Weed Science

www.cambridge.org/wsc

Glyphosate sensitivity of selected weed species commonly found in maize fields

María-Concepción Escorial[1], María-Cristina Chueca[2], Andrés Pérez-Fernández[3] and Iñigo Loureiro[1]

[1]Researcher, Department of Plant Protection, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain; [2]Senior Researcher, Department of Plant Protection, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain and [3]Contracted Researcher, Department of Plant Protection, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain

Research Article

Cite this article: Escorial M-C, Chueca M-C, Pérez-Fernández A, Loureiro I (2019) Glyphosate sensitivity of selected weed species commonly found in maize fields. Weed Sci. **67**: 633–641. doi: 10.1017/wsc.2019.54

Received: 21 May 2019
Revised: 30 July 2019
Accepted: 20 September 2019

Associate Editor:
Te-Ming Paul Tseng, Mississippi State University

Keywords:
Dicotyledonous weeds; dose–response assays; monocotyledonous weeds; sensitivity index; *Zea mays*

Author for correspondence:
Iñigo Loureiro, Department of Plant Protection, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Ctra. de La Coruña, km 7,5, 28040 Madrid, Spain.
Email: loureiro@inia.es

Abstract

Glyphosate resistance has evolved worldwide. Glyphosate is also the most used herbicide in Spain, and current changes in herbicide usage patterns can increase the risk of glyphosate resistance development. The objective of this study was to assess the glyphosate sensitivity of different selected weed species important in Spanish maize (*Zea mays* L.) fields. To this end, dose–response experiments were conducted under controlled conditions in a growth chamber to examine variation in glyphosate sensitivity among populations of five grass weed species and eight broadleaf weed species that are commonly found in the maize fields in Castilla y León, the biggest maize-growing region in Spain. The glyphosate doses that caused growth reduction by 50% (GR_{50}) were calculated for each weed population. No populations were resistant to glyphosate. In addition, baseline values of glyphosate sensitivity were determined for each weed species. The GR_{50} baseline values ranged from 10.25 to 53.23 g ai ha^{-1} for the dicotyledonous weed species and from 16.05 to 66.34 g ai ha^{-1} for the monocotyledonous weed species. The ratio between the GR_{50} values of the least and most sensitive populations was used to determine the SI_{50} (sensitivity index at 50% growth reduction) for each weed species. The SI_{50} values showed a 1.4- to 3.3-fold difference in sensitivity for dicotyledonous weed species and 1.4- to 2.4-fold difference for monocotyledonous weed species. The sensitivity index was also calculated as the ratio between the GR_{50} values of the least sensitive population and the baseline GR_{50} value estimated for a range of susceptible populations (SI_{50}b). SI_{50}b values showed a 1.2- to 1.6-fold difference in sensitivity for dicotyledonous weed species and 1.1- to 1.2-fold difference for monocotyledonous weed species. The sensitivity data generated in this study provide a reference for determining time-dependent changes in glyphosate sensitivity in the commonly found weeds in the maize fields of Castilla y Léon.

Introduction

The presence of weeds in maize (*Zea mays* L.) fields is a major concern for maize growers because their presence diminishes yield and their removal is time-consuming and requires considerable resources. Specifically, the estimated loss in maize production due to weeds is 32%, and this loss is greater than that caused by pests (18%) and pathogens (15%) (Oerke and Dehne 2004). Weeds can also harbor crop pests and diseases that need to be controlled, and consequently increase production costs. Moreover, the presence of weeds makes harvesting more difficult and devalues the crop by reducing its quality. In Spain, maize was cultivated in 2017 in Mediterranean semiarid conditions under flood or sprinkler irrigation on about 330,000 ha, most of which are located in Castilla y León (26%), Aragón (25%), Extremadura (14%), and Cataluña (11%). The annual maize production in Spain is about 4×10^9 kg with an average yield of 10,000 kg ha^{-1} (MAPA 2018). Chemical control is the most widely used method for controlling weeds in maize production. In Europe, herbicides are used to control weeds in greater than 90% of maize cultivation areas (Meissle et al. 2010). In general, the currently used herbicides are highly effective, very reliable, and provide broad-spectrum control of weeds without damaging the crop.

Nevertheless, resistance to commonly used herbicides is an emerging problem. Currently, there are fields in the maize cropping areas in Spain in which weed populations of the dicotyledonous species of *Amaranthus*, *Chenopodium*, and *Solanum* genera present problems with control when conventionally used photosystem II–inhibiting herbicides such as terbuthylazine are used. On the other hand, the monocotyledonous weed species of *Echinochloa*, *Sorghum*, *Setaria*, and *Digitaria* genera are becoming resistant to acetolactate synthase (ALS) inhibitors (CPRH 2018). Resistance to ALS- and acetyl CoA carboxylase–inhibiting herbicides, which are widely used for controlling weeds in other annual crops, is also increasing, as evidenced by the resistance of blackgrass (*Alopecurus myosuroides* Huds.) and Italian ryegrass [*Lolium perenne* L.

ssp. *multiflorum* (Lam.) Husnot.] in the United Kingdom (Hicks et al. 2018; Hull et al. 2014) and ripgut brome (*Bromus diandrus* Roth) (Escorial et al. 2011) or rigid ryegrass (*Lolium rigidum* Gaudin) in Spain (Loureiro et al. 2017). Accordingly, farmers are now compelled to use other strategies for weed control before sowing the crop, such as false seedbeds and delayed sowing to promote the early emergence of weed (van der Weide and Bleeker 1998), commonly followed by glyphosate application. However, modeling studies to compare the rates of evolution of glyphosate resistance under crop rotation and annual use of glyphosate pre-sowing have identified increased glyphosate use on stale seedbeds, often in systems with reduced or no-tillage, as a major driver for evolution of glyphosate resistance in Australian populations of *L. rigidum* (Neve et al. 2003).

Of all the herbicides, glyphosate is the most widely used globally, because it has high efficacy against a broad spectrum of weeds (Duke and Powles 2008). Glyphosate use enables the application of new crop production systems, such as conservation agriculture and no-till practices, and new weed management approaches that rely on the cultivation of glyphosate-resistant (GR) crops. However, the cultivation of GR crops increases glyphosate use, which can result in less use of other herbicides, an increased number of weed species that cannot be controlled by glyphosate, weed shifts, and weed resistance to glyphosate (Bonny 2016; García-Ruiz et al. 2018; Johnson et al. 2009). Although glyphosate is viewed as a low-risk herbicide with regard to the evolution of resistance, the emergence of glyphosate-resistant weed populations, especially in monocultures with limited rotation or minimal tillage, could threaten the utility of both glyphosate and GR crops. The results of several surveys among American scientists and farmers revealed that 80% of respondents attributed shifts in the weed species to the use of GR crops (Culpepper 2006; Gibson et al. 2006; Johnson and Gibson 2006). It has also been reported that the extensive and continuous use of glyphosate can promote glyphosate resistance in weeds (Heap and Duke 2017). More recently, Heap (2019) reported that 43 different weed species had developed resistance to glyphosate, although the use of a GR crop did not always account for this development. The infestation of cultivated crops with glyphosate-resistant *Amaranthus* species, especially Palmer amaranth (*Amaranthus palmeri* S. Watson), has become one of the biggest weed problems in U.S. agriculture (WSSA 2016).

The reduced herbicide rates to control weeds have been applied in more than 50% of the areas cultivated in maize in the Netherlands, and more than 80% of the maize cultivation areas in Denmark, Germany, and France (Meissle et al. 2010). However, the use of low herbicide doses can result in the rapid evolution of herbicide resistance because of the development of non–target site resistance (Manalil et al. 2011; Norsworthy et al. 2012). Neve and Powles (2005) claimed that low application rates of a herbicide could accelerate the evolution of herbicide resistance in a weed population with broad genetic diversity, such as *L. rigidum* in Australia. Collavo and Sattin (2014) also reported the development of glyphosate resistance in *Lolium* spp. due to the continuous low-dose application of glyphosate to cereals in Italy, which could be attributed to both target-site and non–target site mechanisms.

Determining the sensitivity of target pests to an active substance is advantageous, because it gives baseline information about the level of resistance to a particular plant protection product in a pest population (EPPO 2015). Sensitivity data also enable comparisons to be made between the same and different populations at various times to detect any sensitivity shifts and resistance development (Moss 2001). These data are especially important for detecting non–target site resistance when less sensitive weed populations may be selected and resistance slowly evolves in each subsequent generation (Gressel 2011). Differential sensitivity to glyphosate has been identified in several dicotyledonous weed species, such as common lambsquarters (*Chenopodium album* L.) (Westhoven et al. 2008), *Amaranthus* spp. (Norsworthy et al. 2008; Patzoldt et al. 2002; Smith and Hallett 2006; Volenberg et al. 2007), *Erigeron* spp. (González-Torralva et al. 2010), and kochia [*Bassia scoparia* (L.) A. J. Scott] (Waite et al. 2013). Differential glyphosate sensitivity has also been identified in monocotyledonous weed species, such as quackgrass [*Elymus repens* (L.) Gould] (Espeby et al. 2014), *A. myosuroides* (Davies and Neve 2017), *L. rigidum*, and *B. diandrus* (Barroso et al. 2010).

Against this background, we undertook an investigation whose aims were (1) to assess the response to glyphosate of the most commonly found weeds in the maize fields of Castilla y León and (2) to generate sensitivity data to establish the basis for monitoring the response to glyphosate across a range of weed populations before this herbicide is used extensively in the maize fields of this region.

Materials and Methods

Plant Material

The investigation comprised glyphosate dose–response assays that used seeds from 85 different populations of eight dicotyledonous or broadleaf weed species and five monocotyledonous or grass weed species. The eight dicotyledonous weed species were velvetleaf (*Abutilon theophrasti* Medik.), five populations; redroot pigweed (*Amaranthus retroflexus* L.), nine populations; *C. album*, 10 populations; jimsonweed (*Datura stramonium* L.), eight populations; common purslane (*Portulaca oleracea* L.), 10 populations; black nightshade (*Solanum nigrum* L.), five populations; two species of *Xanthium*, namely spiny cocklebur (*Xanthium spinosum* L.), four populations, and common cocklebur (*Xanthium strumarium* L.), six populations. The five monocotyledonous weed species were large crabgrass [*Digitaria sanguinalis* (L.) Scop.], 10 populations; barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], five populations; and three *Setaria* spp., namely adherent bristlegrass [*Setaria adhaerens* (Forssk.) Chiov.], five populations, bristly foxtail [*Setaria verticillata* (L.) P. Beauv.], three populations, and green foxtail [*Setaria viridis* (L.) P. Beauv.], five populations.

Seed Sampling

Seeds were collected in field surveys conducted randomly during 2013 and 2014 in maize fields of provinces with the largest areas of maize cultivation in Castilla y León, namely León (64,547 ha), Zamora (18,507 ha), Salamanca (18,230 ha), and Valladolid (9,082 ha) (Figure 1). Sampling was done by extensive driving through the region and stopping at 10-km intervals to sample the nearest maize area. The sample sites were georeferenced using a global positioning system. A total of 59 field sites were visited. At each site, mature seeds from 25 to 50 plants that were randomly selected from different patches in the maize field were collected for each weed species. The seed from each weed species in the same maize field was bulked to form a population. Each seed sample contained at least 10,000 mature seeds. The seeds from each weed sample were placed in paper bags, dried at room temperature in the laboratory, manually cleaned and threshed, and stored at room temperature until use. One hundred and seventy-six seed samples from 13 weed species were collected. Only 87 weed samples with

https://doi.org/10.1017/wsc.2019.54 Published online by Cambridge University Press

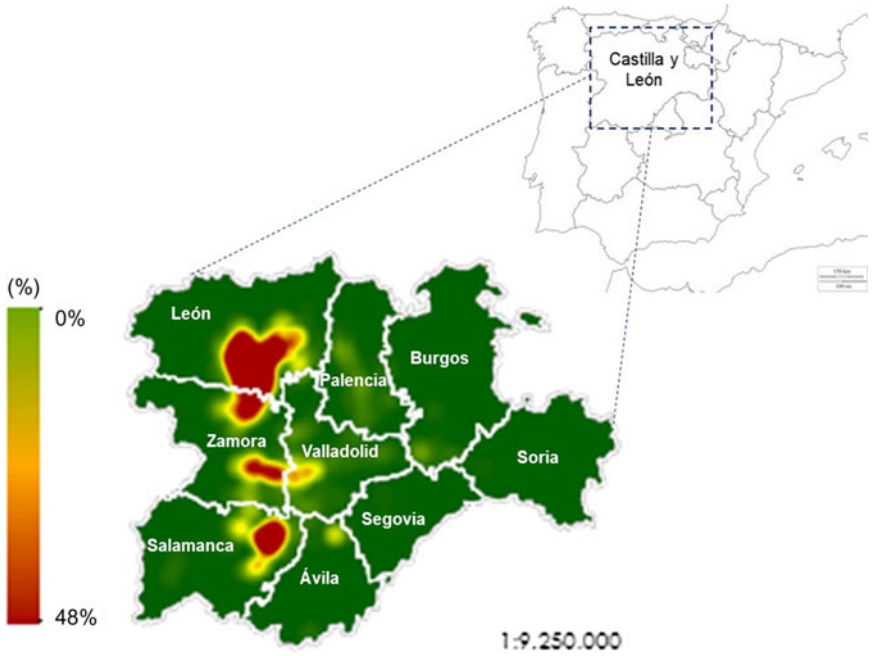

Figure 1. Percentage of the maize cropping area in 2016 in Castilla León, Spain. Data were obtained from the Agricultural Statistics, Studies and Planning Service of the Department of Agriculture and Livestock of the Regional Government of Castilla y Léon, 2016.

good germination (> 70%) with a minimum of five and a maximum of 10 populations per species were used in the study. We are unable to guarantee that the sampled weed populations were not previously exposed to glyphosate, because this herbicide is one of the most widely used herbicides in Spain. However, we assumed that the sampled populations were not exposed to glyphosate, because it is not commonly used in conventional maize farming.

Glyphosate Dose–response Assays

The glyphosate dose–response assays were conducted in a growth chamber under a 16-h photoperiod and 300 µE m^{-2} s^{-1} photosynthetically active radiation and 8 h of darkness at 30 ± 2 C/16 ± 1 C (day/night).

The seeds from each population were first pre-germinated in trays, and the germinated seedlings were then transplanted at an early seedling stage to 200-ml plastic pots filled with a 75% soil: mulch:sand (1:1:1) and 25% vermiculite mixture, at a rate of 3 uniform seedlings per pot. For *Xanthium* species, the seeds were sown directly in the plastic pots at a rate of 1 plant per pot. When the plantlets of the monocotyledonous weeds were at the 2- to 3-leaf stage (BBCH 12-13) or the plantlets of the dicotyledonous weeds were at the 2- to 4-leaf stage (BBCH 12-14), glyphosate (Roundup®, 360 g ai L^{-1}, Monsanto Agricultura, Madrid, España) was applied at doses of 0, 16.8, 33.6, 67.5, 135, 270, and 1,080 g ai ha^{-1}. Three replicates of five pots were made for each population and dose, and each dose–response assay was repeated twice. The glyphosate treatments were applied using an automatic sprayer (Devries Manufacturing, Hollandale, MN, USA) equipped with a TeeJet® 8002-E flat-fan nozzle (TeeJet Technologies, Orléans, France) that was calibrated to spray 175 L ha^{-1} at 130 kPa.

Once treated, the plants were returned to the growth chamber and watered as required throughout the experiment. At 15 d after treatment (DAT) for all weed species, the aboveground plant parts were first cut down and weighed, and then dried in an oven at 80 C for 48 h, and weighed again. For the development of herbicide dose–response curves, doses should cover the whole range of plant responses, from almost no apparent effects to complete kill of the plants, so the aboveground fresh weight for the *Xanthium* spp. was measured at 21 DAT to ensure that the full effects of the herbicide were visible.

Data Analysis

For determining the dose–response curve of each weed species' population for the different glyphosate doses, the dry-weight parameter was first transformed to a percentage of the untreated control and a log-logistic model (Seefeldt et al. 1995) was then fit to estimate I_{50} values (the effective dose for 50% growth reduction = GR_{50}) according to Equation 1.

$$y = C + \left[(D - C)/\left(1 + (x/I_{50})^{b}\right)\right] \quad (1)$$

where C is the lower limit and corresponds to the mean response at highest glyphosate dose, D is the upper limit and corresponds to the response of the control, and b is the slope of the curve around the GR_{50}. These parameters were estimated by curve fitting with an iterative adjustment approach using the Table Curve® 2D program v. 5.01 (Systat Software, San José, CA, USA). A dose–response curve for each weed species was then generated using the data from all populations, and the mean GR_{50} value (baseline) for each weed species was estimated. The 95% confidence intervals (CI95%) for GR_{50} were calculated. The ratio between the GR_{50} values of the least and most sensitive populations was used to determine the SI_{50} (sensitivity index at 50% efficacy) for each weed species.

The percentage values of dry weight after herbicide treatment were arcsine square-root transformed before a two-way ANOVA using the general linear model procedure. The population effect was considered as the random effect, and glyphosate dose was considered to be the fixed effect. When the *F*-test was significant

https://doi.org/10.1017/wsc.2019.54 Published online by Cambridge University Press

Table 1. GR_{50} values for each population of the selected dicotyledonous weed species.

Dicotyledonous weed species populations sensitivity to glyphosate							
		Glyphosate (g ai ha^{-1})				Glyphosate (g ai ha^{-1})	
Species	Population[a]	GR_{50}	SE	Species	Population[a]	GR_{50}	SE
ABUTH	20	40	5.4	SOLNI	10	33	3.7
	48	49	17.4		33	18	0.1
	56	50	4.2		68	17	0.6
	75	83	15.2		94	21	0.3
	92	49	10.4		96	23	0.2
AMARE	46	13	0.3	CHEAL	30	39	2.6
	57	19	0.9		65	32	1.9
	67	17	0.1		81	35	0.9
	82	18	0.6		86	27	1.5
	87	12	0.8		91	43	3.1
	127	21	0.2		123	38	1.8
	133	19	0.2		129	41	0.6
	149	10	0.3		144	44	0.6
	154	13	0.2		148	42	1.3
					155	34	1.0
POROL	35	24	0.5	DATST	34	10	0.7
	98	19	0.4		44	10	0.6
	121	21	0.4		70	10	0.4
	152	21	0.9		89	11	0.6
	172	32	2.2		137	10	1.3
	177	27	0.3		147	14	0.8
	187	28	0.7		167	12	1.5
	188	33	2.4		204	4	0.2
	202	29	0.3				
	208	32	0.2				
XANST	22	23	1.9	XANSP	49	48	11.7
	31	25	1.7		54	42	4.2
	36	22	5.3		83	38	0.7
	53	27	0.7		90	36	1.2
	61	20	0.9				
	76	21	0.7				

[a]The identification numbers of populations correspond with their numbers in the collection of the Weed Control Group of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). The GR_{50} values are displayed as mean ± standard error (SE) and were estimated by the log-logistic equation used to calculate the glyphosate dose that caused a 50% growth reduction in the bioassays conducted under controlled growth chamber conditions.

at $P = 0.05$, the mean dry weight of the populations and glyphosate doses were compared using the Newman-Keuls test. All statistical analyses were done using computerized statistical software (Statgraphics Centurion XVI.II, StatPoint, Herndon, VA, USA).

Results and Discussion

Establishing the baseline sensitivity of a weed population to herbicides is critical for monitoring the development of herbicide resistance and managing this resistance in weed populations (Moss 2001; Paterson et al. 2002; Ulber et al. 2013). Sensitivity data for a particular herbicide may be considered as a baseline when they are obtained from a weed population that has not been previously exposed to that herbicide or to herbicides with the same mode of action (EPPO 2015). Although GR crops have not been authorized for cultivation in the European Union, glyphosate is the most commonly used herbicide (Benbrook 2016), and also in Spain (MAPA 2013). The use of glyphosate is mainly associated with reduced-tillage or no-till farming systems (Wiese et al. 2018).

The weed species we selected for our surveys are among the most prevalent weed species in maize-growing regions in Spain (San Martín et al. 2015) and elsewhere in Europe (Dewar 2009; Jensen et al. 2011). Analyses of glyphosate dose–response curves were performed separately for each weed population of the different species of weeds, and the GR_{50} values were determined. The GR_{50} values were used as a measure of sensitivity. The applied doses were appropriate for describing the dose–response curves for all weed species. Table 1 displays the GR_{50} values for each population of the dicotyledonous weed species, and Table 2 displays the GR_{50} values for each population of the monocotyledonous weed species. Our results reveal that all populations of the weed species selected to determine glyphosate sensitivity are susceptible to this herbicide. The GR_{50} values ranged from 4 g ai ha^{-1} (one population of *D. stramonium*) to 83 g ai ha^{-1} (one population of *A. theophrasti*), and both these values are much lower than the recommended glyphosate dose of 540 g ai ha^{-1}. The GR_{50} values for the different populations of the same species were relatively similar. Table 3 displays the SI_{50}, which is a measure of the variability of the response among the weed populations. For dicotyledonous weed species, the SI_{50} values showed a 1.4- to 3.3-fold difference in sensitivity, and there was a 1.4- to 2.4-fold difference in sensitivity for monocotyledonous weed species (Table 3).

For monocotyledonous weed species, the ANOVA results revealed no significant differences in glyphosate response among populations ($P > 0.05$). For the dicotyledonous weed species *A. theophrasti*, *D. stramonium*, and *S. nigrum* and the two *Xanthium* species, the ANOVA did not show any significant differences ($P > 0.05$) in the glyphosate response among populations. We found significant differences in sensitivity among the studied populations of *A. retroflexus* ($F_{(9,124)} = 3.69$, $P = 0.0004$)

https://doi.org/10.1017/wsc.2019.54 Published online by Cambridge University Press

Table 2. GR_{50} values for each population of the selected monocotyledonous weed species.

Monocotyledonous weed species populations sensitivity to glyphosate							
		Glyphosate (g ai ha^{-1})				Glyphosate (g ai ha^{-1})	
Species	Population[a]	GR_{50}	SE	Species	Population[a]	GR_{50}	SE
ECHCG	41	71	6.4	SETAD	110	28	0.9
	75	75	3.6		120	26	1.2
	109	63	1.5		175	32	6.2
	151	76	3.2		195	32	3.1
	153	49	3.3		205	31	2.7
DIGSA	101	33	1.6	SETVI	108	34	1.8
	112	33	0.5		182	27	0.4
	136	39	0.3		186	35	2.6
	150	36	0.4		198	25	0.4
	159	36	1.2		200	40	0.9
	179	44	1.0	SETVE	40	20	0.9
	184	40	1.7		100	9	0.6
	199	42	0.8		102	16	0.7
	203	41	0.9				
	212	38	0.5				

[a]The identification number of populations corresponds with their numbers in the collection of the Weed Control Group of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). The GR_{50} values are displayed as mean ± standard error (SE) and were estimated by the log-logistic equation used to calculate the glyphosate dose that caused a 50% growth reduction in the bioassays conducted under controlled growth chamber conditions.

Table 3. Indices of glyphosate sensitivity of selected weed species.[a]

Dicotyledonous weed species	SI_{50}	$SI_{50}b$	Monocotyledonous weed species	SI_{50}	$SI_{50}b$
Abutilon theophrasti	2.1	1.6	*Digitaria sanguinalis*	1.4	1.2
Amaranthus retroflexus	2.2	1.3	*Echinochloa crus-galli*	1.6	1.1
Chenopodium album	1.6	1.2	*Setaria adhaerens*	1.2	1.1
Datura stramonium	3.3	1.4	*Setaria verticillata*	2.4	1.2
Portulaca oleracea	1.6	1.2	*Setaria viridis*	1.6	1.2
Solanum nigrum	2.0	1.5			
Xanthium strumarium	1.4	1.2			
Xanthium spinosum	1.4	1.2			

[a]The SI_{50} (sensitivity index at 50% growth reduction) values for each weed species are displayed as the ratio between the GR_{50} values of the least and most sensitive populations. The $SI_{50}b$ values for each weed species were calculated as the ratio between the GR_{50} value of the least sensitive population and the baseline GR_{50} value estimated for a range of susceptible populations of the same species.

and *C. album* ($F_{(9,124)} = 3.33$, $P = 0.001$). However, the SI_{50} values for these species were 2.1 and 1.6 for *A. retroflexus* and *C. album*, respectively (Table 3). These SI_{50} values are consistent for susceptible weed populations, as small resistance indices (< 3) can occur among susceptible populations due to natural variation in intrapopulation sensitivity to herbicides (EHRAC 2017; Espeby et al. 2011; Patzoldt et al. 2002; Schulz et al. 2014). However, this variation can also be due to differences in the selection pressure exerted through the recurrent use of the same herbicide or mode of action (Claerhout et al. 2015; Kniss et al. 2007). It could be possible that those weed populations with the highest GR_{50} values may be in a more advanced stage of selection for glyphosate resistance. It has been reported that the rate at which herbicide resistance evolves in weed populations is influenced by biological and genetic factors, which are inherent to each weed species, and by selection pressure, which can be manipulated by the cropping system and the weed management strategy (Harker 2013).

The baseline GR_{50} values ($GR_{50}b$) of glyphosate sensitivity for the main dicotyledonous and monocotyledonous weed species are displayed in Figures 2 and 3, respectively. The GR_{50b} values for the dicotyledonous weed species ranged from 10.25 g ai ha^{-1} (CI 95% = 9.08 to 11.42) for *D. stramonium*, the most glyphosate-sensitive species, to 53.23 g ai ha^{-1} (CI 95% = 41.96 to 64.51) for *A. theophrasti*, the least sensitive species (Figure 2). We must consider that the evaluation of the herbicide treatment for the *Xanthium* spp. was extended by 1 wk, so despite being a similar response, it might not be comparable with responses of the rest of the weed species. For monocotyledonous weed species, the $GR_{50}b$ values ranged from 16.05 g ai ha^{-1} (CI 95% = 13.22 to 18.88) for *S. verticillata*, the most sensitive species, to 66.34 g ai ha^{-1} (CI 95% = 59.86 to 72.83) for *E. crus-galli*, the least sensitive species (Figure 3).

When the baseline $GR_{50}b$ values for these species (instead of the GR_{50} value of the most sensitive population) were used to calculate the sensitivity index among populations, the differences in glyphosate sensitivity diminished to a maximum of 1.6-fold instead of 3.3-fold for dicotyledonous weed species (Table 3). When this calculation was done for monocotyledonous weed species, the differences in glyphosate sensitivity diminished to a maximum of 1.2-fold, instead of 2.4-fold (Table 3). Therefore, baseline data should take into account the natural variation of the sensitivity of weed populations and may be a more useful parameter than the value of the most sensitive population for establishing sensitivity indices.

The GR_{50} values for the response to glyphosate estimated in this study are consistent with those reported in other studies. Tharp et al. (1999) studied the response of several annual weed species to glyphosate and found GR_{50} values of 96 g ai ha^{-1} for giant foxtail (*Setaria faberi* Herrm.), 120 g ai ha^{-1} for *C. album* and *D. sanguinalis*, and 160 g ai ha^{-1} for *E. crus-galli*. Boutin et al. (2004) reported GR_{50} values for the response to glyphosate at 29.2 and 18.5 g ai ha^{-1} for cornflower (*Centaurea cyanus* L.) and corn poppy (*Papaver rhoeas* L.), respectively, while White and Boutin (2007) reported a GR_{50} of 77 g ai ha^{-1} for *S. nigrum*. Our GR_{50} values also agree with those estimated in a Danish study on the sensitivity to glyphosate of six non-target plant species and 10 crop species (Strandberg et al. 2012). Those authors reported GR_{50} values that ranged from 29 to 130.9 g ai ha^{-1} for the non-target weeds common yarrow (*Achillea millefolium* L.) and herb-robert

https://doi.org/10.1017/wsc.2019.54 Published online by Cambridge University Press

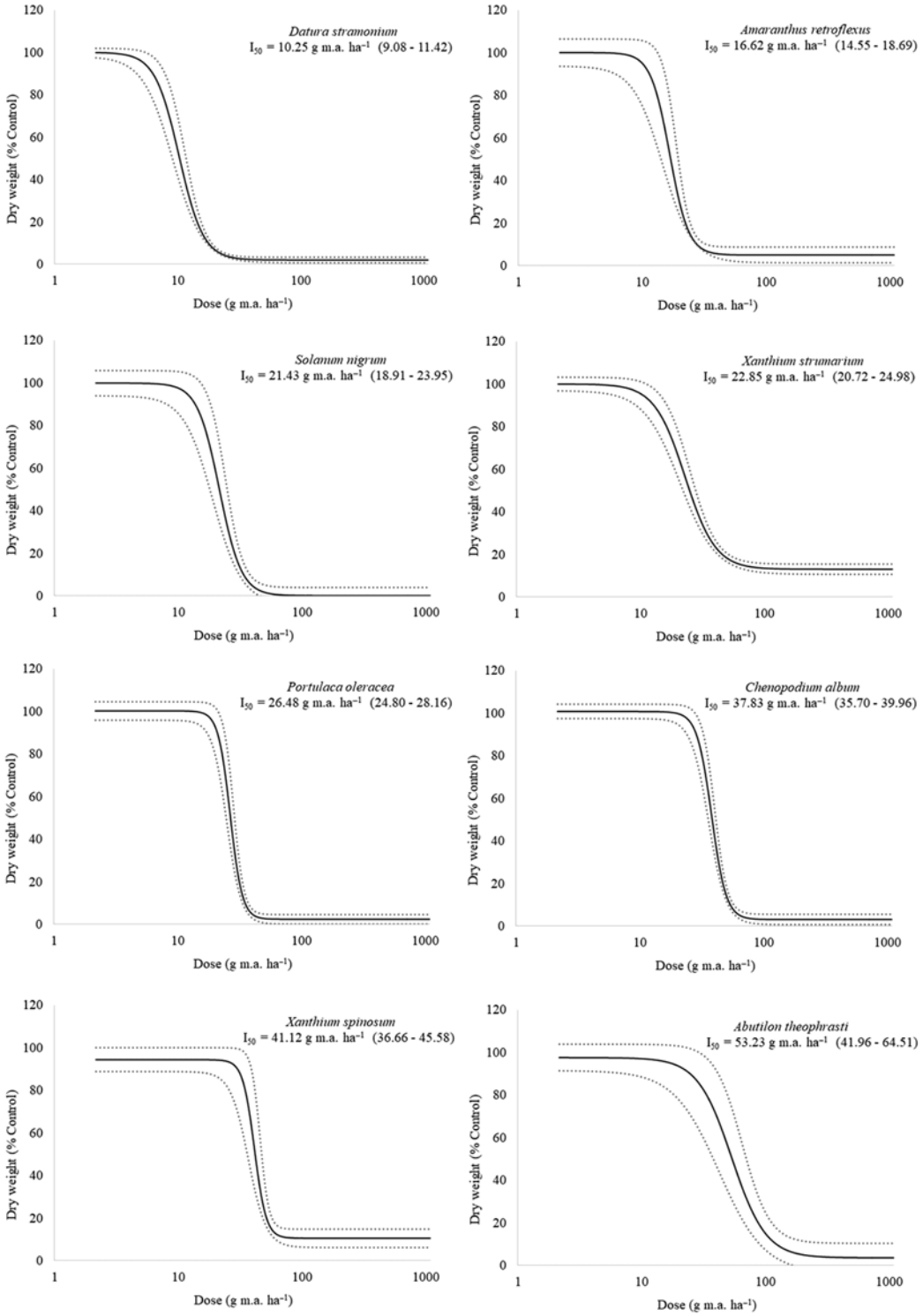

Figure 2. Effect of glyphosate on the growth of eight dicotyledonous weed species. The black line represents the dose–response curve fit to the mean aboveground dry biomass values in response to increasing glyphosate doses from all the populations assessed in each species. Dotted gray lines represent the 95% confidence interval (CI) for the dose.

(*Geranium robertianum* L.), respectively. For crop species, they reported GR_{50} values that ranged from 1.6 g ai ha^{-1} for common sunflower (*Helianthus annuus* L.) to 84.6 g ai ha^{-1} for onion (*Allium cepa* L.). However, it should be noted that GR_{50} values depend to a large extent on the experimental conditions. Tharp et al. (1999) showed that the GR_{50} values for the response to glyphosate of *A. theophrasti* varied from 28 to 120 g ai ha^{-1} depending on the growth stage of the plants. Ou et al. (2018) reported GR_{50} values for the response to glyphosate of two *B. scoparia* populations which varied from 42 to 67 g ha^{-1} to 171 to 187 g ha^{-1} when the assays were conducted at 25/15 C (day/night temperature) and 32.5/22.5 C, respectively. The GR_{50}

https://doi.org/10.1017/wsc.2019.54 Published online by Cambridge University Press

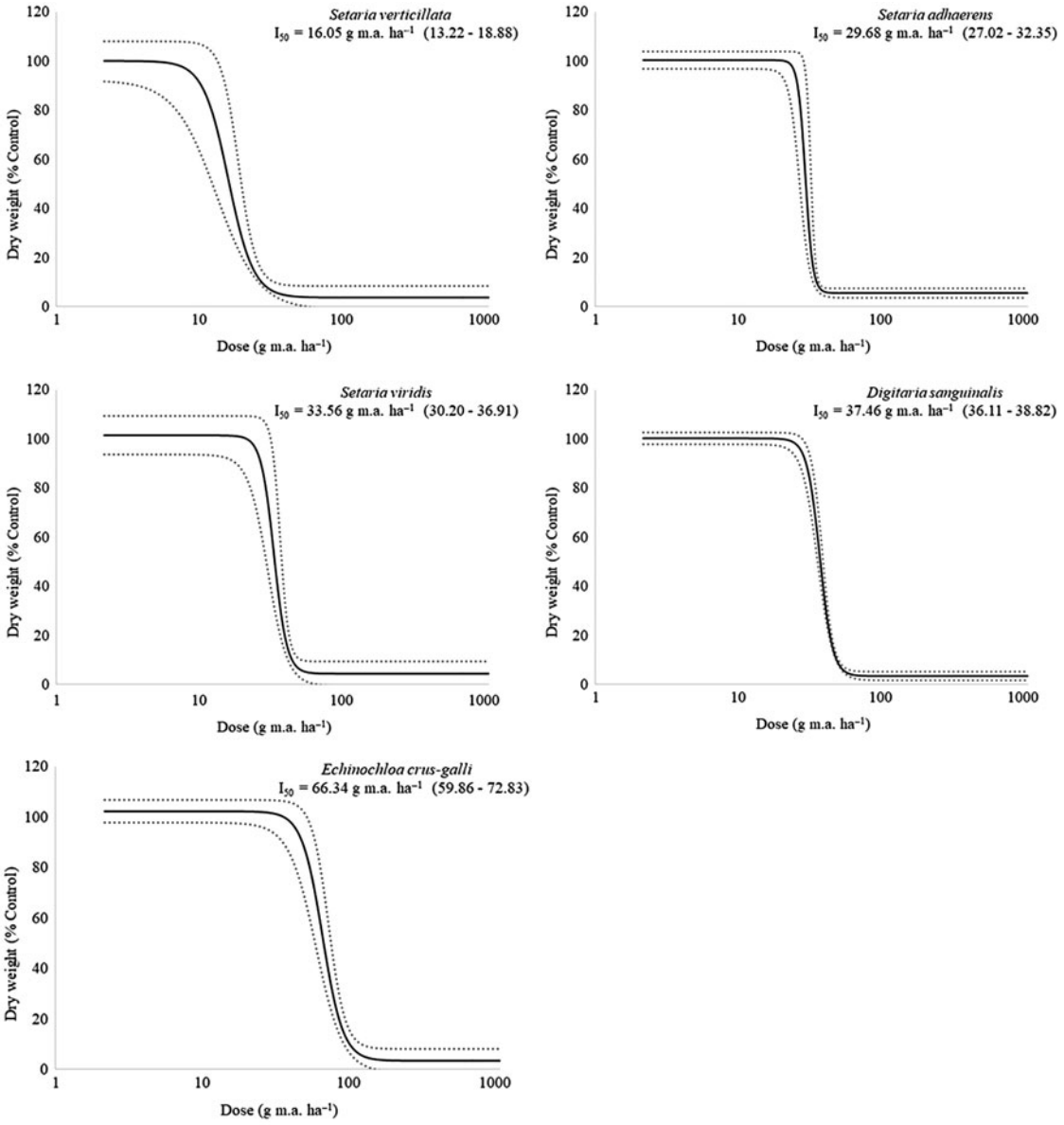

Figure 3. Effect of glyphosate on the growth of five monocotyledonous weed species. The black line represents the dose–response curve fit to the mean aboveground dry biomass values in response to increasing glyphosate doses from all the populations assessed in each species. Dotted gray lines represent the 95% confidence interval (CI) for the dose.

values should be only compared when the experiments have been carried out under similar conditions of plant growth (temperature, relative humidity, and light intensity), phenological stage of the plants, herbicide products used, or the timing of evaluation of the experiment.

Herbicide resistance is increasing worldwide, with an increasing number of cases of cross- and multiple resistance (Hicks et al. 2018; Loureiro et al. 2017; Peterson et al. 2018; Powles 2014), thereby limiting the number of available herbicide options for weed control for farmers. Glyphosate resistance is not an exception. Although surveying and characterizing herbicide sensitivity variation across a large number of populations with dose–response experiments can be expensive and time-consuming, such proactive monitoring studies are essential for identifying shifts in herbicide sensitivity and response before widespread development of herbicide resistance. The sensitivity data generated in this study provide an important reference for determining any time-dependent changes in glyphosate sensitivity of the commonly found weed species in the maize fields. Therefore, subsequent monitoring of the glyphosate sensitivity of these weeds will be needed to ensure the continued use of glyphosate and to minimize and delay the development of resistance.

Acknowledgments. This work was supported by the Spanish Ministry of Agriculture, Food and Environment (MAGRAMA), under grant EG13-75. No conflicts of interest have been declared.

References

Barroso J, Loureiro I, Escorial MC, Chueca MC (2010) The response of *Bromus diandrus* and *Lolium rigidum* to dalapon and glyphosate I: baseline sensitivity. Weed Res 50:312–319

https://doi.org/10.1017/wsc.2019.54 Published online by Cambridge University Press

Benbrook CM (2016) Trends in glyphosate herbicide use in the United States and globally. Environ Sci Eur 28:1–15
Bonny S (2016) Genetically modified herbicide-tolerant crops, weeds, and herbicides: overview and impact. Environ Manage 57:31–48
Boutin C, Elmegaard N, Kjær C (2004) Toxicity testing of fifteen non-crop plant species with six herbicides in a greenhouse experiment: implications for risk assessment. Ecotoxicology 23:349–369
Claerhout S, Reheul D, De Cauwer B (2015) Sensitivity of *Echinochloa crus-galli* populations to maize herbicides: a comparison between cropping systems. Weed Sci 55:470–481
Collavo A, Sattin M (2014) First glyphosate-resistant *Lolium* spp. biotypes found in a European annual arable cropping system also affected by ACCase and ALS resistance. Weed Res 54:325–334
[CPRH] Comité Prevención Resistencia Herbicidas (2018) Home page. http://semh.net/grupos-de-trabajo/cprh. Accessed: March 6, 2019
Culpepper AS (2006) Glyphosate-induced weed shifts. Weed Technol 20: 277–281
Davies LR, Neve, P (2017) Inter-population variability and adaptive potential for reduced glyphosate sensitivity in *Alopecurus myosuroides*. Weed Res 57:323–332
Dewar AM (2009) Weed control in glyphosate-tolerant maize in Europe. Pest Manag Sci 65:1047–1058
Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325
Escorial MC, Loureiro I, Rodriguez-Garcia E, Chueca C (2011) Population variability in the response of ripgut brome (*Bromus diandrus*) to sulfosulfuron and glyphosate herbicides. Weed Sci 59:107–112
Espeby LA, Fogelfors H, Milberg P (2011) Susceptibility variation to new and established herbicides: examples of inter-population sensitivity of grass weeds. Crop Prot 30:429–435
Espeby LA, Fogelfors H, Sjödal S, Milberg P (2014) Variation in *Elymus repens* susceptibility to glyphosate. Acta Agric Scand Sect B Soil Plant Sci 64: 211–219
[EHRAC] European Herbicide Resistance Action Committee (2017) European Guidelines to Conduct Herbicide Resistance Tests. http://hracglobal.com/europe/files/docs/Europe_Guidelines_Herbicide_Resistance-tests_13Oct17.pdf. Accessed: April 6, 2019
[EPPO] European and Mediterranean Plant Protection Organization (2015) Efficacy evaluation of plant protection products. Resistance risk analysis. PP1/213 (3). EPPO Bulletin 45:371–387
García-Ruiz E, Loureiro I, Farinós GP, Gómez P, Gutiérrez E, Sánchez FJ, Escorial MC, Ortego F, Chueca MC, Castanera P (2018) Weeds and ground-dwelling predators' response to two different weed management systems in glyphosate-tolerant cotton: a farm-scale study. PLoS ONE 13:e0191408
Gibson KD, Johnson WG, Hillger DE (2006) Farmer perceptions of weed problems in corn and soybean rotation systems. Weed Technol 20: 751–755
González-Torralva F, Cruz-Hipólito H, Bastida F, Muelleder N, Smeda RJ, De Prado R (2010) Differential susceptibility to glyphosate among the *Conyza* weed species in Spain. J Agric Food Chem 58:4361–4366
Gressel J (2011) Low pesticide rates may hasten the evolution of resistance by increasing mutation frequencies. Pest Manag Sci 67:253–257
Harker KN (2013) Slowing weed evolution with integrated weed management. Can J Plant Sci 93:759–764
Heap I (2019) The International Survey of Herbicide Resistant Weeds. www.weedscience.org. Accessed: February 18, 2019
Heap I, Duke SO (2017) Overview of glyphosate-resistant weeds worldwide. Pest Manag Sci 74:1040–1049
Hicks HL, Comont D, Coutts SR, Crook L, Hull R, Norris K, Neve P, Childs DZ, Freckleton RP (2018) The factors driving evolved herbicide resistance at a national scale. Nat Ecol Evol 2:529–536
Hull R, Tatnell LV, Cook SK, Moss SR (2014) Current status of herbicide-resistant weeds in the UK. Asp Appl Biol 127:261–272
Jensen PK, Bibard V, Czembor E, Dumitru S, Foucart G, Froud-Williams RJ, Jensen JE, Saavedra M, Sattin M, Soukup J, Palou AT, Thibord JB, Voegler W, Kudsk P (2011) Survey of Weeds in Maize Crops in Europe. Slagelse, Denmark: Aarhus University. https://www.researchgate.net/publication/232775702_survey_of_weeds_in_maize_cops_in_europe. Accessed: April 4, 2018
Johnson WG, Davis VM, Kruger GR, Weller SC (2009) Influence of glyphosate-resistant cropping systems on weed species shifts and glyphosate-resistant weed populations. Eur J Agron 31:162–172
Johnson WG, Gibson KD (2006) Glyphosate-resistant weeds and resistance management strategies: an Indiana grower perspective. Weed Technol 20:768
Kniss, AR, Miller SD, Westra PH, Wilson RG (2007) Glyphosate susceptibility in common lambsquarters (*Chenopodium album*) is influenced by parental exposure. Weed Sci 55:572–577
Loureiro I, Escorial C, Hernández-Plaza E, González-Andújar JL, Chueca MC (2017) Current status in herbicide resistance in *Lolium rigidum* in winter cereal fields in Spain: evolution of resistance 12 years after. Crop Prot 102:10–18
Manalil SBR, Renton M, Powles SB (2011) Rapid evolution of herbicide resistance by low herbicide dosages. Weed Sci 59:210–217
Meissle M, Mouron P, Musa T, Bigler F, Pons X, Vasileiadis VP, Otto S, Antichi D, Kiss J, Pálinkás Z, Dorner Z, van der Weide R, Groten J, Czembor E, Adamczyk J, *et al.* (2010) Pests, pesticide use and alternative options in European maize production: current status and future prospects. J Appl Entomol 134:357–375
[MAPA] Ministerio de Agricultura Pesca y Alimentación (2013) Encuesta de Utilización de Productos Fitosanitarios. https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/estadisticas-medios-produccion/fitosanitarios.aspx. Accessed: March 6, 2019
[MAPA] Ministerio de Agricultura Pesca y Alimentación (2018) Superficies y producciones anuales de cultivo de acuerdo con el Reglamento (CE) 543/2009. https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura. Accessed: March 6, 2019
Moss SR (2001) Baseline sensitivity to herbicides: a guideline to methodologies. Pages 769–774 *in* Proceedings of British Crop Protection Conference—Weeds. Brighton, UK: British Crop Production Council
Neve P, Diggle AJ, Smith FP, Powles SB (2003) Simulating evolution of glyphosate resistance in *Lolium rigidum* II: past, present and future glyphosate use in Australian cropping. Weed Res 43:418–427
Neve P, Powles SB (2005) Recurrent selection with reduced herbicide rates results in the rapid evolution of herbicide resistance in *Lolium rigidum*. Theor Appl Genet 110:1154–1166
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
Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide-resistance: best management practices and recommendations. Weed Sci 60:31–62
Oerke EC, Dehne HW (2004) Safeguarding production-losses in major crops and the role of crop protection. Crop Prot 23:275–285
Ou J, Stahlman PW, Jugulam M (2018) Reduced absorption of glyphosate and decreased translocation of dicamba contribute to poor control of kochia (*Kochia scoparia*) at high temperature. Pest Manag Sci 74: 1134–1142
Paterson EA, Shenton ZL, Straszewski AE (2002) Establishment of the baseline sensitivity and monitoring response of *Papaver rhoeas* populations to florasulam. Pest Manag Sci 58:964–966
Patzoldt WL, Tranel PJ, Hager AG (2002) Variable herbicide response among Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) populations. Crop Prot 21:707–712
Peterson MA, Collavo A, Ovejero R, Shivraind V, Walsh MJ (2018) The challenge of herbicide resistance around the world: a current summary. Pest Manag Sci 74: 2246–2259
Powles S (2014) Global herbicide resistance challenge. Pest Manag Sci 70: 1305–1305
San Martín C, Andújar D, Fernández-Quintanilla C, Dorado J (2015) Spatial distribution patterns of weed communities in corn fields of central Spain. Weed Sci 63:936–945

https://doi.org/10.1017/wsc.2019.54 Published online by Cambridge University Press

Schulz A, Mathiassen SK, de Mol F (2014) Approaches to early detection of herbicide resistance in *Apera spica-venti* regarding intra- and inter-field situations. J Plant Dis Prot 121:138–148

Seefeldt SS, Jensen SE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationship. Weed Technol 9:218–227

Smith DA, Hallett SG (2006) Variable response to glyphosate in common waterhemp from different parts of the Midwestern USA. Weed Technol 20:18–23

Strandberg B, Mathiassen SK, Bruus M, Kjær C, Damgaard C, Andersen HV, Bossi R, Løfstrøm P, Larsen SE, Bak J, Kudsk P (2012) Effects of Herbicides on Non-target Plants: How Do Effects in Standard Plant Test Relate to Effects in Natural Habitats? Copenhagen: Danish Environmental Protection Agency. Pestic Res no. 137

Tharp BE, Schabenberger O, Kells JJ (1999) Response of annual weed species to glufosinate and glyphosate. Weed Technol 13: 542–547

Ulber L, Nordmeyer H, Zwerger P (2013) Resistance risk assessment within herbicide authorisation—a call for sensitivity data. Pest Manag Sci 69:160–164

Van der Weide RY, Bleeker P (1998) Effects of sowing time, false seedbed and pre-emergence harrowing in silage maize. Page 1 *in* Proceedings of the 3rd European Weed Research Society Workshop on Physical Weed Control. Wye, UK: European Weed Research Society. http://www.ewrs.org/pwc/doc/1998_Wye.pdf. Accessed: June 30, 2019

Volenberg DS, Tranel PJ, Hager AG, Patzoldt W L (2007) Responses of contemporary and historical waterhemp (*Amaranthus tuberculatus*) accessions to glyphosate. Weed Sci 55:327–333

Waite J, Thompson CR, Peterson DE, Currie RS, Olson BLS, Stahlman PW, *et al.* (2013) Differential kochia (*Kochia scoparia*) populations response to glyphosate. Weed Sci 61:193–200

[WSSA] Weed Science Society of America (2016) WSSA Survey Ranks Palmer amaranth as the Most Troublesome Weed in the U.S., Galium as the Most Troublesome in Canada. http://wssa.net/2016/04/wssa-survey-ranks-palmer-amaranth-as-the-most-troublesome-weed-in-the-u-s-galium-as-the-most-troublesome-in-canada. Accessed: March 6, 2019

Westhoven AM, Kruger GR, Gerber CK, Stachler JM, Loux MM, Johnson WG (2008) Characterization of selected common lambsquarters (*Chenopodium album*) biotypes with tolerance to glyphosate. Weed Sci 56: 685–691

White AL, Boutin C (2007) Herbicidal effects on non-target vegetation: investigating the limitations of current pesticide registration guidelines. Environ Toxicol Chem 26:34–43

Wiese A, Schulte M, Theuvsen L, Steinmann HH (2018) Interactions of glyphosate use with farm characteristics and cropping patterns in Central Europe. Pest Manag Sci 74:1155–1165

https://doi.org/10.1017/wsc.2019.54 Published online by Cambridge University Press