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Effect of Temperature on Germination Characteristics of Glyphosate-Resistant and Glyphosate-Susceptible Kochia (*Kochia scoparia*)

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Glyphosate-resistant (GR) kochia is an increasing concern for growers across the U.S. Great Plains and Canadian prairies. Integrated strategies to mitigate resistance will require an improved understanding of the seed germination dynamics of GR kochia populations. Experiments were conducted to characterize the germination of GR vs. glyphosate-susceptible (GS) kochia populations under different constant (5 to 35 C) and alternating (5/10 to 30/35 C) temperatures. Seven GR and two GS populations were collected from wheat–fallow fields in northern Montana. Selected lines of GR and GS were obtained after three generations of recurrent group selection in the greenhouse. The GR-selected lines had 4.1 to 10.8 average *EPSPS* copies compared with a single *EPSPS* gene copy for the GS selected lines. Four out of seven GR selected lines had lower final germination (*d* parameter) and took more time to complete 50% cumulative germination (I_{50} values) under all constant and alternating temperatures, compared with the GS selected lines. Those GR selected lines also had a delayed germination initiation (I_{10} values), particularly at lower temperatures (5 to 10 C constant or 5/10 C alternating). In contrast, the final germination (*d*) of the other three GR selected lines did not differ from GS lines at a majority of temperatures tested. The I_{50} values of those GR lines were also comparable to GS lines under a majority of the temperatures. There was no significant correlation of observed percent cumulative germination and *EPSPS* gene copy number of selected kochia lines. The temperature-dependent dormancy and altered germination characteristics of the four GR kochia lines reflect the common selection of resistance and avoidance (glyphosate or other preseeding treatments) mechanisms. This is most likely attributed to long-term, intensive cropping practices and less diverse weed control methods, rather than a fitness cost or pleiotropic effect of multiple copies of the *EPSPS* gene.
Nomenclature: Glyphosate, kochia; *Kochia scoparia* (L.) Schrad.
Key words: 5-enolpyruvyl-shikimate-3-phosphate synthase (*EPSPS*) gene amplification, germination dynamics, glyphosate resistance, seed dormancy, wheat–fallow system.

Kochia is a problematic, summer annual broadleaf weed, prevalent in cropland and noncropland throughout the U.S. Great Plains, including Montana and in Canada (Friesen et al. 2009). Kochia is a monoecious, C_4 diploid ($2n = 18$) that possesses several unique biological characteristics, including early seedling emergence, rapid growth, and high tolerance to abiotic stresses (heat, cold, salt, drought) (Friesen et al. 2009; Schwinghamer and Van Acker 2008). Because of its protogynous flowering, kochia exhibits a high degree of outcrossing and pollen-mediated gene flow (Beckie et al. 2016; Mengistu and Messersmith 2002; Stallings et al. 1995). Kochia is a prolific seed producer (>100,000 seeds plant^{-1}) and disperses its seeds long distances through wind-mediated tumbling of the matured plant (Baker et al. 2010; Beckie et al. 2016; Christoffoleti et al. 1997; Kumar and Jha 2015a). Consequently, kochia manifests considerable genetic diversity within a field population (Mengistu and Messersmith 2002; Stallings et al. 1995).

Herbicide-resistant (HR) kochia is an increasing problem for growers in the U.S. Great Plains and Canada (Heap 2016). To date, kochia has developed resistance to four different herbicide sites of action, including photosystem II (PS II) inhibitors (atrazine; Group 5), acetolactate synthase (ALS) inhibitors (sulfonylurea and imidazolinone herbicides; Group 2), synthetic auxins (dicamba and fluroxypyr; Group 4), and 5-enolpyruvyl-shikimate-3-phosphate synthase (*EPSPS*) inhibitor (glyphosate; Group 9) (Heap 2016). Glyphosate-resistant (GR) kochia was first identified in western Kansas in 2007 and has also been reported from 10 other states in the U.S. Great Plains, including Montana, and in three Canadian provinces (Hall et al. 2014; Kumar et al. 2014, 2015; Waite et al. 2013; Wiersma et al. 2015). A majority of these GR kochia cases have been reported from no-till

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wheat–fallow fields with a history of intensive glyphosate use in chemical fallow, often at low rates, resulting in poor overall weed control.

The *EPSPS* gene amplification (increased *EPSPS* copy number) confers glyphosate resistance in GR kochia populations reported from U.S. Great Plains and Canada (Godar et al. 2015; Kumar et al. 2015; Wiersma et al. 2015). GR kochia plants from Colorado, North Dakota, South Dakota, and Kansas showed 3 to 10 times more *EPSPS* gene copies relative to susceptible plants (one copy of *EPSPS* gene) (Godar et al. 2015; Wiersma et al. 2015). In Montana, GR kochia biotypes with 3- to 15-fold increase in *EPSPS* gene copies have been reported (Kumar et al. 2015; Kumar and Jha 2015b). A cytogenetic study on GR kochia from Kansas indicated that the increase in *EPSPS* gene copies was possibly due to an unequal crossover during meiosis, resulting in tandem gene duplication, and the extra *EPSPS* gene copies followed a single locus inheritance (Jugulam et al. 2014).

Novel herbicide-resistance alleles may alter other unrelated physiological processes that are directly associated with the fitness attributes of an HR weed biotype (Vila-Aiub et al. 2009). A fitness cost is generally defined as the reduction of fitness caused by the negative pleiotropic effects of a resistance allele on seed germination, emergence, vegetative growth, phenology, and fecundity characteristics of an HR relative to an herbicide-susceptible (HS) weed biotype (Vila-Aiub et al. 2009, 2015). In the literature, no fitness cost due to *EPSPS* gene amplification has been reported in GR Palmer amaranth (*Amaranthus palmeri* S. Wats.) biotypes from Georgia (Giacomini et al. 2014; Vila-Aiub et al. 2014). Similarly, no differences in vegetative growth (plant height, plant width, primary branches, total leaf area, shoot dry weights) and reproductive (harvest index and 1,000-seed weights) characteristics have been found between glyphosate-susceptible (GS) and GR kochia biotypes (Kumar and Jha 2015b).

Less information is available in the literature on seed germination characteristics of HR and HS weed biotypes, and variable results have been observed, depending on the resistance trait and the weed species. For example, Mapplebeck et al. (1982) documented that seeds of triazine-resistant birdsrape mustard (*Brassica campestris* L.) had a slower germination rate (12 to 18 h later) compared with seeds from susceptible plants. In contrast, an *ALS*-resistant prickly lettuce (*Lactuca serriola* L.) biotype germinated much faster than the susceptible biotype after 2.5 d of incubation; however, both biotypes had cumulative germination of ≥98% after 7 d at 20 C (Alcocer-Ruthling et al. 1992). Dyer et al. (1993) reported that ALS-resistant kochia seeds germinated faster than the susceptible seeds at 4.6 C but not at higher temperatures. Thompson et al. (1994) also found that ALS-resistant kochia seeds from Kansas and North Dakota had faster germination rate (12 to 70 h early) and total cumulative germination (100 to 300 h earlier) than the susceptible seeds at 8 C. In a recent study, dicamba-resistant kochia lines had lower cumulative germination than the susceptible line at a majority of constant temperatures from 10 to 35 C and alternating temperatures from 5/10 to 30/35 C (Kumar and Jha 2016). Nevertheless, there is a lack of information in the literature on the germination characteristics of kochia populations with resistance to glyphosate conferred by *EPSPS* gene amplification. Also, it is unclear whether there is any relationship between increased glyphosate resistance levels (as a result of increased *EPSPS* gene copies) and seed dormancy in GR kochia.

Therefore, the objectives of this research were to: (1) determine the relative *EPSPS* gene copy number (glyphosate resistance mechanism) to confirm glyphosate resistance in selected GR vs. GS kochia populations collected from wheat–fallow fields in Montana, (2) compare the seed germination characteristics of those populations under different constant and alternating temperatures, and (3) investigate a possible link between the *EPSPS* gene copy number and observed cumulative (total) germination of those GR and GS kochia populations at each temperature.

Materials and Methods

Plant Material. Kochia seeds were collected from 8 to 10 fully matured putative GR plants in the chemical fallow phase of wheat–fallow rotation in Hill, Liberty, and Toole counties of north-central Montana in the fall of 2012. Seeds from GR plants in a field were composited into a single sample, referred to as a population. Three GR populations collected from Hill County were designated as MT2 (45°54′58.55″N, 108°14′36.66″W), MT4 (45°54′59.39″N, 108°14′37.87″W), and MT9 (45°54′54.76″N, 108°14′44.15″W); a GR population from Liberty County was designated as MT37 (48°41′49.44″N, 111°13′0.49″W); and GR populations from Toole County were designated as MT70 (48°38′33.15″N, 112°4′56.06″W), MT74 (48°44′41.30″N, 112°0′45.70″W),

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and MT75 (48°33′51.77″N, 111°59′30.79″W). Similarly, two GS kochia populations, designated as GS1 (45°54′54.80″N, 108°14′44.74″W) and GS2 (45°54′53.50″N, 108°14′38.78″W), were collected from two different wheat–fallow fields in Hill County; these two fields were in close proximity to the fields where the Hill County GR populations were collected. The sampled fields had a history (>8 yr) of at least three applications of glyphosate per year for weed control in the summer fallow prior to winter wheat planting in the fall.

After threshing and cleaning with an air-propelled column blower, kochia seeds from each population were separately sown on germination flats (53 by 35 by 10 cm) containing commercial potting mix (VermiSoil™, Vermicrop Organics, 4265 Duluth Avenue, Rocklin, CA) in a greenhouse at the Montana State University Southern Agricultural Research Center (MSU-SARC) near Huntley, MT. The greenhouse conditions were maintained at 25/23 C day/night temperatures and 16/8 h day/night photoperiods supplemented with metal-halide lamps (400 mmol m^{-2} s^{-1}). At least 200 kochia plants (8 to 10 cm tall) from each population were treated with 435 (0.5X), 870 (1X), and 1,740 (2X) g ae ha^{-1} of glyphosate (Roundup PowerMax®, Monsanto Company, St. Louis, MO) plus 20 g L^{-1} ammonium sulfate (AMS) in a stationary spray chamber fitted with an even flat-fan nozzle (TeeJet 8001EXR, Spraying System Company, Wheaton, IL) calibrated to deliver 94 L ha^{-1} of spray solution at 276 kPa. Plants from all GR populations survived the 2X rate of glyphosate 21 d after treatment (DAT); confirming the resistance to glyphosate. In contrast, plants from both GS1 and GS2 populations failed to survive the 1X rate of glyphosate 21 DAT (unpublished data).

Development of GR and GS Selected Lines. Kochia plants from each GR population that survived the 2X glyphosate rate were transplanted into 20 L plastic pots (two to three plants per pot) containing the commercial potting mix in the greenhouse as previously described. A group of 20 phenotypically uniform plants of each GR and GS population were selected. Plants were watered as needed to avoid moisture stress and fertilized (Miracle-Gro water-soluble fertilizer [24–8–16], Scotts Miracle-Gro Products, 14111 Scottslawn Road, Marysville, OH) on a biweekly basis to maintain good growth. Prior to flower initiation, groups of three to four GR or GS plants (to avoid inbreeding depression) were covered with pollination bags (DelStar Technologies, 601 Industrial Drive, Middletown, DE) to restrict cross-pollination between GR and GS kochia. Fully matured seeds were collected from the GR plants, and the progeny seedlings were further subjected to two more generations of recurrent group selection (to eliminate heterozygosity) using the 2X rate of glyphosate. Seeds of each of the two GS populations were also obtained after three generations of recurrent group selection without glyphosate under restricted cross-pollination; a subsample of each GS population at each generation was grown and sprayed to confirm susceptibility to the 1X rate of glyphosate. This procedure enabled the development of selected lines from individual GR and GS populations, referred to as GR and GS selected lines, respectively. The selected lines were subsequently used for determining *EPSPS* gene copy numbers and for germination experiments. This methodology not only reduced within-population variability but also minimized the seed germination differences due to differences in maternal environment (Fenner 1991; Thompson et al. 1994). Generally, kochia seed does not exhibit a high degree of primary dormancy and is capable of germinating rapidly (within 24 h) under favorable conditions (Dyer et al. 1993; Everitt et al. 1983; Thompson et al. 1994; Zorner et al. 1984).

***EPSPS* Gene Copy Number.** Laboratory experiments were conducted in the fall of 2014 to determine the *EPSPS* gene copy number of the seven GR and two GS selected kochia lines and were repeated in time (run 1 and run 2). Seeds of each selected line were sown on germination flats as described earlier. Three plants per line were sampled, and a 100 mg sample of young leaf tissue per plant was collected when plants attained 8 to 10 cm height. The genomic DNA (gDNA) was extracted as per the DNeasy plant mini kit (Qiagen Inc., Valencia, CA) standard protocol. The gDNA quantity and quality were checked using an Epoch 2 spectrophotometer (BioTek™ Synergy™ 2 multimode microplate reader, Winooski, VT), and the gDNA integrity was ensured using 1% agarose gel electrophoresis. High-quality gDNA samples (260/280 ratio of ≥1.8) were used for determining the *EPSPS* gene copy number and were processed for use in a quantitative PCR (qPCR) assay as described in Kumar et al. (2015).

For the qPCR assay, the *ALS* gene was chosen as a reference gene due to its stability across kochia populations (Godar et al. 2015; Kumar et al. 2015; Wiersma et al. 2015). For both the *EPSPS* and *ALS* primer pairs (described in Kumar et al. 2015),

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primer efficiency curves were generated. The primer efficiency for the *EPSPS* gene was 100% and for the *ALS* gene it was 101%. The qPCR reaction was carried out in a CFX Connect Real-Time qPCR detection system (Bio-Rad Laboratories, Hercules, CA) using a previously established protocol (Kumar et al. 2015). The qPCR reaction efficiency was 102% ($R^2 = 0.99$, slope = –3.22). The *EPSPS*:*ALS* gene copy number was determined by the comparative C_T method ($\Delta C_T = C_T ALS - C_T EPSPS$). The relative increase in the *EPSPS* gene copy number in a GR selected line was calculated as $2^{\Delta CT}$. Each sample was run in triplicate (three technical replications) to calculate the mean and standard error of the increase in the relative *EPSPS* copy number.

Germination Experiments. Seeds of GR and GS selected kochia lines were stored in paper bags at 4 C until being used in germination experiments. Germination assays were conducted in the fall of 2014 (2 mo after harvest of selected lines) and repeated in time (run 1 and run 2). Germination characteristics of GR and GS selected lines were compared at seven different constant temperatures: 5, 10, 15, 20, 25, 30, and 35 C; and six different alternating temperatures: 5/10, 10/15, 15/20, 20/25, 25/30, and 30/35 C at a 12 h/12 h cycle. The temperature regimes were chosen based on previous studies on kochia seed germination (Dyer et al. 1993; Everitt et al. 1983; Kumar and Jha 2016; Thompson et al. 1994). Treatments were arranged in a completely randomized design with three replications.

Fifty, fully intact seeds were placed with even spacing between two filter-paper layers (Whatman®, Grade 2, Sigma-Aldrich, St. Louis, MO) and moistened with 5 ml of distilled water in a 10-cm-diameter petri dish (Sigma-Aldrich). Light is not required for seed germination of kochia (Everitt et al. 1983); therefore, petri dishes were placed in the dark inside incubators (VMR International, Sheldon Manufacturing, Cornelius, OR) set at either constant or alternating temperatures. A seed with a visible and uncoiled radicle tip (length of protruding radicles greater than or equal to seed diameter) was considered germinated (Dyer et al. 1993). Germination was quantified daily for 15 d, after which germination ceased. Any nongerminated seed was tested for viability by soaking the seed in a 10 g L^{-1} solution of tetrazolium chloride for 24 h at room temperature (Kumar and Jha 2015a; Sbatella and Wilson 2010). A seed with a red-stained embryo was considered viable (Thompson et al. 1994). Seed germination was expressed as a percentage of total viable seeds, and cumulative germination percentages were calculated.

Statistical Analyses. Data on percent cumulative germination were subjected to ANOVA using PROC MIXED in SAS (v. 9.3, SAS Institute, Cary, NC) to test the significance of experimental run, selected line, temperature (constant or alternating), and their interactions. Transformation of data did not improve homogeneity of variance; therefore, analysis was performed on nontransformed data. A three-parameter log-logistic model (Equation 1) was fit to describe the percent cumulative germination dynamics of each selected kochia line over time at different constant and alternating temperatures, using the 'drc' package in R software (R Core Team 2015):

$$Y = \{d/1 + \exp[b(\log t - \log I_{50})]\} \qquad [1]$$

where Y is the percent cumulative germination over time t; d is the final cumulative germination (%); I_{50} is the incubation time (h) to reach 50% cumulative germination; b denotes the slope around the inflection point "I_{50}." The slope parameter (b) indicates the germination rate of each selected line over time (i.e., slope with a larger negative value indicates more rapid germination of that selected kochia line). Parameter estimates, standard errors, and I_{50} values of GS and GR selected lines of kochia at each temperature treatment were determined using the 'drc' package in R software. Parameter estimates of GS vs. GR selected lines at each temperature treatment were compared with an approximate *t*-test using the *compParm* statement in the 'drc' package (Ritz et al. 2015). Correlation analyses were performed between *EPSPS* gene copy number and observed percent cumulative germination of GR and GS selected lines at each constant/alternating temperature using PROC CORR in SAS.

Results and Discussion

***EPSPS* Gene Copy Number.** The main effect of experimental run or experimental run by treatment interaction was not significant; therefore, data on *EPSPS* gene copy number from both runs were combined. Results from the qPCR assay revealed that GR selected lines from Hill County, e.g., MT2, MT4, and MT9, had an average of 9.6, 4.1, and 10.8 relative *EPSPS* copies, respectively. The GR selected line MT37 from Liberty County had an average of 6.8 relative *EPSPS* copies. The *EPSPS* gene copies for the GR selected lines MT70, MT74, and MT75 from Toole County averaged 5.0, 5.7, and 10.5, respectively. In contrast, all kochia plants from GS1 and GS2 selected lines had only one relative *EPSPS* gene copy. The *EPSPS* gene amplification with similar magnitudes (4- to 10-fold) has also been reported in previously confirmed GR kochia populations from Montana (Kumar et al. 2015). The levels of *EPSPS* gene amplification observed in the current study are also comparable to those reported (3- to 11-fold) in GR kochia populations from other U.S. Great Plains states, including Kansas, Colorado, South Dakota, and North Dakota (Godar et al. 2015; Wiersma et al. 2015). A few kochia populations recently identified from eastern Colorado and Alberta, Canada, had 15 to 20 *EPSPS* gene copies (T Gaines, personal communication). In previous research, there was a positive linear relationship between *EPSPS* gene copy number and glyphosate resistance level (based on I_{50} values) in GR kochia populations collected from wheat–fallow fields in Montana (Kumar et al. 2015). In that study, plants with 4 to 10 *EPSPS* gene copies demonstrated 4.6- to 11-fold levels of resistance (based on R/S ratios from whole-plant dose–response assays). Similarly, GR kochia plants from Kansas with 6.7 to 8.3 *EPSPS* gene copies had higher levels of resistance compared with those with 4.2 to 4.9 *EPSPS* gene copies (Godar et al. 2015).

Seed Germination in Response to Temperature. The main effect of experimental run or interactions involving experimental run were not significant; therefore, germination data were pooled over runs. Seeds of all selected kochia lines (GR or GS) had ≥96% viability at all constant or alternating temperatures tested (unpublished data). Lack-of-fit tests ($P > 0.05$) indicated that the nonlinear regression model (Equation 1) acceptably described the percent cumulative germination data for all selected line and incubation temperature combinations. The four GR selected lines of MT37 (Liberty), MT70 (Toole), MT74 (Toole), and MT75 (Toole) differed in their germination responses compared with the other three GR lines of MT2 (Hill), MT4 (Hill), and MT9 (Hill) and with the two GS lines of GS1 (Hill) and GS2 (Hill) (Tables 1 to 4). The differential response could possibly be because the populations

Table 1. Estimated parameters *d* and *b* (± SE) obtained from the log-logistic model fit to describe the cumulative germination pattern of glyphosate-susceptible (GS) and glyphosate-resistant (GR) kochia selected lines over time (h) under different constant temperatures.[a,b]

		Temperature (C)						
Population	Parameter	5	10	15	20	25	30	35
GS1	*d*	90.5 (2.8)	99.0 (0.7)	97.8 (2.1)	102.5 (1.7)	104.3 (9.0)	98.8 (0.8)	100.1 (1.3)
GS2	*d*	91.3 (4.4)	101.4 (1.0)	101.8 (3.8)	95.4 (1.2)	108.7 (7.8)	97.9 (1.5)	99.4 (2.4)
MT2	*d*	97.6 (1.8)	101.5 (1.6)	97.8 (1.3)	99.8 (0.9)	104.9 (6.2)	98.6 (0.7)	102.9 (1.7)
MT4	*d*	74.2 (2.8)*	86.7 (1.6)*	104.2 (4.1)	95.3 (4.2)	97.3 (2.0)	101.1 (1.5)	102.7 (2.2)
MT9	*d*	76.7 (4.7)*	83.0 (2.3)*	100.7 (5.5)	91.7 (5.6)	90.2 (2.3)	101.0 (3.8)	97.7 (1.4)
MT37	*d*	67.8 (2.5)*	71.1 (1.7)*	74.5 (5.4)*	78.2 (5.3)*	77.3 (5.2)*	79.4 (3.1)*	80.0 (1.7)*
MT70	*d*	57.2 (2.6)*	61.3 (2.2)*	67.4 (5.9)*	71.3 (9.0)*	74.9 (4.2)*	77.5 (3.4)*	78.3 (2.1)*
MT74	*d*	56.8 (1.7)*	62.9 (5.9)*	72.1 (4.7)*	74.6 (7.2)*	78.4 (5.7)*	81.3 (3.5)*	78.1 (1.8)*
MT75	*d*	57.2 (1.4)*	63.5 (4.7)*	73.2 (6.7)*	76.4 (8.6)*	79.3 (5.9)*	80.5 (4.9)*	82.7 (2.6)*
GS1	*b*	−2.0 (0.2)	−1.9 (0.5)	−1.7 (0.2)	−1.5 (0.2)	−0.6 (0.3)	−1.3 (1.0)	−1.8 (0.2)
GS2	*b*	−2.5 (0.2)	−2.3 (0.2)	−1.3 (0.1)	−2.1 (0.4)	−0.7 (0.2)	−1.4 (0.2)	−1.2 (0.1)
MT2	*b*	−2.4 (0.3)	−2.3 (0.1)	−3.0 (0.3)*	−3.1 (0.7)*	−0.5 (0.2)	−3.7 (0.9)*	−1.3 (0.2)
MT4	*b*	−1.9 (0.4)	−1.7 (0.2)	−1.7 (0.2)	−1.6 (0.2)	−1.6 (0.2)*	−1.9 (0.2)	−1.3 (0.1)
MT9	*b*	−1.4 (0.4)*	−1.3 (0.1)*	−1.4 (0.1)	−0.9 (0.2)	−1.6 (0.2)*	−0.9 (0.2)	−1.6 (0.1)
MT37	*b*	−1.5 (0.3)*	−1.5 (0.1)*	−1.7 (0.3)	−1.1 (0.2)	−1.1 (0.2)	−1.2 (0.2)	−1.6 (0.2)
MT70	*b*	−1.3 (0.3)*	−1.5 (0.2)*	−1.2 (0.3)	−0.7 (0.2)	−1.3 (0.2)	−1.2 (0.1)	−1.6 (0.2)
MT74	*b*	−1.5 (0.8)*	−1.2 (0.1)*	−1.4 (0.5)	−1.0 (0.2)	−1.2 (0.2)	−1.3 (0.1)	−1.6 (0.2)
MT75	*b*	−1.4 (0.5)*	−1.1 (0.2)*	−1.4 (0.2)	−0.8 (0.2)	−0.9 (0.2)	−1.0 (0.1)	−1.3 (0.1)

[a] Abbreviations: GS1 and GS2 are GS kochia lines from Hill County, MT; MT2, MT4, and MT9 are GR kochia lines from Hill County, MT; MT37 is GR kochia line from Liberty County, MT; and MT70, MT74, and MT75 are GR kochia lines from Toole County, MT.

[b] *d* is the final percent germination; *b* is the slope parameter, indicating the germination rate of a selected line over time.

* Denotes a significant difference for the regression parameter (*d* or *b*) between a GR selected line and GS1 or GS2 selected line within a temperature treatment according to approximate *t*-test (Ritz et al. 2015).

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Table 2. Estimated parameters *d* and *b* (± SE) obtained from the log-logistic models that were fit to describe the cumulative germination pattern of glyphosate-susceptible (GS) and glyphosate-resistant (GR) kochia selected lines over time (h) under different alternating temperatures.[a,b]

		Temperature (C)					
Population	Parameter	5/10	10/15	15/20	20/25	25/30	30/35
GS1	*d*	95.9 (3.3)	101.1 (3.0)	99.3 (2.1)	96.4 (1.5)	98.7 (0.9)	100.2 (1.0)
GS2	*d*	101.1 (6.4)	94.5 (2.7)	101.4 (2.2)	97.6 (1.9)	95.7 (1.1)	95.0 (1.7)
MT2	*d*	100.1 (4.0)	91.2 (4.2)	102.8 (2.1)	101.5 (1.2)	96.2 (1.1)	97.5 (1.0)
MT4	*d*	59.6 (3.8)*	92.2 (3.3)	95.8 (3.3)	99.4 (1.6)	89.7 (1.2)	97.6 (1.3)
MT9	*d*	56.2 (2.5)*	70.5 (4.2)*	83.4 (4.3)*	90.9 (1.7)	89.7 (2.3)	91.6 (1.4)
MT37	*d*	55.7 (1.6)*	62.2 (6.0)*	67.8 (4.6)*	73.7 (2.2)*	79.0 (1.4)*	78.5 (1.5)*
MT70	*d*	52.6 (1.6)*	64.1 (4.1)*	66.6 (5.1)*	71.8 (2.3)*	72.9 (1.0)*	79.0 (1.5)*
MT74	*d*	53.5 (2.3)*	61.1 (2.1)*	67.9 (5.0)*	73.1 (1.6)*	76.6 (1.3)*	77.5 (1.4)*
MT75	*d*	53.3 (2.8)*	62.3 (4.8)*	78.5 (4.5)*	81.0 (2.2)*	82.0 (1.4)*	84.2 (1.5)*
GS1	*b*	−1.5 (0.1)	−1.2 (0.1)	−1.5 (0.2)	−1.5 (0.2)	−2.3 (0.7)	−2.9 (0.8)
GS2	*b*	−1.1 (0.1)	−1.3 (0.2)	−1.6 (0.1)	−1.2 (0.2)	−2.1 (0.6)	−1.6 (0.3)
MT2	*b*	−1.6 (0.2)*	−1.2 (0.3)	−1.7 (0.1)	−1.7 (0.2)	−2.1 (0.8)	−3.3 (0.6)*
MT4	*b*	−1.4 (0.2)	−1.4 (0.2)	−1.4 (0.2)	−1.7 (0.1)	−2.2 (0.2)	−2.2 (0.2)
MT9	*b*	−1.6 (0.3)*	−1.3 (0.1)	−1.4 (0.2)	−1.7 (0.1)	−1.2 (0.2)	−1.9 (0.3)
MT37	*b*	−1.8 (1.0)*	−1.1 (0.2)	−0.9 (0.2)	−1.5 (0.1)	−1.9 (0.2)	−1.8 (0.3)
MT70	*b*	−2.2 (0.9)*	−1.3 (0.2)	−1.3 (0.2)	−1.5 (0.1)	−2.6 (0.3)	−1.9 (0.2)
MT74	*b*	−2.3 (0.6)*	−1.1 (0.3)	−1.2 (0.3)	−1.6 (0.2)	−2.2 (0.2)	−2.2 (0.2)
MT75	*b*	−1.5 (0.3)	−1.0 (0.2)	−1.4 (0.2)	−1.7 (0.1)	−2.0 (0.2)	−1.9 (0.2)

[a] Abbreviations: GS1 and GS2 are GS kochia lines from Hill County, MT; whereas, MT2, MT4, and MT9 are GR kochia lines from Hill County, MT; MT37 is GR kochia line from Liberty County, MT; and MT70, MT74, and MT75 are GR kochia lines from Toole County, MT.

[b] *d* is the final percent germination; *b* is the slope parameter, indicating the germination rate of a selected line over time.

* Denotes a significant difference for the regression parameter (*d* or *b*) between a GR selected line and GS1 or GS2 selected line within a temperature treatment according to approximate *t*-test (Ritz et al. 2015).

were geographically distinct (separate wheat–fallow fields) and might not have been exposed to identical agronomic selection practices.

The GR selected lines of MT37, MT70, MT74, and MT75 had lower final germination (*d* parameter) at lower constant temperatures of 5 and 10 C, and they also had lower final germination at higher constant temperatures of 15 to 35 C compared with the GS lines (Table 1). However, the final germination (*d* parameter) of the GR selected line of MT2 did not differ from the GS lines, either at lower or higher constant temperatures. Additionally, GR selected lines of MT4 and MT9 had final germinations similar to GS lines at 15 to 35 C. These results indicate that the viable seeds of the four GR lines from Liberty and Toole counties were relatively dormant compared with GS and three GR lines from Hill County in response to temperatures tested. Furthermore, those four GR lines showed a temperature-sensitive dormancy response, lower final germination at lower temperatures and higher at higher temperatures (Table 1). In contrast, the GS and other GR lines (especially MT2) were relatively nondormant, with >90% final germination across all temperatures.

Similarly, at all alternating temperatures tested (5/10 to 30/35 C), the GR selected lines of MT37, MT70, MT74, or MT75 had considerably less final germination than the GS selected lines (Table 2). Consistent with the constant temperature treatments, the final germination of GR lines of MT2, MT4, and MT9 did not differ from GS lines at the majority of the alternating temperatures tested.

The rate of germination (slope, *b* parameter) of GR selected lines (except MT2 and MT4) was different from GS1 or GS2 selected line at 5 and 10 C (Table 1). GR selected lines of MT37, MT70, MT74, or MT75 had lower germination rates at 5 and 10 C relative to GS lines. However, no differences in the germination rate between any of those four GR lines and GS lines were evident at temperatures from 15 to 35 C. Conversely, GR selected lines of MT2, MT4, or MT9 germinated faster than the GS1 or GS2 selected line at one or more of the temperatures ranging from 15 to 30 C.

At alternating temperatures, there were no differences in the germination rates (*b*) between GR and GS selected lines, except for a few instances at 5/10 C (Table 2). The GR selected lines of MT2

Table 3. Estimated parameters I_{10} and I_{50} (± SE) obtained from the log-logistic models fit to describe the cumulative germination pattern of glyphosate-susceptible (GS) and glyphosate-resistant (GR) kochia selected lines over time (h) under different constant temperatures.[a,b]

		Temperature (C)						
Population	Parameter	5	10	15	20	25	30	35
GS1	I_{10}	21 (2.7)	16 (1.1)	18 (1.2)	10 (1.1)	7 (0.2)	5 (1.2)	7 (1.0)
GS2	I_{10}	18 (2.9)	18 (1.2)	16 (1.1)	12 (1.2)	8 (1.1)	4 (1.1)	6 (0.8)
MT2	I_{10}	24 (3.0)	21 (1.3)	16 (0.9)	10 (2.3)	7 (0.02)	8 (1.7)	5 (1.0)
MT4	I_{10}	25 (5.3)	23 (1.7)	17 (1.2)	8 (1.1)	9 (1.5)	6 (1.1)	6 (1.0)
MT9	I_{10}	62 (3.0)*	42 (2.2)*	15 (1.4)	6 (1.2)	9 (1.6)	5 (0.8)	7 (1.1)
MT37	I_{10}	53 (5.2)*	39 (2.4)*	19 (1.4)	7 (1.9)	7 (2.2)	7 (1.6)	10 (1.6)
MT70	I_{10}	42 (3.9)*	36 (1.9)*	15 (3.0)	7 (1.3)	8 (2.2)	7 (1.6)	11 (1.9)
MT74	I_{10}	51 (3.6)*	39 (2.7)*	17 (2.4)	19 (3.2)*	15 (1.7)*	12 (1.7)*	10 (1.3)
MT75	I_{10}	46 (2.8)*	35 (2.8)	18 (2.6)	7 (2.0)	5 (0.9)	5 (1.2)	7 (1.0)
GS1	I_{50}	65 (4.0)	36 (1.9)	33 (0.9)	22 (1.6)	20 (0.7)	19 (1.7)	21 (1.3)
GS2	I_{50}	69 (5.7)	42 (3.3)	37 (1.2)	21 (1.7)	22 (0.4)	21 (0.8)	24 (1.7)
MT2	I_{50}	73 (2.8)	40 (1.8)	36 (1.2)	20 (1.3)	19 (0.8)	21 (1.5)	18 (1.5)
MT4	I_{50}	112 (5.5)*	73 (5.1)*	39 (2.0)	27 (2.4)	22 (2.0)	20 (1.3)	22 (1.7)
MT9	I_{50}	139 (8.5)*	97 (5.4)*	56 (3.5)*	28 (3.5)	25 (2.3)	21 (1.1)	20 (1.4)
MT37	I_{50}	176 (7.4)*	87 (7.1)*	59 (2.4)*	47 (6.6)*	48 (3.1)*	39 (2.6)*	38 (2.4)*
MT70	I_{50}	157 (10.4)*	94 (7.9)*	52 (5.9)*	51 (5.2)*	43 (3.6)*	41 (3.0)*	46 (3.2)*
MT74	I_{50}	147 (4.3)*	97 (9.0)*	61 (4.7)*	59 (7.7)*	58 (4.6)*	55 (3.0)*	39 (2.3)*
MT75	I_{50}	156 (5.5)*	87 (6.6)*	63 (3.7)*	54 (6.7)*	48 (3.9)*	39 (1.5)*	36 (2.6)*

[a] Abbreviations: GS1 and GS2 are GS kochia lines from Hill County, MT; whereas, MT2, MT4, and MT9 are GR kochia lines from Hill County, MT; MT37 is GR kochia line from Liberty County, MT; and MT70, MT74, and MT75 are GR kochia lines from Toole County, MT.

[b] I_{10} and I_{50} refer to the incubation period (h) required to reach 10 and 50% of cumulative germination by a selected line, respectively.

* Denotes a significant difference for the regression parameter (I_{10} or I_{50}) between a GR selected line and GS1 or GS2 selected line within a temperature treatment according to approximate *t*-test (Ritz et al. 2015).

and MT9 germinated faster than the GS2 selected line at 5/10 C.

The fitted model also provided two other biological parameters, i.e., the incubation time (h) to reach 10 and 50% cumulative germination (I_{10} and I_{50} values, respectively) for each selected kochia line. Compared with GS, all GR selected lines showed a delay in germination initiation (higher I_{10} values) at constant temperatures of 5 and 10 C, except GR selected lines of MT2 and MT4 (Table 3). The I_{10} values between these two GR lines (MT2 and MT4) and GS selected lines did not differ at 5 or 10 C. At constant temperatures ≥15 C, no differences were evident in the I_{10} values among the majority of GR and GS selected lines.

At an alternating temperature of 5/10 C, seeds of all GR selected lines except MT2 took more time to initiate germination (greater I_{10} values) compared with GS selected lines (Table 4). Consistent with the constant temperature data, there were no differences in I_{10} values among a majority of the GR and GS selected lines at alternating temperatures ≥10/15 C.

In general, kochia seeds (GR and GS) germinated more rapidly (took less time) at constant temperatures ≥15 C than at low temperatures of 5 or 10 C, indicated by the lower I_{50} values at temperatures of 15 C or higher (Table 3). Kochia can germinate over a wide range of temperatures from 5 to 30 C, with an optimum temperature of 15 to 25 C (≥50% germination within 24 h) (Everitt et al. 1983; Jami Al-Ahmadi and Kafi 2007). GR selected lines of MT37, MT70, MT74, and MT75 germinated more slowly (greater I_{50} values) than GS lines at all constant temperatures tested. In other words, the germination period of these four GR selected lines would be more prolonged compared with the GS selected lines. However, the I_{50} values for GR selected line MT2 did not differ from GS lines at any of the constant temperatures. Also, the I_{50} values of GR selected lines of MT4 and MT9 were comparable to GS lines at temperatures ≥20 C.

At all alternating temperatures tested, the I_{50} values of GR selected lines of MT37, MT70, MT74, and MT75 were higher than GS selected

Table 4. Estimated parameters I_{10} and I_{50} ($\pm$ SE) obtained from the log-logistic models that were fit to describe the cumulative germination pattern of glyphosate-susceptible (GS) and glyphosate-resistant (GR) kochia selected lines over time (h) under different alternating temperatures.[a,b]

		Temperature (C)					
Population	Parameter	5/10	10/15	15/20	20/25	25/30	30/35
GS1	I_{10}	10 (1.3)	9 (1.0)	6 (1.4)	6 (0.6)	7 (0.9)	7 (1.0)
GS2	I_{10}	8 (1.4)	11 (1.2)	10 (1.5)	5 (0.9)	6 (1.1)	8 (0.7)
MT2	I_{10}	12 (2.3)	13 (1.2)	11 (2.1)	6 (1.1)	5 (1.1)	7 (1.0)
MT4	I_{10}	23 (3.1)*	15 (2.3)	8 (1.3)	12 (1.0)	4 (1.2)	6 (1.3)
MT9	I_{10}	27 (2.5)*	16 (1.2)	12 (1.3)	12 (1.7)	6 (2.3)	8 (0.4)
MT37	I_{10}	35 (3.6)*	14 (2.0)	8 (1.6)	11 (1.2)	10 (1.4)	9 (1.1)
MT70	I_{10}	36 (1.6)*	15 (2.1)	11 (2.1)	11 (1.3)	11 (1.0)	8 (1.1)
MT74	I_{10}	42 (2.3)*	26 (2.2)*	12 (1.6)	10 (1.6)	11 (1.3)	10 (1.2)
MT75	I_{10}	24 (2.8)*	11 (4.8)	13 (2.5)	13 (1.9)	10 (1.4)	11 (1.3)
GS1	I_{50}	60 (3.9)	45 (2.8)	33 (2.0)	21 (1.6)	14 (1.4)	15 (1.7)
GS2	I_{50}	59 (4.5)	55 (3.1)	36 (2.2)	19 (1.8)	12 (1.7)	14 (2.1)
MT2	I_{50}	82 (5.5)*	50 (6.5)	39 (2.2)	20 (1.3)	12 (1.4)	17 (1.0)
MT4	I_{50}	110 (8.5)*	53 (4.5)	38 (3.1)	24 (1.8)	17 (1.4)	15 (1.5)
MT9	I_{50}	104 (7.2)*	75 (4.9)*	40 (5.8)	26 (2.0)	14 (2.3)	18 (1.8)
MT37	I_{50}	117 (9.0)*	90 (5.0)*	70 (6.7)*	47 (3.5)*	32 (2.3)*	26 (2.1)*
MT70	I_{50}	125 (8.7)*	82 (3.8)*	58 (4.7)*	46 (3.3)*	31 (1.6)*	27 (2.1)*
MT74	I_{50}	127 (7.6)*	107 (7.5)*	70 (7.1)*	39 (2.6)*	40 (2.3)*	29 (2.4)*
MT75	I_{50}	108 (5.8)*	86 (6.1)*	66 (5.0)*	49 (3.1)*	35 (2.0)*	30 (1.7)*

[a] Abbreviations: GS1 and GS2 are GS kochia lines from Hill County, MT; whereas, MT2, MT4, and MT9 are GR kochia lines from Hill County, MT; MT37 is GR kochia line from Liberty County, MT; and MT70, MT74, and MT75 are GR kochia lines from Toole County, MT.

[b] I_{10} and I_{50} refer to the incubation period (h) required to reach 10 and 50% of cumulative germination by a selected line, respectively.

* Denotes a significant difference for the regression parameter (I_{10} or I_{50}) between a GR selected line and GS1 or GS2 selected line within a temperature treatment according to approximate *t*-test (Ritz et al. 2015).

lines (Table 4), further indicating the relatively slow germination pattern of those four GR vs. GS lines. Nevertheless, the I_{50} values for the GR selected lines of MT2 and MT4 did not differ from any of the GS lines at the majority of the alternating temperatures tested, and those of GR MT9 line were comparable to GS lines at temperatures ≥15/20 C.

Relationship between *EPSPS* Gene Copy Number and Cumulative Germination. There was no significant correlation between the *EPSPS* gene copy number and observed percent cumulative germination of GR and GS selected lines at any of the constant (r = −0.154 to −0.391; P-value = 0.159 to 0.548) or alternating (r = −0.045 to −0.235; P-value = 0.091 to 0.824) temperatures tested. Therefore, the reduced final germination or temperature-dependent reduction of germination and a slower germination pattern of GR selected lines MT37, MT70, MT74, and MT75 relative to GS lines are less likely attributed to the pleiotropic effects of the *EPSPS* gene amplification.

The glyphosate resistance and temperature-mediated seed dormancy in the four GR selected lines reflect coselection of resistance and avoidance imposed by decades of intensive cropping practices. Long-term, intensive crop management practices are used in the no-till, dryland cereal belt of Montana and other U.S. Great Plains states and in Canada, with heavy reliance on nonselective herbicides, predominantly glyphosate, applied preseeding. If germination is delayed, as evident in the four GR selected lines, particularly at lower temperatures (5 to 10 C constant or 5/10 C alternating), then parts of the weed population may avoid preseeding control. This would also allow these GR kochia cohorts to survive against tillage during early-spring seedbed preparation or against early-spring frosts, thereby, increasing the proportion of GR plants in the population. Kochia generally exhibits little seed dormancy and seed persistence of less than 2 yr in the soil (Everitt et al. 1983; Zorner et al. 1984; JA Dille, personal communication); therefore, the defense against preseeding control methods may be

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more important considering the transient nature of the seedbank. Previous researchers have also documented the impact of long-term crop management practices on selection of dormancy and herbicide resistance traits in other HR weed populations (Owen et al. 2010; Shergill et al. 2015). Management practices in an intensive cropping system, including the continuous use of selective/nonselective herbicides or soil-residual herbicides with longer selection, tend to favor the survival of late-emerging (delayed germination) weed cohorts (avoiding preseeding or early in-crop weed control), and relatively dormant weed cohorts dominate the weed seedbank over time (Fleet and Gill 2012; Owen et al. 2015; Sbatella and Wilson 2010).

Additionally, the GR trait may allow those kochia cohorts to survive subsequent glyphosate applications in chemical fallow/postharvest wheat stubble or in-crop (in GR corn [*Zea mays* L.], sugar beet [*Beta vulgaris* L.], or soybean [*Glycine max* L.]). This also highlights the importance of diverse in-crop and late-season weed control methods to prevent those late-emerging GR kochia populations from replenishing the soil seedbank. Although these GR populations originated in the dryland, cereal-based cropping systems, GR kochia has also been reported from GR corn, sugar beet, and soybean fields in the U.S. Great Plains (Heap 2016). Evident from this research, selection by herbicides/cropping practices can lead to more than one response, including an alteration in the time of germination and possibly seed dormancy in weed populations, which implicates the need to adopt more diversified cropping and weed control practices.

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Literature Cited

Alcocer-Ruthling M, Thill DC, Shafii B (1992) Differential competitiveness of sulfonylurea resistant and susceptible biotypes of prickly lettuce (*Lactuca serriola*). Weed Technol 6:303–309

Baker DV, Withrow JR, Brown CS, Beck KG (2010) Tumbling: use of diffuse knapweed (*Centaurea diffusa*) to examine an understudied dispersal mechanism. Invasive Plant Sci Manag 3:301–309

Beckie H, Blackshaw R, Hall L, Johnson E (2016) Pollen- and seed-mediated gene flow in kochia (*Kochia scoparia*). Weed Sci 64:624–633

Christoffoleti PJ, Westra PB, Moore F (1997) Growth analysis of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci 45:691–695

Dyer WE, Chee PW, Fay PK (1993) Rapid germination of sulfonylurea-resistant *Kochia scoparia* L. accessions is associated with elevated seed levels of branched chain amino acids. Weed Sci. 41:18–22

Everitt JH, Alaniz MA, Lee JB (1983) Seed germination characteristics of *Kochia scoparia*. J Range Manage 36:646–648

Fenner M (1991) The effects of the parent environment on seed germinability. Seed Sci Res 1:75–84

Fleet B, Gill G (2012) Seed dormancy and seedling recruitment in smooth barley (*Hordeum murinum* ssp. glaucum) populations in southern Australia. Weed Sci 60:394–400

Friesen LF, Beckie HJ, Warwick SI, Van Acker RC (2009) The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. Can J Plant Sci 89:141–167

Giacomini D, Westra P, Ward SM (2014) Impact of genetic background in fitness cost studies: an example from glyphosate-resistant Palmer amaranth. Weed Sci 62:29–37

Godar AS, Stahlman PW, Jugulam M, Dille JA (2015) Glyphosate-resistant kochia (*Kochia scoparia*) in Kansas: EPSPS gene copy number in relation to resistance levels. Weed Sci 63:587–595

Hall LM, Beckie HJ, Low R, Shirriff SW, Blackshaw RE, Kimmel N, Neeser C (2014) Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta. Can J Plant Sci 94:127–130

Heap I (2016) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: May 15, 2016

Jami Al-Ahmadi M, Kafi M (2007) Cardinal temperatures for germination of *Kochia scoparia* (L.). J Arid Environ 68:308–314

Jugulam M, Niehues K, Godar AS, Koo DH, Danilova T, Friebe B, Sehgal S, Varanasi VK, Wiersma A, Westra P, Stahlman PW, Gill BS (2014) Tandem amplification of a chromosomal segment harboring 5-enolpyruvylshikimate-3-phosphate synthase locus confers glyphosate resistance in *Kochia scoparia*. Plant Physiol 166:1200–1207

Kumar V, Jha P, Reichard N (2014) Occurrence and characterization of kochia (*Kochia scoparia*) accessions with resistance to glyphosate in Montana. Weed Technol 28: 122–130

Kumar V, Jha P, Giacomini D, Westra E, Westra P (2015) Molecular basis of evolved resistance to glyphosate and acetolactate synthase-inhibitor herbicides in kochia (*Kochia scoparia*) accessions from Montana. Weed Sci 63:758–769

Kumar V, Jha P (2015a) Influence of herbicides applied postharvest in wheat stubble on control, fecundity, and progeny fitness of *Kochia scoparia* in the US Great Plains. Crop Prot 71:144–149

Kumar V, Jha P (2015b) Growth and reproduction of glyphosate-resistant and susceptible populations of *Kochia scoparia*. PLoS One 11:e0147779

Kumar V, Jha P (2016) Differences in germination, growth, and fecundity characteristics of dicamba-fluroxypyr-resistant and susceptible *Kochia scoparia*. PLoS One 11:e0161533

Mapplebeck LR, Souza Machado V, Grodzinski B (1982) Seed germination and seedling growth characteristics of atrazine susceptible and resistant biotypes of *Brassica campestris*. Can J Plant Sci 62:733–739

Mengistu LW, Messersmith CG (2002) Genetic diversity of kochia. Weed Sci 50:498–503

Owen MJ, Goggin DE, Powles SB (2015) Intensive cropping systems select for greater seed dormancy and increased herbicide resistance levels in *Lolium rigidum* (annual ryegrass). Pest Manag Sci 71:966–971

Owen MJ, Michael PJ, Renton M, Steadman KJ, Powles SB (2010) Towards large-scale prediction of *Lolium rigidum* emergence. II. Correlation between dormancy and herbicide resistance levels suggests an impact of cropping systems. Weed Res 51:133–141

R Core Team (2015) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, http://www.R-project.org. Accessed June 12, 2016

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-response analysis using R. PLoS One 10:e0146021

Sbatella GM, Wilson RG (2010) Isoxaflutole shifts kochia (*Kochia scoparia*) populations in continuous corn. Weed Technol 24:392–396

Schwinghamer TD, Van Acker RC (2008) Emergence timing and persistence of kochia (*Kochia Scoparia*). Weed Sci 56:37–41

Shergill LS, Fleet B, Preston C, Gill G (2015) Incidence of herbicide resistance, seedling emergence, and seed persistence of smooth barley (*Hordeum glaucum*) in south Australia. Weed Technol 29:782–792

Stallings GP, Thill DC, Mallory-Smith CA, Shafii B (1995) Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci 43:95–102

Thompson CR, Thill DC, Shafii B (1994) Germination characteristics of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). Weed Sci 42:50–56

Vila-Aiub MM, Goh SS, Gaines TA, Han H, Busi R, Yu Q, Powles SB (2014) No fitness cost of glyphosate resistance endowed by massive EPSPS gene amplification in *Amaranthus palmeri*. Planta 239:793–801

Vila-Aiub MM, Gundel PE, Preston C (2015) Experimental methods for estimation of plant fitness costs associated with herbicide-resistance genes. Weed Sci 63:203–216

Vila-Aiub MM, Neve P, Powles SB (2009) Fitness costs associated with evolved herbicide resistance alleles in plants. New Phytol 184:751–767

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

Wiersma AT, Gaines TA, Preston C, Hamilton JP, Giacomini D, Buell CR, Leach JE, Westra P (2015) Gene amplification of 5-enol-pyruvylshikimate-3-phosphate synthase in glyphosate-resistant *Kochia scoparia*. Planta 241:463–474

Zorner PS, Zimdahl RL, Schweizer EE (1984) Effect of depth and duration of seed burial on kochia (*Kochia scoparia*). Weed Sci 32:602–607

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