Author Contributions

C. Landau, and A. Hager and M.M. Williams designed the experimental approach and wrote the manuscript. WIth the exception of C. Landau and M.M. Williams, all authors performed the experiments. C. Landau analyzed and interpreted the data. All authors were involved with project planning and discussion.

Data Availability

Some data used in the analysis are protected under nondisclosure agreements. Truncated data can be obtained from the corresponding author at christopher.landau@usda.gov upon reasonable request.

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