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Differential Germination Characteristics of Dicamba-Resistant Kochia (*Bassia scoparia*) Populations in Response to Temperature

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Abstract

Dicamba-resistant (DR) kochia [*Bassia scoparia* (L.) A. J. Scott] has been reported in six U.S. states and one Canadian province. To develop effective *B. scoparia* control tactics, it is necessary to understand the seed germination pattern of DR *B. scoparia*. The objective of this study was to compare the germination characteristics of DR versus dicamba-susceptible (DS) *B. scoparia* populations from Montana and Kansas under constant (5 to 35 C) and/or alternating temperatures (5/10 to 30/35 C). DR *B. scoparia* lines from Montana were generated after three generations of recurrent selection of field-collected populations with dicamba. Seeds of DR or DS lines from Kansas were obtained after one generation of restricted self-pollination. DR *B. scoparia* lines from both Montana and Kansas had a lower maximum cumulative germination than the DS lines across all temperature treatments. A majority of DR *B. scoparia* lines from Montana showed a temperature-mediated seed germination response, with a higher thermal requirement (30 to 35 C or 25/30 to 30/35 C) to attain the maximum cumulative germination compared with DS lines. Germination rates at 5 to 30 C were lower for DR versus DS *B. scoparia* lines from Kansas. All DR lines from Montana took more time than DS lines to initiate germination at 5 and 10 C or 5/10 and 20/25 C. Similarly, there was a delayed onset of germination of the DR versus DS line from Kansas at 5, 10, 15, and 20 C. Furthermore, the DR *B. scoparia* from both Kansas and Montana had a slower germination pattern relative to the DS *B. scoparia*. Diversified crop rotations using winter wheat (*Triticum aestivum* L.), fall-sown cover crops, or early-spring planted crops (e.g., wheat or barley [*Hordeum vulgare* L.]) that are competitive against late-emerging *B. scoparia* in conjunction with strategic tillage and late-season weed control tactics should be used to facilitate depletion of DR *B. scoparia* seedbanks.

Introduction

Kochia [*Bassia scoparia* (L.) A. J. Scott] is among the most troublesome, summer annual weeds in semiarid to arid regions of the U.S. Great Plains (Friesen et al. 2009). *Bassia scoparia* manifests several biological attributes, including early seedling emergence in the spring, rapid growth behavior (C₄ plant), tolerance to biotic and abiotic stresses (heat, cold, salt, and drought), and limited seed persistence (1 to 2 yr) in the soil (Dille et al. 2017; Friesen et al. 2009; Kumar et al. 2018; Schwinghamer and Van Acker 2008). It produces protogynous and monoecious flowers, which enable a high degree of outcrossing and pollen-mediated gene flow (Beckie et al. 2016; Mengistu and Messersmith 2002; Stallings et al. 1995). *Bassia scoparia* has a high fecundity potential (>100,000 seeds plant⁻¹) and disperses its seed long distances via wind-mediated tumbling of the mature plant (Baker et al. 2010; Christoffoleti et al. 1997; Kumar and Jha 2015d). Consequently, *B. scoparia* exhibits high genetic and phenotypic diversity within and among populations (Kumar and Jha 2015b; Mengistu and Messersmith 2002; Stallings et al. 1995).

Frequent reports on herbicide-resistant (HR) *B. scoparia* pose a concern for growers in the U.S. Great Plains. Currently, *B. scoparia* populations resistant to at least four different herbicide sites of action, including photosystem 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 inhibitor (glyphosate; Group 9) have been documented (Heap 2018; Kumar et al. 2014). Recently, a *B. scoparia* population from Kansas was found to be resistant to all four of these herbicide sites of action (Varanasi et al. 2015). Dicamba-resistant (DR) *B. scoparia* was first identified from wheat (*Triticum aestivum* L.) fields in north-central Montana in 1994 (Cranston et al. 2001; Heap 2018). Since then, a total of six U.S. states and one Canadian

province have confirmed cases of DR *B. scoparia* (Crespo et al. 2014; Jha et al. 2015; Heap 2018; Kumar and Jha 2016; Preston et al. 2009; Varanasi et al. 2015; Westra 2016). These reports on DR *B. scoparia* are predominantly from croplands (wheat fields or cornfields [*Zea mays* L.]), but a few cases have also been reported in non-cropland situations (chemical fallow fields or roadsides).

In the past two decades, several studies have been conducted to elucidate the mechanism(s) of dicamba resistance in DR *B. scoparia*. One such study conducted by Cranston et al. (2001) reported no differences in dicamba uptake, translocation, and metabolism between dicamba-susceptible (DS) and DR *B. scoparia* biotypes. In a subsequent study, differences in root growth inhibition and gravitropism were observed in a few DR *B. scoparia* biotypes, with possible involvement of the auxin signaling pathway (Goss and Dyer 2003).

In a study conducted to evaluate the fitness penalty associated with the DR trait in *B. scoparia*, a reduced cumulative germination of DR versus DS *B. scoparia* lines derived from within a single field population has been reported from Montana (Kumar and Jha 2016). To remove heterozygosity, those lines were developed after three generations of recurrent group selection with the 2X rate of dicamba ($1X = 280 \text{ g ae ha}^{-1}$). Furthermore, the DR *B. scoparia* lines had reduced vegetative growth (plant height, plant width, primary branches, total leaf area, basal stem diameter, shoot dry weights) and fecundity (total seed number and 1,000-seed weight) characteristics compared with the DS line (Kumar and Jha 2016).

Variable results on seed germination characteristics of HR versus herbicide-susceptible biotypes have been reported in different weed species. For instance, seeds of a triazine-resistant birdsrape mustard (*Brassica rapa* L.) biotype had a slower germination rate (12 to 18 h later) compared with a susceptible biotype (Mapplebeck et al. 1982). In contrast, seeds of a prickly lettuce (*Lactuca serriola* L.) biotype resistant to ALS inhibitors had a faster germination rate than the susceptible biotype after 2.5 d of incubation; however, both biotypes attained the maximum cumulative germination of $\geq 98\%$ after 7 d at 20 C (Alcocer-Ruthling et al. 1992). Dyer et al. (1993) found that ALS-resistant *B. scoparia* seeds germinated faster than susceptible seeds at 4.6 C, but not at higher temperatures. Similarly, Thompson et al. (1994) observed a faster germination rate (12 to 70 h early) and cumulative germination (100 to 300 h earlier) of ALS-resistant *B. scoparia* seeds from Kansas and North Dakota, compared with ALS-susceptible seeds at 8 C. In contrast, delayed germination as an escape mechanism has been reported in *B. scoparia* populations from cornfields in Nebraska, where isoxaflutole was continuously used to control *B. scoparia* for >8 yr (Sbatella and Wilson 2010). In a recent study, Kumar and Jha (2017) also found that four out of seven glyphosate-resistant (GR) *B. scoparia* populations had a delayed germination initiation, took more time to complete 50% maximum cumulative germination (I_{50} values), and had a lower final germination under both constant and alternating temperatures, compared with the glyphosate-susceptible (GS) populations. The previous study on temperature requirements for germination of DR versus DS *B. scoparia* was conducted using a single field population from Montana (Kumar and Jha 2016); however, it is unknown whether the results are applicable to geographically distinct DR *B. scoparia* populations. An improved understanding of germination characteristics of field-evolved DR populations in the Northern and Central Great Plains is much needed to develop ecologically based weed management plans (beyond herbicide use) for mitigating the resistant weed seedbank. Therefore, the main

objective of this research was to compare the germination characteristics of selected DR and DS *B. scoparia* lines from the Northern (Montana) and Central (Kansas) Great Plains in response to constant and/or alternating temperatures.

Materials and Methods

Plant Material

Montana *Bassia scoparia* Lines

Seed collections from Montana were a part of the statewide field survey on HR *B. scoparia* conducted in 2013. Fully matured seeds were collected from 10 putative DR plants that survived a field-use rate of dicamba in a wheat or a chemical fallow field (wheat-fallow rotation) in Chouteau, Glacier, and Liberty Counties of northern Montana in the fall of 2013. The fields sampled for DR *B. scoparia* had a history (>10 yr) of dicamba use in the chemical fallow or wheat phase of the rotation. Seeds from DR plants in each field were composited into a single sample, referred to as a population. One DR population collected from Liberty County was designated as MT16 (48°74'27.64"N, 110°88'43.95"W), two DR populations from Glacier County were designated as MT52 (48°67' 81.05"N, 112°25'82.72"W) and MT59 (48°52'32.56"N, 112°20' 09.11"W), and a DR population from Chouteau County was designated as MT101 (48°04'07.02"N, 110°42'38.99"W). Similarly, two DS *B. scoparia* populations, designated as DS1 (48° 69'44.80"N, 110°83'44.74"W) and DS2 (48°64'53.50"N, 112° 49'38.78"W), were collected from two different wheat fields (within a 5-km radius of fields where DR populations were collected) in Liberty County and Glacier County, respectively, in MT.

After threshing and cleaning, *B. scoparia* seeds were separately sown on germination flats (53 by 35 by 10 cm) containing a 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 $25/20 \pm 2$ C day/night temperatures and 16/8-h day/night photoperiods supplemented with metal-halide lamps ($550 \text{ mmol m}^{-2} \text{ s}^{-1}$). To confirm dicamba resistance, about 100 *B. scoparia* plants (8- to 10-cm tall) from each population were treated with 140 (1/2X), 280 (1X), and 560 (2X) g ha^{-1} of dicamba (Clarity®, BASF, Research Triangle Park, NC) in a cabinet spray chamber. *Bassia scoparia* plants from all DR populations survived the 2X rate of dicamba at 28 d after treatment (DAT), thus confirming the resistance to dicamba (unpublished data). In contrast, *B. scoparia* plants from both DS populations failed to survive even the 1/2X rate of dicamba at 28 DAT (unpublished data).

Bassia scoparia plants from each DR population that survived the 2X rate of dicamba were transplanted into 20-L plastic pots (2 to 3 plants pot^{-1}) containing the commercial potting mix, maintained in the greenhouse, and subjected to three generations of recurrent group selection (each generation with dicamba at 560 g ha^{-1}) to remove heterozygosity, as described in Kumar and Jha (2017). Seeds of each DS population were also obtained after three generations of recurrent group selection without dicamba under restricted cross-pollination. A subsample of each DS population at each generation was grown and sprayed to confirm susceptibility to the 1X rate of dicamba. This procedure enabled the development of nearly homogenous lines from individual DR and DS populations, referred to as DR and DS selected lines,

respectively. Generally, *B. scoparia* 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). Dose–response studies indicated that the DR *B. scoparia* lines MT16, MT52, MT59, and MT101 had 4.8-, 4.3-, 7.1-, and 3.3-fold resistance to dicamba, respectively, relative to the DS1 and DS2 lines (P. Jha, C. A. Lim, V. Kumar, unpublished data).

Kansas *Bassia scoparia* Lines

Seeds of a DR *B. scoparia* population were collected from the research farm at the Kansas State University Agricultural Research Center (KSU-ARC), near Hays, KS, in the fall of 2015. Fully matured seeds were obtained from 6 to 8 *B. scoparia* plants that survived 560 g ha⁻¹ of dicamba in a chemical fallow field. This sampled field was under continuous wheat–sorghum–fallow rotation for >10 yr, with frequent use (at least twice in a year) of dicamba. Seeds of the DS *B. scoparia* population were collected from pastureland with no previous history of dicamba use, within 2 km of the field where the DR population was collected. The DR and DS *B. scoparia* populations from Kansas were designated as KS-110 and KS-S, respectively.

Seeds of KS-110 and KS-S *B. scoparia* populations were separately sown on 10 by 10 cm plastic pots containing commercial potting mix (Miracle-Gro® potting mix, Miracle-Gro Lawn Products, Inc., 14111 Scottslawn Rd., Marysville, OH 43041) in a greenhouse at the KSU-ARC. To confirm dicamba resistance, approximately 50 *B. scoparia* plants (8- to 10-cm tall) from each population were treated with the 2X rate (560 g ha⁻¹) of dicamba in a cabinet spray chamber. All *B. scoparia* plants from the KS-110 population survived the 2X rate at 28 DAT, thus confirming the resistance to dicamba (unpublished data). In contrast, *B. scoparia* plants from the KS-S population had 100% mortality at 28 DAT with the 1X rate of dicamba (unpublished data). The KS-110 plants that survived the 2X rate of dicamba were grown under pollen isolation conditions to obtain progeny seeds that were used in subsequent germination experiments. Similarly, seeds of the KS-S population were generated by group selfing over one generation under pollen isolation conditions. This procedure enabled the development of nearly homogenous lines from individual KS-110 and KS-S populations, referred to as KS-110 and KS-S selected lines, respectively. In dicamba dose–response studies, the KS-110 population had an 8.2-fold resistance to dicamba based on the shoot dry weight reduction (GR₅₀ values) response (V. Kumar, R. P. Engel, P. W. Stahlman, unpublished data).

Germination Experiments

Seeds of DR and DS selected *B. scoparia* lines from Montana and DR and DS lines from Kansas were stored in paper bags at 4 C until being used in germination experiments. All germination experiments on Montana selected lines were initiated in the fall of 2015 (2 mo after development of nearly homogenous selected lines) at the MSU-SARC. Germination characteristics of KS-110 and KS-S lines from Kansas were studied at the KSU-ARC, in the fall of 2017.

Previous studies have shown that *B. scoparia* or forage kochia [*Bassia prostrata* (L.) A. J. Scott] can germinate over a wide range of temperatures (5 to 30 C) with optimum temperatures of 20 to 25 C (Everitt et al. 1983; Kumar and Jha 2017; Romo and Kaferkamp 1987; Thompson et al. 1994; Young et al. 1981). An

optimum alternating (day/night regime) temperature for forage *B. prostrata* was 20/15 C (Young et al. 1981). Germination characteristics of selected DR and DS *B. scoparia* lines from Montana 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 14/10-h cycle. Seeds from Kansas lines were tested only under constant temperatures of 5, 10, 15, 20, 25, and 30 C. Treatments were arranged in a completely randomized design with four replications, and all experiments were repeated in time.

Fifty fully intact seeds per population were evenly placed between two layers of filter paper (Whatman® Grade 2, Sigma-Aldrich, St Louis, MO 63178) moistened with 5 ml of distilled water in a 10-cm-diameter petri dish (Sigma-Aldrich). The filter papers were kept moist during the entire study period by adding distilled water as and when needed. Light is not required for germination of *B. scoparia* seed (Everitt et al. 1983); therefore, the petri dishes were placed in the dark inside incubators (VMR International, Sheldon Manufacturing, Cornelius, OR 97113) set at either constant or alternating temperatures. *Bassia scoparia* seed with a visible, uncoiled radicle tip (length of protruding radicles ≥ seed diameter) was considered germinated (Dyer et al. 1993; Young et al. 1981). The number of germinated seeds in each petri dish was counted on a daily basis. No further germination occurred after 14 d of incubation; however, germination counts were continued until 28 d of incubation. Subsequently, non-germinated seeds were tested for viability using a 10 g L⁻¹ tetrazolium chloride solution for 24 h (Kumar and Jha 2017; Sbatella and Wilson 2010). Seed with a red-stained embryo examined under a dissecting microscope (10-fold magnification) was considered viable (Thompson et al. 1994). Seed germination (on a daily basis) was expressed as the percentage of total viable seeds for each selected *B. scoparia* line at each temperature. The percent cumulative germination over a 14-d incubation period was calculated based on the daily germination counts.

Statistical Analyses

The experimental run by treatment interaction was not significant for Montana selected lines or Kansas lines; therefore, data were pooled over experimental runs. Data on percent cumulative germination of each *B. scoparia* line were subjected to ANOVA using PROC MIXED in SAS (SAS® v. 9.3, SAS Institute, Cary, NC 27513) to test the significance of *B. scoparia* line, temperature (constant or alternating), and their interactions. The ANOVA assumptions for normality of residuals and homogeneity of variance were tested using PROC UNIVARIATE in SAS, and all data met both the assumptions. To model the percent cumulative germination pattern of each *B. scoparia* line over time, a three-parameter log-logistic model (Equation 1) was fit at different constant or alternating temperatures, using the ‘drc’ package in R software (Ritz and Streibig 2005; Ritz et al. 2015):

$$y = \{d / 1 + \exp [b(\log h - \log t_{50})]\} \quad [1]$$

where y is the percent cumulative germination over time h ; d is the maximum percent cumulative germination; t_{50} is the incubation time to reach 50% cumulative germination (h); and b denotes the slope around the inflection point “ t_{50} .” Slope parameter (b) indicates the germination rate of each selected line over time (i.e., a slope with a large negative value suggests a rapid germination of that selected *B. scoparia* line). The Akaike information criterion was used to select the nonlinear three-

Table 1. Estimated parameters *d* and *b* obtained from the three-parameter log-logistic models fit to describe the cumulative germination response of dicamba-susceptible (DS) and dicamba-resistant (DR) *Bassia scoparia* selected lines from Montana over time (h) under different constant temperatures.

Selected line ^a	Parameter ^b	Temperature ^c —C—						
		5	10	15	20	25	30	35
DS1	<i>d</i>	90.5	99.0	97.7	100.0	100.0	98.8	100.0
DS2	<i>d</i>	97.2	100.0	100.0	95.4	100.0	97.9	99.4
MT16	<i>d</i>	43.1*	43.3*	71.8*	76.3*	71.2*	86.5*	82.2*
MT52	<i>d</i>	45.1*	54.3*	61.4*	65.2*	59.8*	71.7*	74.2*
MT59	<i>d</i>	41.1*	47.1*	52.2*	56.7*	56.2*	66.6*	64.4*
MT101	<i>d</i>	47.8*	62.3*	76.7*	88.8*	90.9*	85.7*	90.4*
DS1	<i>b</i>	-1.9	-2.5	-1.7	-1.6	-0.6	-1.2	-1.8
DS2	<i>b</i>	-1.6	-1.7	-1.3	-2.0	-0.7	-1.4	-1.2
MT16	<i>b</i>	-2.7*	-1.4*	-1.7	-0.8*	-0.9	-1.3	-1.1*
MT52	<i>b</i>	-3.4*	-1.2*	-1.8	-0.8*	-0.9	-1.0	-1.1*
MT59	<i>b</i>	-2.1	-1.0*	-1.4	-1.1*	-0.6	-0.9	-1.2*
MT101	<i>b</i>	-1.7	-0.6*	-1.1*	-0.7*	-0.7	-0.9	-1.1*

^aDS1 and DS2 are DS *B. scoparia* lines from Liberty County, MT; whereas, MT16 is a DR *B. scoparia* line from Liberty County, MT; MT52 and MT59 are DR *B. scoparia* lines from Glacier County, MT; and MT101 is a DR *B. scoparia* line from Chouteau County, MT.

^b*d* is the maximum cumulative germination; *b* is the slope parameter, indicating the germination rate of a selected line over time.

^cAn asterisk (*) denotes a significant difference for the regression parameter (*d* or *b*) between a DR selected line and DS1 or DS2 selected line within a temperature treatment, according to approximate *t*-test (Ritz et al. 2015).

parameter model (Ritz and Spiess 2008). A lack-of-fit test ($P > 0.05$) further indicated that the selected nonlinear regression model (Equation 1) had acceptably described the percent cumulative germination data for each *B. scoparia* line (Ritz and Streibig 2005). Parameter estimates, SEs, and t_{10} , t_{50} , and t_{90} values (incubation time required for 10%, 50%, and 90% of the maximum cumulative seed germination [parameter *d*] to occur) of DS and DR selected *B. scoparia* lines at each temperature treatment were determined using the 'drc' package in R software (Ritz and Streibig 2005). Parameter estimates of DS and DR selected lines at each temperature were compared with the approximate *t*-test using the *compParm* statement in the 'drc' package (Ritz et al. 2015).

Results and Discussion

Seeds from DR and DS *B. scoparia* selected lines from Montana were >98% viable across all constant and alternating temperatures tested (unpublished data). Lack-of-fit tests indicated that the three-parameter log-logistic (Equation 1) model adequately described ($P > 0.05$) the percent cumulative germination data for each *B. scoparia* line and temperature tested. The germination characteristics (parameter estimates *d*, *b*, t_{10} , and t_{50}) of DR selected lines were different from the DS1 or DS2 selected lines from Montana under both constant and alternating temperatures (Tables 1–4).

The maximum cumulative germination (*d* parameter) of all DR selected lines from Montana (MT16, MT52, MT59, MT101) was lower than the DS1 or DS2 selected lines across all constant or alternating temperatures (Tables 1 and 2). At low constant temperatures of 5 to 15 C, the maximum cumulative germination of DR selected lines ranged from 41% to 77% compared with 90% to 100% for the DS selected lines. Except for the MT101 line, the maximum germination of DR selected lines did not exceed 77% at constant temperatures of 20 and 25 C, which are considered

optimum for germination of *B. scoparia* (Jami Al-Ahmadi and Kafi 2007). In general, the maximum cumulative germination of DR lines (except MT101) was higher at high temperatures of 30

Table 2. Estimated parameters *d* and *b* obtained from the three parameter log-logistic models that were fit to describe the cumulative germination response of dicamba-susceptible (DS) and dicamba-resistant (DR) *Bassia scoparia* selected lines from Montana over time (h) under different alternating temperatures.

Selected line ^a	Parameter ^b	Temperature ^c —C—					
		5/10	10/15	15/20	20/25	25/30	30/35
DS1	<i>d</i>	95.9	100.0	99.3	96.4	98.7	100.0
DS2	<i>d</i>	100.0	94.5	100.0	97.6	95.7	95.0
MT16	<i>d</i>	23.0*	44.8*	68.3*	78.4*	89.8*	90.7*
MT52	<i>d</i>	44.6*	59.7*	61.0*	69.7*	75.5*	78.9*
MT59	<i>d</i>	32.3*	35.1*	59.2*	63.0*	88.4*	90.3*
MT101	<i>d</i>	53.7*	70.2*	75.6*	78.3*	83.3*	84.5*
DS1	<i>b</i>	-1.5	-1.2	-1.5	-1.5	-2.9	-3.9
DS2	<i>b</i>	-1.1	-1.3	-1.6	-1.2	-2.1	-1.6
MT16	<i>b</i>	-1.7	-1.5	-1.2	-1.2	-1.6	-1.6*
MT52	<i>b</i>	-1.0	-0.9	-1.2	-1.4	-1.6	-1.6*
MT59	<i>b</i>	-1.3	-1.6	-1.3	-1.3	-1.5	-1.2*
MT101	<i>b</i>	-1.8	-1.2	-1.4	-1.5	-1.3	-1.5*

^aDS1 and DS2 are DS *B. scoparia* lines from Liberty County, MT; whereas, MT16 is a DR *B. scoparia* line from Liberty County, MT; MT52 and MT59 are DR *B. scoparia* lines from Glacier County, MT; and MT101 is a DR *B. scoparia* line from Chouteau County, MT.

^b*d* is the maximum cumulative germination; *b* is the slope parameter, indicating the germination rate of a selected line over time.

^cAn asterisk (*) denotes a significant difference for the regression parameter (*d* or *b*) between a DR selected line and DS1 or DS2 selected line within a temperature treatment, according to approximate *t*-test (Ritz et al. 2015).

Table 3. Estimated parameters t_{10} and t_{50} obtained from the three-parameter log-logistic models fit to describe the cumulative germination response of dicamba-susceptible (DS) and dicamba-resistant (DR) *Bassia scoparia* selected lines from Montana over time (h) under different constant temperatures.

Selected line ^a	Parameter ^b	Temperature ^c —C—						
		5	10	15	20	25	30	35
DS1	t_{10}	21*	8	8	5	2	5	8
DS2	t_{10}	18	5	9	6	3	3	3
MT16	t_{10}	70*	34*	26*	14*	8	6	7
MT52	t_{10}	61*	23*	30*	11*	5	7	8
MT59	t_{10}	50*	23*	22*	14*	4	6	11
MT101	t_{10}	32*	22*	8	4	2	2	6
DS1	t_{50}	65	20	30	22	7	12	29
DS2	t_{50}	69	17	42	18	13	14	18
MT16	t_{50}	154*	122*	95*	91*	69*	67*	50*
MT52	t_{50}	115*	128*	100*	133*	46*	56*	52*
MT59	t_{50}	142*	176*	64*	157*	150*	64*	69*
MT101	t_{50}	112*	239*	57*	65*	23*	21	40*

^aDS1 and DS2 are DS *B. scoparia* lines from Liberty County, MT; whereas MT16 is a DR *B. scoparia* line from Liberty County, MT; MT52 and MT59 are DR *B. scoparia* lines from Glacier County, MT; and MT101 is a DR *B. scoparia* line from Chouteau County, MT.

^b t_{10} and t_{50} refer to the incubation time (h) required to reach 10% and 50% of cumulative germination by a selected line, respectively.

^cAn asterisk (*) denotes a significant difference for the regression parameter (t_{10} or t_{50}) between a DR selected line and DS1 or DS2 selected line within a temperature treatment, according to approximate *t*-test (Ritz et al. 2015).

and 35 C (64% to 90%) compared with 5 to 25 C (41% to 76%), indicating a high thermal requirement for germination of DR versus DS *B. scoparia* seeds. Similarly, at alternating temperatures of 5/10 and 10/15 C, the maximum cumulative germination of DR lines, namely, MT16, MT52, and MT59, did not exceed 60% compared with 94% to 100% germination of DS selected lines. The maximum germination of any of the DR selected lines did not exceed 79% at 15/20 and 20/25 C, while it was as much as 91% at the high alternating temperature of 30/35 C. In contrast, the maximum cumulative germination of DS selected lines was almost similar (90% to 100%) across all constant or alternating temperatures tested.

The germination rate (*b* parameter) of all DR selected lines, namely, MT16, MT52, MT59, and MT101, was different from either the DS1 or DS2 selected line, at least at constant temperatures of 10, 20, or 35 C (Table 1). The lower negative values of the *b* parameter for DR compared with DS selected lines at those temperatures indicated a lower germination rate (slower in dormancy release) of DR versus DS *B. scoparia*. There were no differences in the germination rates between DR and DS selected lines under alternating temperatures, except at 30/35 C (Table 2). The lower negative values of the *b* parameter for all DR versus DS1 selected lines at 30/35 C indicated that the DR selected lines had a slower germination rate than the DS1 line at that high alternating temperature.

Two other biological parameters, that is, the time (h) to reach 10% and 50% maximum cumulative germination (t_{10} and t_{50} values, respectively), were also estimated from the fitted models for the selected *B. scoparia* lines from Montana. Compared with DS1 and DS2 selected lines, all four DR selected lines exhibited

Table 4. Estimated parameters t_{10} and t_{50} obtained from the three-parameter log-logistic models that were fit to describe the cumulative germination response of dicamba-susceptible (DS) and dicamba-resistant (DR) *Bassia scoparia* selected lines from Montana over time (h) under different alternating temperatures.^{a,b}

Selected line ^a	Parameter ^b	Temperature ^c —C—						
		5/10	10/15	15/20	20/25	25/30	30/35	
DS1	t_{10}	14	7	6	4	9	11	
DS2	t_{10}	7	11	9	2	4	4	
MT16	t_{10}	47*	18	10	9*	12*	11*	
MT52	t_{10}	43*	12	11	13*	10	10	
MT59	t_{10}	19	18	14	13*	17*	17*	
MT101	t_{10}	32*	14	13	14*	3	4	
DS1	t_{50}	60	45	26	17	19	20	
DS2	t_{50}	59	55	36	14	10	16	
MT16	t_{50}	132*	75*	60*	19	44*	41*	
MT52	t_{50}	260*	148*	60*	58*	40*	36*	
MT59	t_{50}	101*	109*	95*	62*	72*	101*	
MT101	t_{50}	107*	77*	62*	57*	16	17	

^aDS1 and DS2 are DS *B. scoparia* lines from Liberty County, MT; whereas, MT16 is a DR *B. scoparia* line from Liberty County, MT; MT52 and MT59 are DR *B. scoparia* lines from Glacier County, MT; and MT101 is a DR *B. scoparia* line from Chouteau County, MT.

^b t_{10} and t_{50} refers to the incubation period (h) required to reach 10% and 50% of maximum cumulative germination by a selected line, respectively.

^cAn asterisk (*) denotes a significant difference for the regression parameter (t_{10} or t_{50}) between a DR selected line and DS1 or DS2 selected line within a temperature treatment, according to approximate *t*-test (Ritz et al. 2015).

delayed germination initiation (higher t_{10} values) at low constant temperatures of 5 and 10 C (Table 3). Delayed germination of all DR versus DS lines was also observed at 15 and 20 C, except for MT101. No differences in the t_{10} values were observed among DR and DS *B. scoparia* lines at constant temperatures ≥ 25 C. At alternating temperatures of 5/10 and 20/25 C, seeds of a majority of DR selected lines had greater t_{10} values (took more time to initiate germination) compared with the DS selected *B. scoparia* lines (Table 4). However, no differences in the t_{10} values were observed among DR and DS selected *B. scoparia* lines at alternating temperatures of 10/15 and 15/20 C. In general, seeds of all DR selected lines from Montana (MT16, MT52, MT59, MT101) had significantly higher t_{50} values compared with the DS selected lines under a majority of the constant temperatures tested, further indicating that DR lines germinated more slowly than DS lines (Table 3). Similarly, the t_{50} values of DR selected lines were greater than those of DS selected lines across a majority of alternating temperatures (Table 4).

Differences in the germination characteristics (*d*, *b*, t_{10} , t_{50} , and t_{90}) between KS-110 (DR) and KS-S (DS) *B. scoparia* lines were also evident across the constant temperatures tested (Table 5). Seeds of the KS-110 line (83% to 88%) had lower maximum cumulative germination (*d* parameter) compared with the KS-S line (96% to 100%) at 5, 10, 15, 20, 25, and 30 C. In contrast to DR lines from Montana, the maximum cumulative germination (*d* parameter) of KS-110 and KS-S lines did not differ from low (5 C) to high (30 C) temperatures tested. The KS-110 line showed lower germination rate (*b* parameter) than the KS-S line, indicated by lower negative values of the *b* parameter across all constant temperatures, except at 20 C. Based on the fitted

Table 5. Influence of constant temperatures on the estimated regression parameters (d , b , t_{10} , t_{50} , and t_{90}) obtained from the three-parameter log-logistic models fit to describe the cumulative germination response of dicamba-susceptible (KS-S) and dicamba-resistant (KS-110) *Bassia scoparia* lines from Kansas over time (h) under different constant temperatures.

Temperature —C—	Population ^a	Regression parameter estimates ^b				
		d	b	t_{10}	t_{50}	t_{90}
5	KS-S	100.0	-1.9	28	142	379
	KS-110	84.0*	-1.3*	50*	155*	510*
10	KS-S	96.7	-3.5	30	88	143
	KS-110	84.0*	-2.5*	48*	113*	365*
15	KS-S	100.0	-3.0	35	82	171
	KS-110	88.8*	-1.7*	49*	126*	332*
20	KS-S	99.0	-2.3	27	69	179
	KS-110	83.7*	-2.1	44*	124*	278*
25	KS-S	100.0	-3.5	36	67	124
	KS-110	83.8*	-2.5*	35	158*	254*
30	KS-S	98.3	-4.0	41	56	77
	KS-110	79.1*	-2.1*	38	80*	226*

^aKS-S, dicamba-susceptible *B. scoparia* line from Hays, KS; KS-110, dicamba-resistant *B. scoparia* from Hays, KS.

^b d is the maximum-cumulative germination; b is the slope parameter, indicating the germination rate of a selected line over time. t_{10} is the time (h) of incubation required for 10% maximum cumulative seed germination; t_{50} is the time of incubation required for 50% maximum cumulative seed germination; t_{90} is the time of incubation required for 90% maximum cumulative germination. An asterisk (*) denotes a significant difference for the regression parameters (d , b , t_{10} , t_{50} , and t_{90}) between KS-110 and KS-S *B. scoparia* lines at each temperature treatment, according to approximate t -test (Ritz et al. 2015).

nonlinear model, t_{10} and t_{50} values (incubation time needed to achieve 10% and 50% maximum cumulative germination, respectively) for KS-S and KS-110 lines differed at the majority of constant temperatures tested. Seeds of the KS-S line took less time (about 14 to 22 fewer hours) than the KS-110 line to achieve 10% maximum cumulative germination (t_{10}) at 5, 10, 15, and 20 C. However, differences in the t_{10} values for KS-S and KS-110 lines were not evident at 25 (36 vs. 35) or 30 C (41 vs. 38). Furthermore, the KS-S line germinated 13 to 91 h sooner than the KS-110 line to achieve 50% maximum cumulative germination (t_{50} values) across 5 to 30 C temperatures. Similarly, based on estimated t_{90} values, seeds of the KS-110 line took 99 to 222 more hours to complete 90% maximum cumulative germination compared with the KS-S line across the tested temperature regimes (Table 5). It is important to note that the KS-S line might have undergone different selection processes as imposed by the management practices in the pasture field over the years that could possibly influence the germination characteristics.

These results indicate a coselection of dicamba resistance and temperature-mediated seed dormancy in these DR *B. scoparia* lines. Reduced maximum cumulative germination, lower germination rate, and slower or delayed germination behavior observed in DR compared with DS *B. scoparia*, especially at low temperatures, would all act as escape mechanisms for early-season DR *B. scoparia* cohorts and would ensure survival of those cohorts against preplanting weed control practices (burndown herbicides or tillage) or against early-spring frosts common in the U.S. Great Plains region. Similarly, differences in germination characteristics of GR and GS *B. scoparia* populations were attributed to the common selection of resistance and avoidance

(glyphosate and other preplanting treatments) mechanisms (Kumar and Jha 2017). Intensive weed management practices such as prolonged use of selective/nonselective herbicides normally favor the survival of late-emerging (delayed germination) weed cohorts, and the dormant weed cohorts dominate the weed seedbank over time (Fleet and Gill 2012; Owen et al. 2015; Sbatella and Wilson 2010). Additionally, resistance to dicamba would allow *B. scoparia* cohorts to survive subsequent in-crop herbicide applications, which would potentially increase the DR:DS ratio of the *B. scoparia* seedbank. Results from this study indicate that the DR *B. scoparia* lines will have a more gradual or prolonged emergence period than the DS *B. scoparia* lines. A slower/delayed germination of the majority of DR *B. scoparia* lines, even at temperatures >25 C, further indicates that those late-emerging seedlings will provide greater interference in late-planted crops such as soybean [*Glycine max* (L.) Merr.], sorghum [*Sorghum bicolor* (L.) Moench ssp. *bicolor*], sunflower (*Helianthus annuus* L.), or dry bean (*Phaseolus vulgaris* L.) in this region. Nonetheless, DR *B. scoparia* lines exhibiting temperature-dependent seed dormancy, especially those from Montana, will be quite difficult to manage. This potentially emphasizes the need for more aggressive, season-long weed control.

The rapid development of GR *B. scoparia* across 10 U.S. Great Plains states and 3 Canadian provinces has escalated the use of dicamba for *B. scoparia* control (Vink et al. 2012; Foster and Griffin 2018). The commercialization of DR soybean will further increase the use of dicamba for in-crop broadleaf weed control. If dicamba-use stewardship is not properly implemented, this will certainly increase the selection pressure for further development and spread of DR weeds such as *B. scoparia* in this region. For DR *B. scoparia* management, more intensive and diversified weed control programs need to be employed, especially ones that can exploit the seed dormancy in *B. scoparia*. Considering *B. scoparia*'s delayed onset of germination and seed persistence of less than 2 yr in the soil (Dille et al. 2017), it is possible that techniques such as pre-irrigation and stale seedbed may work well to stimulate emergence and deplete the *B. scoparia* seedbank in irrigated sugar beet (*Beta vulgaris* L.), corn, or dry bean/soybean grown in this region. However, if the thermal requirement is also greater, as is evident, particularly in Montana DR *B. scoparia* lines, a stale-seedbed approach may not be effective, as it would not be economically feasible to delay the planting date further to stimulate enough *B. scoparia* seeds to germinate. In that scenario, diversified crop rotations with the inclusion of winter wheat, fall-sown cover crops, or early-spring planted crops such as wheat or barley (*Hordeum vulgare* L.) should enhance crop competitiveness against late-emerging DR *B. scoparia* seedlings. Furthermore, the earlier harvest (July/August) of these crops compared with corn, soybean, or dry bean (October/November) would allow additional tools to prevent late-season additions to the weed seedbank. In dryland wheat-fallow or wheat-sorghum-fallow rotations, an approach to deplete DR *B. scoparia* seedbanks would be the use of a strategic tillage operation (once in 3 yr) with a sweep plow during the summer fallow phase of the rotation (Kettler et al. 2000). With the current low grain commodity prices and increasing cost of managing HR weeds, the use of strategic tillage in an otherwise no-till system could be an economical way of managing the DR *B. scoparia* seedbank. Therefore, growers should integrate all possible weed control tactics, including effective soil-residual PRE and POST herbicide mixtures (multiple sites of action), fall-sown cover crops, addition of fall-sown rotational crops, late-season herbicides, mowing, cutting, or

tillage, to prevent replenishment of DR *B. scoparia* seedbanks (Dille et al. 2017; Kumar et al. 2014; Kumar and Jha 2015a, 2015c, 2015d).

Results from this study will have direct implications for selecting tillage timings, planting dates, and effective crop rotations to ecologically manage the DR *B. scoparia* seedbank. Data from this research will also aid in developing resistance simulation models to predict evolution and population dynamics of DR *B. scoparia* in the field (Neve et al. 2010; Vila-Aiub et al. 2009, 2015). Future research should focus on quantifying the effect of cover crops, tillage, and crop competition on germination and emergence dynamics of DR *B. scoparia* lines in the field to develop effective, multitactic weed management programs.

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