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Short title: MHR *B. scoparia*

Multiple herbicide resistance among kochia (*Bassia scoparia*) populations in southcentral Great Plains

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Abstract

Multiple herbicide-resistant (MHR) kochia [*Bassia scoparia* (L.) A. J. Scott] is a concern for farmers in the Great Plains. A total of 82 *B. scoparia* populations were collected from western Kansas (KS), western Oklahoma (OK), and High Plains of Texas (TX) during fall of 2018 and 2019 (from the various locations), and their herbicide resistance status was evaluated. The main objectives were to (1) determine the distribution and frequency of resistance to atrazine, chlorsulfuron, dicamba, fluroxypyr, and glyphosate; and (2) characterize the resistance levels to glyphosate, dicamba, and/or fluroxypyr in selected *B. scoparia* populations. Results indicated that 33, 100, 48, 30, and 70% of the tested *B. scoparia* populations were potentially resistant (≥20% survival frequency) to atrazine, chlorsulfuron, dicamba, fluroxypyr, and glyphosate, respectively. A three-way premixture of dichlorprop/dicamba/2,4-D provided 100% control of all the tested populations. Dose-response studies further revealed that KS-9 and KS-14 *B. scoparia* populations from KS were 5- to 10-fold resistant to dicamba, 3- to 6-fold resistant to fluroxypyr, and 4- to 5-fold resistant to glyphosate as compared to the susceptible (KS-SUS) population. Similarly, OK-10 and OK-11 populations from OK were 10- to 13-fold resistant to dicamba, 3- to 4-fold resistant to fluroxypyr and glyphosate compared to the OK-SUS population. TX-1 and TX-13 *B. scoparia* populations from TX were 2- to 4-fold resistant to dicamba and TX-1 was 5-fold resistant to glyphosate compared to the TX-SUS population. These results confirm the first report of dicamba and fluroxypyr-resistant *B. scoparia* from Oklahoma, and glyphosate and dicamba-resistant *B. scoparia* from TX. These results imply that adopting effective integrated weed management strategies (chemical and non-chemical) is required to mitigate the further spread of MHR *B. scoparia* in the region.

Keywords: Multiple herbicide resistance; U.S. Great Plains

Introduction

Kochia [*Bassia scoparia* (L.) A. J. Scott] is a problematic summer annual broadleaf weed across cropland and noncropland areas in the U.S. Great Plains (Kumar et al. 2019a). *Bassia scoparia* can emerge early in the spring and exhibits an extended emergence period from mid-February through mid-June (Dille et al. 2017; Kumar et al. 2018). Small *B. scoparia* seedlings have leaves densely covered in short, fine, white hairs (Friesen et al. 2009). These leaf hairs can reduce foliar absorption of herbicides by suspending droplets above the leaf cuticle, which can reduce the effectiveness of herbicides (Friesen et al. 2009). *Bassia scoparia* is highly invasive and tolerant to various abiotic stresses (heat, cold, drought, and salinity) (Christoffoleti et al. 1997; Friesen et al. 2009). *Bassia scoparia* flowers are inconspicuous (without petals) and protogynous (stigmas emerge before anther development), facilitating cross-pollination between plants. A single *B. scoparia* plant can produce >100,000 seeds dispersed over long distances by wind via a tumbling mechanism (Friesen et al. 2009; Kumar et al. 2019a).

Bassia scoparia is a highly competitive weed in agronomic crops because of its early emergence and stress tolerance ability. It can cause yield reductions in many field crops, including corn (*Zea mays* L.), grain sorghum [*Sorghum bicolor* (L.) Moench ssp. *bicolor*], soybean [*Glycine max* (L.) Merr.], sugarbeet (*Beta vulgaris* L.), sunflower (*Helianthus annuus* L.), alfalfa (*Medicago sativa* L.), canola (*Brassica napus* L.), and spring cereals (Geddes and Sharpe 2022; Kumar and Jha 2015; Lewis and Gulden 2014; Wicks et al. 1994, 1997). The amount of yield loss depends on the *B. scoparia* density. For instance, *B. scoparia* density of 184 plants m^{-2} caused a 95% yield reduction in grain sorghum (Wicks et al. 1994). Similarly, yield losses of 23 to 77% in soybean at 20 to 135 plants m^{-2} (Geddes and Sharpe 2022; Wicks et al. 1997), 60% in sugarbeet at 268 plants m^{-2} (Kumar and Jha 2015), and 62 to 95% in sunflower at 34 to 905 plants m^{-2} (Lewis and Gulden 2014) have been reported.

Repeated use of herbicides with the same site of action (SOA) has resulted in the evolution of herbicide-resistant *B. scoparia* (Kumar et al. 2019a; Sharpe et al. 2023; Westra et al. 2019). Currently, *B. scoparia* populations have evolved resistance to five different SOAs globally, including inhibitors of acetolactate synthase (ALS) (Group 2), photosystem (PS) II (Group 5), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Group 9), protoporphyrinogen oxidase (PPO) (Group 14), and synthetic auxins (Group 4) (Heap 2024). Since the first discovery of glyphosate-resistant (GR) *B. scoparia* in western Kansas in 2007 (Godar et al. 2015; Wiersma et al. 2015), GR populations have been reported from 10 states in the U.S. and 4 provinces across the Canadian prairies (Beckie et al. 2013; Godar et al. 2015; Kumar et al. 2019a; Sharpe et al. 2023; Westra et al. 2019). Multiple herbicide resistance to ALS inhibitors, glyphosate, dicamba, or fluroxypyr has been reported from the northern and central Great Plains, including Kansas, Colorado, Montana, as well as the provinces of Alberta, Manitoba, and Saskatchewan in Canada (Beckie et al. 2019; Geddes et al. 2022a; Heap 2024; Kumar et al. 2015; Westra et al. 2019). Furthermore, a single *B. scoparia* population with multiple resistance to chlorsulfuron (Group 2), dicamba (Group 4), atrazine (Group 5), and glyphosate (Group 9) has previously been reported from Kansas (Heap 2024). The status of distribution and frequency of MHR *B. scoparia* in the southcentral Great Plains (SGP), especially Oklahoma and Texas, is unknown. Therefore, the objectives of this study were to (1) determine the frequency and distribution of resistance to atrazine, chlorsulfuron, dicamba, fluroxypyr, and glyphosate among *B. scoparia* populations from KS, OK, and TX, and (2) characterize the level of resistance to glyphosate, dicamba, and/or fluroxypyr in selected populations from KS, OK, and TX.

Materials and Methods

Seed Source. Mature seeds from a total of 82 different *B. scoparia* populations were randomly collected during the fall of 2018 and 2019 from pasture, wheat stubble, fallow, corn, cotton, and grain sorghum production fields in western Kansas (n=19), western Oklahoma (n=13), and the Texas High Plains (n=50) (Supplementary Table 1). At each site, branches with seeds from *B. scoparia* were collected from 15 to 20 different plants and bulked. All the collection sites for these populations were geo-referenced. Seeds were manually threshed from each population and cleaned with a combination of sieves and an air column blower and stored in plastic bags at 4 C prior to conducting greenhouse experiments.

Single-Dose Experiment

Experiments were conducted in a greenhouse at Kansas State University Agricultural Research Center (KSU-ARC) near Hays, KS during the fall of 2021 and repeated in spring of 2022. Seeds from each *B. scoparia* population were separately sown (10 seeds per cell) in 50-cell plastic trays (54- by 28- by 10-cm) containing commercial potting mixture (Miracle-Gro® Moisture Control® Potting Mix, Miracle-Gro Lawn Products, Scottslawn Road, Marysville, OH). Greenhouse

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conditions were maintained at 25/23 ± 3 C day/night temperature and a 16/8 h (day/night) photoperiod supplemented with metal-halide lamps (560 μ mol m^{-2} s^{-1}). *Bassia scoparia* seedlings (2- to 3-cm tall) from each population were transplanted in 50-cell plastic trays (54- by 28- by 10-cm) (one seedling per cell) containing the same potting mixture as mentioned above. Seedlings were watered daily to maintain sufficient moisture for growth. Experiments were arranged in a completely randomized design with 50 replications of individual plants, where each tray with 50 seedlings from each population was separately treated with either glyphosate (Roundup PowerMax, Bayer Crop Science, St. Louis, MO) at 1260 g ha^{-1} plus 2% w/v ammonium sulfate (AMS), chlorsulfuron (Glean XP, FMC Corporation, Philadelphia, PA) at 26 g ha^{-1} with 0.25% v/v nonionic surfactant (NIS), atrazine (AAtrex 4L, Syngenta Crop Protection, Greensboro, NC) at 1120 g ha^{-1} with 1% v/v crop oil concentrate (COC), dicamba (Clarity, BASF Corporation, Research Triangle Park, NC) at 560 g ha^{-1} with 0.25% v/v NIS, fluroxypyr (Starane Ultra, Corteva Agriscience, Indianapolis, IN) at 228 g ha^{-1}, and a premixture of dichlorprop/dicamba/2,4-D (Scorch EXT, Nufarm Inc., Alsip, IL) at 374/186/186 g ha^{-1} with 0.25% v/v NIS. A tray of seedlings from each population was treated when plants were 6- to 9-cm tall. All herbicides were applied using a stationary cabinet spray chamber (Research Track Sprayer, De Vries Manufacturing, Hollandale, MN), equipped with an even flat-fan nozzle (TeeJet 8001EXR, Spraying System, Wheaton, IL) calibrated to deliver 132 L ha^{-1} of spray solution at 241 kPa. At 28 days after treatment (DAT), the survival frequency (number of alive seedlings/total number of seedlings treated) was determined for each population for each herbicide. A treated plant was considered dead if the plant showed chlorosis, necrosis, epinasty, stem curling/swelling, and no new regrowth at 28 DAT. We used previously described categories by Owen et al. (2007) and Westra et al. (2019) to classify tested *B. scoparia* populations as either susceptible (<2% survival), having low resistance (2% to 19% survival), or resistant (≥20% survival) to the respective herbicide applied.

Dose-Response Experiments

Based on the results from the single-dose experiment, two putative MHR *B. scoparia* populations (resistant to dicamba, fluroxypyr, and glyphosate) from Kansas (KS-9 and KS-14) and two from Oklahoma (OK-10 and OK-11) were selected for further characterization (Table 1). Seeds of the MHR *B. scoparia* populations (with the highest survival frequency to glyphosate

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and dicamba) from Texas showed poor seed germination; thus, TX-1 (resistant to dicamba and glyphosate) and TX-13 (resistant to glyphosate only) populations were selected for dose-response assays. Similarly, SUS populations (susceptible to glyphosate, dicamba, and fluroxypyr) from Kansas (KS-SUS), Oklahoma (OK-SUS), and Texas (TX-SUS) were also selected (Supplementary Table 1). Greenhouse experiments were conducted as mentioned above, at KSU-ARC near Hays, KS in the fall of 2023 and repeated in the spring of 2024. Field-collected seeds for MHR and SUS *B. scoparia* populations from each respective state were separately sown in 50-cell plastic trays (54- by 28- by 10-cm) containing the same potting mixture as mentioned above. *Bassia scoparia* seedlings (2- to 3-cm tall) from each population were transplanted in 10- by 10-cm square plastic pots (1 seedling per pot) containing the same potting mixture as mentioned above. *Bassia scoparia* seedlings were watered daily to maintain optimum growth. Experiments were conducted in a randomized complete block design with 12 replications (one plant per pot indicates one replication). Dicamba, fluroxypyr, and glyphosate dose-response experiments were conducted for Kansas and Oklahoma populations and only dicamba and glyphosate dose-response experiments were conducted for Texas populations. Dicamba doses were 0, 140, 280, 560 (field-use rate in fallow), 1120, 2240, 4480, 8960, and 17,920 g ae ha^{-1}. The tested doses for fluroxypyr were 0, 57, 114, 228 (field-use rate in wheat), 456, 912, 1824, and 3648 g ae ha^{-1}, and glyphosate doses were 0, 315, 630, 1260 (field-use rate in fallow), 2520, 5040, 10,080, and 20,160 g ae ha^{-1}. The NIS at 0.25% v/v with dicamba and AMS at 2% w/v with glyphosate were used. All selected herbicide doses were applied to 6- to 9-cm tall *B. scoparia* for each population using a stationary cabinet spray chamber as described above. Percent visual control ratings were recorded at 7, 14, and 28 DAT based on injury symptoms corresponding to each herbicide such as chlorosis, epinasty (curling, twisting, and cupping), and necrosis of *B. scoparia* seedlings on a scale of 0% (no control) to 100% (complete control). At 28 DAT, each plant was clipped at the soil surface, placed in a paper bag, and dried at 65 C for 4 days to obtain shoot dry biomass. Dry biomass data from each replication were converted to percent reduction of biomass using Equation 1:

$$\text{Shoot dry biomass reduction (\%)} = \left[\frac{C-T}{C}\right] X\ 100 \qquad \text{Eqn. 1}$$

where C is the shoot dry biomass from the nontreated check treatment (average of 12 replications), and T is the shoot dry biomass from a treated pot.

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Statistical Analyses

The collection sites for 82 *B. scoparia* populations were mapped using QGIS (Version 3.22) to visualize the spatial distribution of resistance for each herbicide and state. Data from dose-response experiments for percent visual control and reduction in shoot dry biomass were subjected to ANOVA using PROC MIXED procedure in SAS 9.3 (SAS Institute, Inc., SAS Campus Drive, Cary, NC). The fixed effects were experimental run, herbicide dose, populations, and their interactions. Replications and all interactions involving replication were considered random effects. The data followed all the ANOVA assumptions as tested by PROC UNIVARIATE in SAS. The experimental run-by-herbicide interaction for each population within the state was non-significant (P value >0.05), therefore, data were pooled across experimental runs for each tested population within a state. Data on percent visual control and reduction of shoot dry biomass for each *B. scoparia* population were regressed over herbicide doses using a three-parameter log-logistic model in R software (Ritz et al. 2015).

$$Y = \{d/1 + exp[b(log\, x - log\, e)]\} \qquad \text{Eqn. 2}$$

where Y is percent control or percent reduction in shoot dry biomass, d is maximum percent control or biomass reduction (upper asymptote, fixed to 100%), b is slope, x is herbicide dose, and e represents herbicide dose needed for 50% control or shoot dry biomass reduction (referred to as LD_{50} or GR_{50} values, respectively). All nonlinear regression parameters and LD_{90} or GR_{90} values (herbicide dose required for 90% *B. scoparia* control or shoot dry biomass reduction, respectively) were estimated using the 'drc' package in *R* software (Ritz et al. 2015). The resistance index for each MHR population was calculated by dividing the LD_{50} or GR_{50} value by the LD_{50} or GR_{50} value of the respective SUS *B. scoparia* population for that state.

Results and Discussion

Single-Dose Experiment. The resistance frequency for atrazine ranged from 0 to 100% with 33% of *B. scoparia* populations classified as resistant, 51% as low resistant, and 16% as susceptible (Figure 1 and Table 2). All tested populations were classified as resistant to chlorsulfuron with a survival frequency ranging from 30 to 100%. The proportion of dicamba resistance among tested *B. scoparia* populations was as follows: 48% classified as resistant, 28% classified as low resistant, and 24% susceptible with a survival frequency ranging from 0 to 96% (Figure 1 and Table 2). For fluroxypyr, 30% of the total tested populations were classified as

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resistant, 40% as low resistant, and 30% as susceptible, with survival frequency ranging from 0 to 100% (Figure 1 and Table 2). Fluroxypyr resistance in *B. scoparia* populations has been reported in several states of the United States (Colorado, Kansas, Montana, North Dakota, and Nebraska) and in provinces of Alberta and Saskatchewan in Canada (Geddes et al. 2021; Heap 2024; Howatt and Ciernia 2014; Kumar et al. 2019; LeClere et al. 2018; Sharpe et al. 2023; Todd et al. 2024). Geddes et al. (2021) reported fluroxypyr resistance in 13% of tested populations from Alberta, Canada. Percent survival or resistance frequency to glyphosate in all tested *B. scoparia* populations ranged from 0 to 97%, with 70% of populations (57 out of 82 total) classified as resistant to glyphosate, 8% classified as low resistant, and 22% as susceptible based on the classification criterion described by Owen et al. (2007) and Westra et al. (2019) (Figure 1 and Table 2). The premixture of dichlorprop/dicamba/2,4-D provided complete control of all the populations. These results are consistent with Dhanda et al. (2023), who previously reported effective control (84 to 90% control) of MHR *B. scoparia* with dichlorprop-p/dicamba/2,4-D mixture.

Considering both 'resistant' and 'low-resistant' categories, 84% of the total tested populations were found to be resistant to atrazine, 100% to chlorsulfuron, 76% to dicamba, 70% to fluroxypyr, and 78% to glyphosate (Table 2). These results indicate a widespread distribution of resistance in *B. scoparia* populations to atrazine, chlorsulfuron, dicamba, fluroxypyr, and glyphosate in the SGP region. About 12% of the total tested populations were found to be three-way resistant (≥20% survival) to atrazine, chlorsulfuron, and glyphosate while 15% of the populations were four-way resistant to atrazine, chlorsulfuron, dicamba, and glyphosate (Figure 2). Only two *B. scoparia* populations from TX were found five-way resistant to atrazine, chlorsulfuron, dicamba, fluroxypyr, and glyphosate (Figure 2). Three-to four-way resistance to atrazine, chlorsulfuron, glyphosate, and/or dicamba/fluroxypyr in *B. scoparia* populations from Kansas has also been reported previously (Kumar et al. 2019a; Varanasi et al. 2015).

These results are consistent with Beckie et al. (2019), who reported ALS-inhibitor resistance in all the surveyed *B. scoparia* populations from Alberta, Canada. They also found that 50% of the tested populations were resistant to glyphosate, 18% to dicamba, and 10% were MHR to ALS inhibitors, dicamba, and glyphosate. Geddes et al. (2022a) documented that 41% of tested *B. scoparia* populations from Alberta, Canada were resistant to both dicamba and fluroxypyr. Geddes et al. (2022b) also reported resistance to glyphosate, fluroxypyr, and dicamba in 78%, 44%, and 28% of the tested *B. scoparia* populations, respectively. Results from a field survey conducted in Montana from 2013 to 2016 indicated that 45 field sites had glyphosate-resistant, 15 sites had dicamba-resistant, and 10 sites had both glyphosate- and dicamba-resistant *B. scoparia* (Kumar et al. 2019a). Westra et al. (2019) reported glyphosate and dicamba resistance in 39 to 60% and 28 to 45% of surveyed *B. scoparia* populations from Colorado, respectively. In that same study, multiple resistance to dicamba and glyphosate was also reported in 14 to 20% of surveyed *B. scoparia* populations (Westra et al. 2019). Results from the present study indicate an increasing rate of resistance in *B. scoparia* as 15% of the populations were found four-way resistant to atrazine, chlorsulfuron, dicamba, and glyphosate.

Dose-Response Experiments

Kansas Populations. Based on the percent control, both MHR *B. scoparia* populations (KS-9 and KS-14) were 4- to 8-fold resistant to dicamba, 2- to 4-fold resistant to fluroxypyr, and 3- to 4-fold resistant to glyphosate as compared to the SUS population (Figure 3; Table 3). Based on percent shoot dry biomass reduction, both MHR populations from KS (KS-9 and KS-14) exhibited 5- to 10-fold resistance to dicamba, 3- to 6-fold resistance to fluroxypyr, and 4- to 5-fold resistance to glyphosate as compared to the SUS population (Figure 3; Table 4). Dicamba at 7,485 g ha^{-1} and 3,489 g ha^{-1} were needed to achieve 90% control for KS-9 and KS-14 populations, respectively, compared to only 514 g ha^{-1} for the SUS population (Figure 3; Table 3). Similarly, about 5 to 7 times higher doses of fluroxypyr and 5 to 6 times higher doses of glyphosate were needed to obtain 90% control of both MHR populations compared to the SUS population. Consistent with percent control, dicamba at 8,677 g ha^{-1} and 3,436 g ha^{-1} were needed to achieve 90% shoot dry biomass reduction of KS-9 and KS-14 populations, respectively, compared to 544 g ha^{-1} for the SUS population (Figure 3; Table 4). Fluroxypyr at 1,787 g ha^{-1} for KS-9 and 2,757 g ha^{-1} for KS-14 were needed to achieve 90% reduction in shoot dry biomass, compared to 253 g ha^{-1} for the SUS population (Figure 3; Table 4). Similarly, glyphosate at 3,635 g ha^{-1} for KS-9 and 4,603 g ha^{-1} KS-14 was needed to achieve 90% shoot dry biomass compared to 646 g ha^{-1} of glyphosate for the SUS population (Figure 3; Table 4). These results are consistent with Kumar et al. (2019b), who previously reported 3- to 15-fold resistance to dicamba and 3- to 9-fold resistance to fluroxypyr from Kansas. Similarly, Godar et al. (2015) also reported 4- to 11-fold resistance to glyphosate in *B. scoparia* populations from Kansas.

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Oklahoma Populations. Based on percent control, the selected MHR populations (OK-10 and OK-11) exhibited 8- to 12-fold resistance to dicamba, 3- to 4-fold resistance to fluroxypyr, and 3- to 4-fold resistance to glyphosate as compared to the SUS population (Figure 4; Table 3). Consistent with percent control, the MHR populations were found 10- to 13-fold resistant to dicamba, 3- to 4-fold resistant to fluroxypyr, and 3- to 4-fold resistant to glyphosate, based on shoot dry biomass reduction as compared to the SUS population (Figure 4; Table 4). Dicamba at 5,628 g ha^{-1} and 5,963 g ha^{-1} were needed to achieve 90% control of OK-10 and OK-11, populations, respectively, compared to only 341 g ha^{-1} for the SUS population (Figure 4; Table 3). Similarly, about 6- to 7-fold higher doses of fluroxypyr and glyphosate were needed to obtain 90% control of both MHR populations from OK compared to the SUS population. Consistent with percent control, 7,527 g ha^{-1} and 9,629 g ha^{-1} of dicamba were needed to achieve 90% shoot dry biomass reduction of OK-10 and OK-11, respectively, compared to 427 g ha^{-1} for the SUS population (Figure 4; Table 4). Fluroxypyr at 1,191 g ha^{-1} and 891 g ha^{-1} were needed to achieve 90% shoot dry biomass reduction of OK-10 and OK-11, respectively, as compared to 179 g ha^{-1} for the SUS population. Similarly, 4,934 to 6,045 g ha^{-1} of glyphosate were needed to achieve 90% shoot dry biomass reduction of both MHR population as compared to 674 g ha^{-1} for the SUS population (Figure 4; Table 4). These results indicate a low-level resistance to glyphosate and fluroxypyr (up to 4-fold) and a moderate to high-level resistance to dicamba (up to 13-fold) among MHR populations from OK. To date, *B. scoparia* populations from OK have only been reported with resistance to ALS inhibitors (chlorsulfuron, metsulfuron-methyl, and sulfometuron-methyl) and to glyphosate (Heap 2024). To our knowledge, the current study revealed the first case of multiple resistance to glyphosate, dicamba, and fluroxypyr in *B. scoparia* populations from OK. Fallow-based cropping systems in western OK, such as winter wheat-fallow and winter wheat-summer crop-fallow are prevalent, and repeated applications of glyphosate and dicamba alone or in mixtures are commonly used for weed control in the fallow phase and at post-harvest time (Manuchehri et al. 2019). This might have resulted in strong selection pressure and ultimately the evolution of herbicide resistance in *B. scoparia* populations. Previous studies have reported 3- to 13-fold glyphosate resistance in *B. scoparia* populations collected from Kansas, Colorado, North Dakota, and South Dakota (Godar et al. 2015; Wiersma et al. 2015). Jha et al. (2015) reported 1.5- to 6.8-fold resistance to dicamba and 1.6- to 4.0-fold resistance to fluroxypyr in *B. scoparia* populations from Montana.

Texas Populations. Based on the percent control and shoot dry biomass reduction, two MHR populations (TX-1 and TX-13) were found 2- to 4-fold resistant to dicamba, and one population (TX-1) was 5-fold resistant to glyphosate compared to the SUS population (Figure 5; Tables 3 and 4). Dose-response studies further revealed that dicamba at 2,272 g ha^{-1} for TX-1 and 1,064 g ha^{-1} for TX-13 were required to achieve 90% control as compared to 557 g ha^{-1} of dicamba for the SUS population. Similarly, a 4-fold higher dose of glyphosate was needed to obtain 90% control of the MHR population from TX compared to the SUS population (Table 3). Consistent with percent control, dicamba at 2,542 g ha^{-1} for TX-1 and 1,838 g ha^{-1} for TX-13 were needed to achieve 90% shoot dry biomass reduction as compared to 604 g ha^{-1} of dicamba for the SUS population (Table 4). For glyphosate, 1,655 g ha^{-1} was required to achieve 90% shoot dry biomass reduction of TX-1 population, compared to the 347 g ha^{-1} rate for the SUS population. These results revealed a low-level resistance to glyphosate and dicamba in selected MHR populations from TX. Previous studies also reported GR *B. scoparia* from the Great Plains, including Colorado (Wiersma et al. 2015), Kansas (Godar et al. 2015), Montana (Kumar et al. 2014), Wyoming (Gaines et al. 2016), Nebraska (Rana and Jhala 2016), North Dakota (Heap 2024), Oklahoma (Heap 2024), South Dakota (Heap 2024), as well as the Canadian provinces of Alberta, Manitoba, and Saskatchewan (Beckie et al. 2015; Heap 2024; Hall et al. 2014). LeClere et al. (2018) reported MHR *B. scoparia* populations to dicamba (8-fold) and fluroxypyr (13-fold) from western Nebraska. Westra et al. (2019) reported multiple resistance to dicamba and glyphosate in 14 to 20% of surveyed *B. scoparia* populations from Colorado. Glyphosate resistance has been reported from ten US states including Colorado, Idaho, Kansas, Montana, Nebraska, North Dakota, Oklahoma, Oregon, South Dakota, and Wyoming whereas dicamba resistance has been reported from six US states, including Colorado, Idaho, Kansas, Montana, Nebraska, and North Dakota (Heap 2024). To date, herbicide resistance in *B. scoparia* populations from TX has been reported to only metsulfuron-methyl (Heap 2024). The present study confirms the first report of multiple resistance to glyphosate and dicamba in *B. scoparia* populations from the High Plains of TX.

Results from this survey revealed widespread occurrence of multiple resistance to atrazine, chlorsulfuron, dicamba, and glyphosate among *B. scoparia* populations from the SGP region. Resistance to fluroxypyr was also evident among one-third of the tested populations. Evolution of glyphosate and dicamba-resistant kochia across KS, OK, TX warrants significant challenge to dicamba-tolerant crops (cotton, soybeans, etc.). These results suggest that sole reliance on these herbicides for *B. scoparia* control should be avoided to prevent further evolution and spread of MHR *B. scoparia* in the region. This study also highlights the critical need for proactive stewardship and adoption of diversified weed control strategies to preserve the long-term effectiveness of these herbicides. Adoption of effective fall or spring applied preemergence herbicides with multiple modes-of-action in conjunction with integrated weed management approaches becomes critical to mitigate the further evolution and spread of multiple resistance among *B. scoparia* populations (Kumar et al. 2019b).

In the present study, some populations showed low levels of resistance (2-3-fold); therefore, it is important to further quantify and identify the underlying mechanism(s) of resistance. However, several previous studies have shown that higher *EPSPS* gene copy number conferred glyphosate resistance in *B. scoparia* populations (Gaines et al. 2016; Godar et al. 2015; Kumar et al. 2019b; Wiersma et al. 2015). Pettinga et al. (2017) reported that a greater synthesis of flavanols would compete with the intercellular transport of dicamba molecules, thus impairing the dicamba translocation and resulting in dicamba resistance in *B. scoparia*. LeClere et al. (2018) reported a point mutation within a highly conserved region of an AUX/IAA protein which conferred cross-resistance to dicamba, 2,4-D, and fluroxypyr in *B. scoparia*. Future studies will investigate the underlying mechanism(s) of resistance and fitness penalty in these MHR *B. scoparia* populations from the SGP region. Declining effective POST herbicide options with the widespread evolution of MHR *B. scoparia* populations warrant the development of integrated weed management strategies that may include diversified competitive crop rotations, occasional or strategic tillage, fall or spring-planted cover crops, use of effective PRE herbicides, herbicide rotations, and multiple herbicide SOA to delay the further evolution and spread of multiple herbicide resistance among *B. scoparia* populations in the region.

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Table 1. Selected putative multiple herbicide-resistant and susceptible (SUS) *Bassia scoparia* populations for dose-response experiment.

Population	Longitude	Latitude	Comments
KS-9	-101.6250	39.7570	Wheat Stubble
KS-14	-101.7200	38.4300	Wheat Stubble
KS-SUS	-99.3256	38.8537	Sorghum Stubble
OK-10	-100.8032	36.9518	Wheat Stubble
OK-11	-101.0475	36.8646	Wheat Stubble
OK-SUS	-97.6039	36.7508	Wheat Stubble
TX-1	-101.5936	35.2661	Cotton Field
TX-13	-102.4100	34.9100	Sorghum Field Edge
TX-SUS	-101.5567	35.2739	Sorghum Field Edge

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Table 2. The number and percentage (in parentheses) of a total of 82 *Bassia scoparia* populations categorized as susceptible, low-level resistant, and resistant to each tested herbicide[a]

Herbicide	Susceptible	Low resistant	Resistant
Atrazine	13 (16)	42 (51)	27 (33)
Chlorsulfuron	0 (0)	0 (0)	82 (100)
Dicamba	20 (24)	23 (28)	39 (48)
Dichlorprop/dicamba/2,4-D	82 (100)	0 (0)	0 (0)
Fluroxypyr	25 (30)	32 (40)	25 (30)
Glyphosate	18 (22)	7 (8)	57 (70)

[a]Populations were classified in three categories based on survival %; <2% = susceptible, 2% to 19% = low resistant, and 20% to 100% = resistant (Owen et al. 2007; Westra et al. 2019)

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Table 3. Regression parameter estimates (Equation 1) for percent control of multiple herbicide-resistant and susceptible (SUS) *Bassia scoparia* populations from western Kansas, western Oklahoma, and Texas High Plains to dicamba, fluroxypyr, and glyphosate under separate dose-response experiments, and calculated resistance index.

		Parameter estimates			Resistance
Herbicide	Population	b (± SE)	LD_{50} (95% CI)	LD_{90} (95% CI)	index
Dicamba	KS-9	-1.3 (0.1)	1533 (1347-1719)	7485 (6103-8868)	7.9
	KS-14	-1.5 (0.1)	874 (773-975)	3489 (2886-4093)	4.5
	KS SUS	-2.2 (0.2)	195 (178-211)	514 (446-582)	-
	OK-10	-1.4 (0.1)	1176 (1010-1341)	5628 (4324-6931)	7.6
	OK-11	-1.8 (0.1)	1867 (1647-2087)	5963 (4680-7245)	12.0
	OK-SUS	-2.7 (0.3)	155 (141-168)	341 (277-404)	-
	TX-1	-1.9 (1.1)	733 (668-797)	2272 (1985-2560)	4.3
	TX-13	-1.8 (0.1)	332 (305-359)	1064 (919-1209)	1.9
	TX-SUS	-1.8 (0.1)	172 (157-185)	557 (474-640)	-
Fluroxypyr	KS-9	-1.3 (0.1)	218 (186-250)	1183 (942-1423)	2.4
	KS-14	-1.3 (0.1)	348 (297-399)	1873 (1480-2266)	3.8
	KS-SUS	-2.2 (0.1)	91 (82-100)	247 (208-285)	-
	OK-10	-1.4 (0.1)	267 (225-307)	1168 (898-1438)	4.0
	OK-11	-1.4 (0.1)	191 (160-222)	900 (704-1096)	2.9
	OK SUS	-2.5 (0.2)	67 (60-73)	157 (129-186)	-
Glyphosate	KS-9	-2.8 (0.3)	1414 (1306-4523)	3052 (2618-3486)	3.4
	KS-14	-2.5 (0.2)	1573 (1453-1693)	3737 (3244-4231)	3.8
	KS-SUS	-4.6 (0.3)	416 (391-441)	665 (600-730)	-
	OK-10	-2.0 (0.1)	1224 (1099-1349)	3659 (3045-4274)	2.9
	OK-11	-2.0 (0.1)	1527 (1375-1678)	4527 (3747-5306)	3.6
	OK-SUS	-5.1 (0.5)	424 (394-454)	648 (579-718)	-
	TX-1	-3.9 (0.5)	756 (697-814)	1324 (1106-1541)	4.6
	TX SUS	-3.0 (0.5)	163 (110-215)	334 (264-404)	-

Abbreviations: KS-9 and KS-14 were multiple herbicide-resistant populations from Kansas; OK-10 and OK-11 were multiple herbicide-resistant populations from Oklahoma; TX-1 and TX-13 were multiple herbicide-resistant populations from Texas; SE, standard error of mean; CI, confidence interval; LD_{50}, effective dose (g ha^{-1}) required for 50% *B. scoparia* control; Resistance index is the ratio of LD_{50} of the resistant population to LD_{50} of the susceptible *B. scoparia* population from each respective state.

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Table 4. Regression parameter estimates (Equation 1) for percent shoot dry biomass reduction of multiple herbicide-resistant and susceptible (SUS) *Bassia scoparia* populations from western Kansas (KS), western Oklahoma (OK), and Texas High Plains (TX) to dicamba, fluroxypyr, and glyphosate under separate dose-response experiments and calculated resistance index.

Herbicide	Population	Parameter estimates			Resistance index
		b (± SE)	GR_{50} (95% CI)	GR_{90} (95% CI)	
Dicamba	KS-9	-1.5 (0.1)	2170 (1972-2368)	8677 (7331-10023)	10.2
	KS-14	-1.8 (0.1)	1046 (958-1135)	3436 (2916-3955)	4.9
	KS SUS	-2.3 (0.2)	213 (196-229)	544 (482-606)	-
	OK-10	-1.2 (0.1)	1387 (1167-1608)	7527 (5653-9400)	10.1
	OK-11	-12 (0.1)	1762 (1473-2051)	9629 (7216-12043)	12.8
	OK-SUS	-1.9 (0.2)	138 (119-157)	427 (327-525)	-
	TX-1	-2.1 (0.1)	921 (841-1000)	2542 (2186-2899)	4.3
	TX-13	-1.5 (0.1)	432 (390-475)	1838 (1523-2115))	2.0
	TX-SUS	-2.0 (0.1)	212 (194-229)	604 (519-690)	-
Fluroxypyr	KS-9	-1.2 (0.1)	287 (232-343)	1787 (1296-2277)	2.9
	KS-14	-1.2 (0.2)	545 (449-641)	2757 (2036-3477)	5.6
	KS-SUS	-2.3 (0.2)	98 (86-111)	253 (207-299)	-
	OK-10	-1.6 (0.1)	303 (268-345)	1191 (910-1472)	4.2
	OK-11	-1.7 (0.1)	248 (212-285)	891 (689-1093)	3.4
	OK SUS	-2.4 (0.2)	73 (66-80)	179 (142-162)	-
Glyphosate	KS-9	-2.8 (0.2)	1685 (1538-1833)	3635 (2948-4323)	3.9
	KS-14	-2.6 (0.2)	1969 (1791-2147)	4603 (3696-5509)	4.6
	KS-SUS	-5.3 (0.5)	427 (393-461)	646 (567-725)	-
	OK-10	-1.7 (0.1)	1431 (1196-1667)	4934 (3902-5966)	3.3
	OK-11	-1.9 (0.2)	1913 (1622-2204)	6045 (4803-7287)	4.4
	OK-SUS	-5.0 (0.3)	435 (388-482)	674 (594-754)	-
	TX-1	-3.1 (0.1)	822 (793-852)	1655 (1363-1947)	5.4
	TX SUS	-2.6 (0.1)	151 (105-198)	347 (264-430)	-

Abbreviations: KS-9 and KS-14 were multiple herbicide-resistant populations from Kansas; OK-10 and OK-11 were multiple herbicide-resistant populations from Oklahoma; TX-1 and TX-13 were multiple herbicide-resistant populations from Texas; SE, standard error of mean; CI, confidence interval; GR_{50}, effective dose (g ha^{-1}) required for 50% shoot dry biomass reduction; Resistance index is the ratio of GR_{50} of the resistant population to GR_{50} of the susceptible *B. scoparia* population from each respective state.

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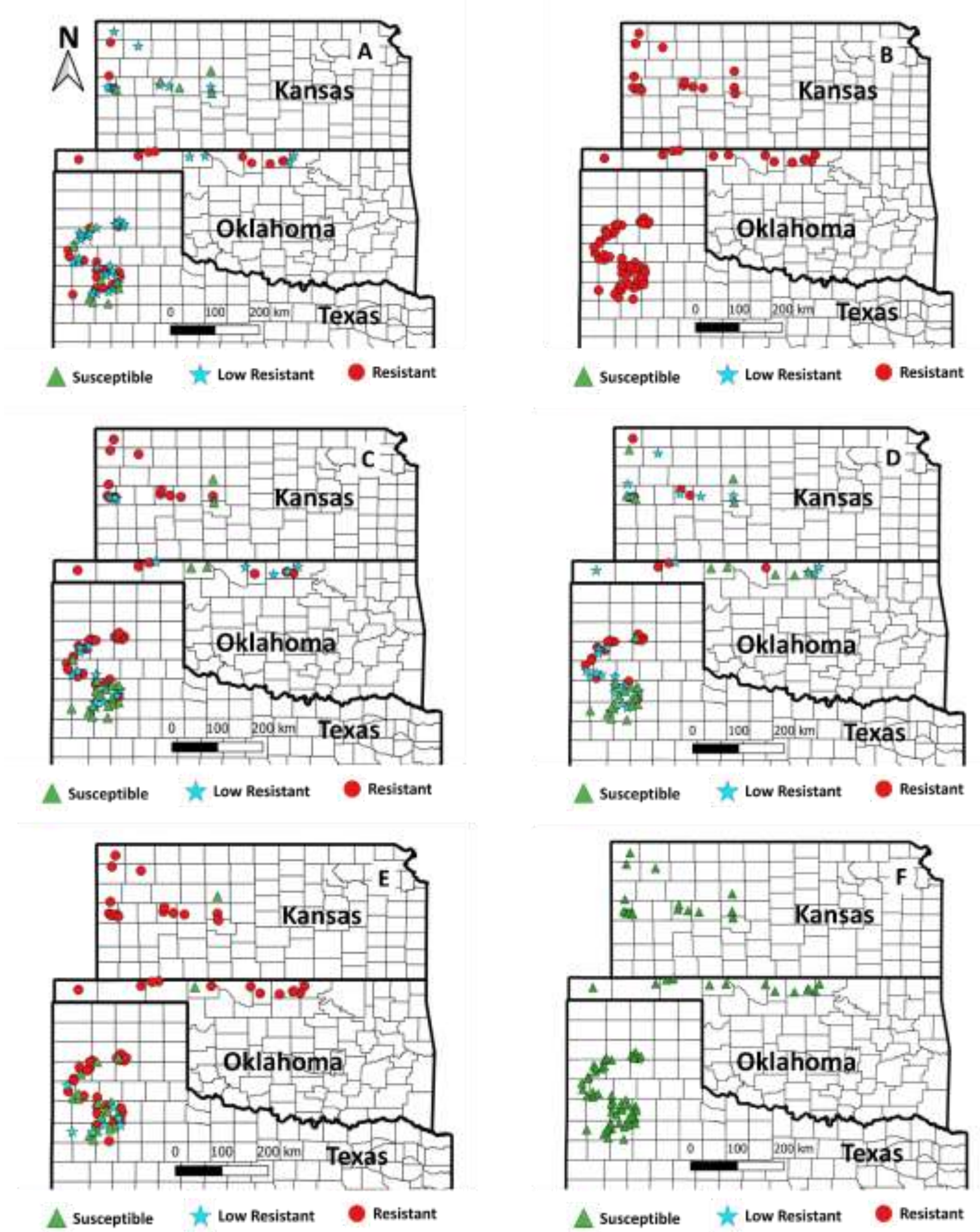

Figure 1. Distribution of *Bassia scoparia* populations resistant to atrazine (A), chlorsulfuron (B), dicamba (C), fluroxypyr (D), glyphosate (E), and dichlorprop/dicamba/2,4-D (F) in western Kansas, western Oklahoma, and Texas High Plains. Populations were classified in three categories based on survival %; <2% = susceptible, 2% to 19% = low resistant, and 20% to 100% = resistant

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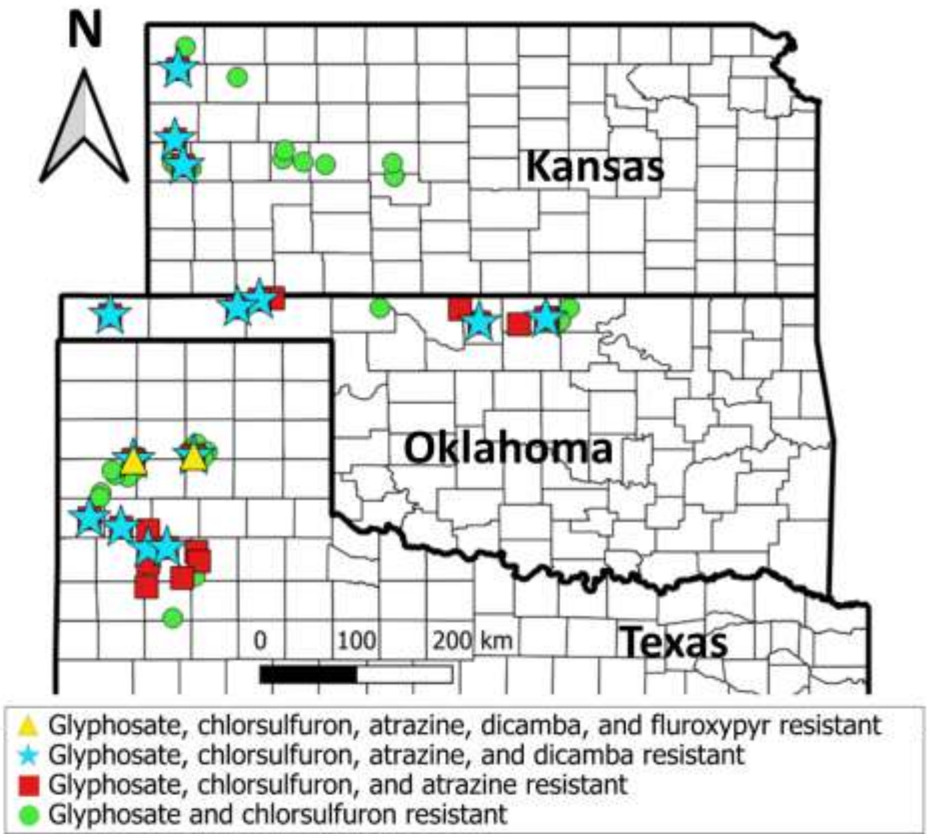

Figure 2. Distribution of multiple herbicide-resistant *Bassia scoparia* populations (out of 82 total) in western Kansas, western Oklahoma, and Texas High Plains

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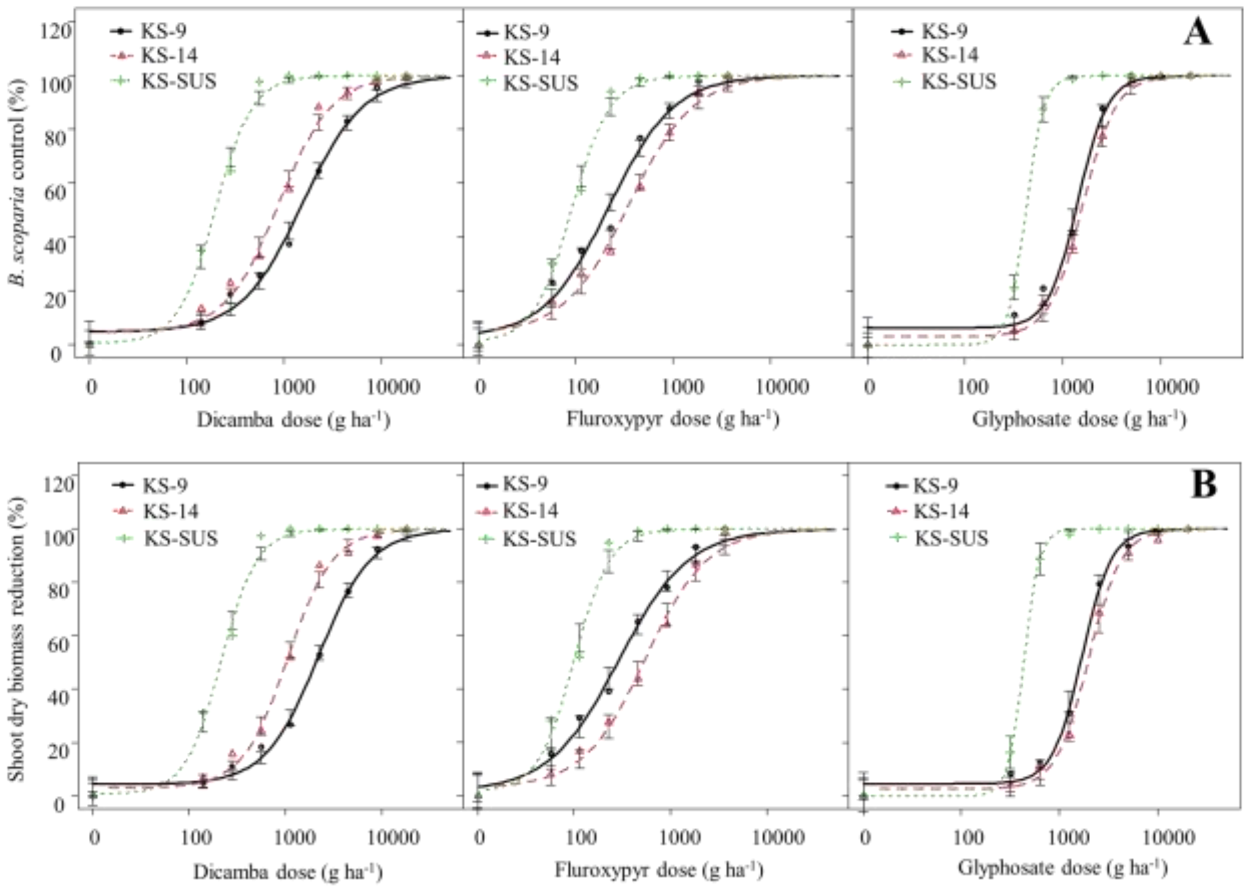

Figure 3. Percent control (A) and shoot dry biomass reduction (B) response of *Bassia scoparia* populations to various doses of dicamba, fluroxypyr, and glyphosate. KS-9 and KS-14 were multiple herbicide-resistant *B. scoparia* populations and KS-SUS was the susceptible population collected from western Kansas

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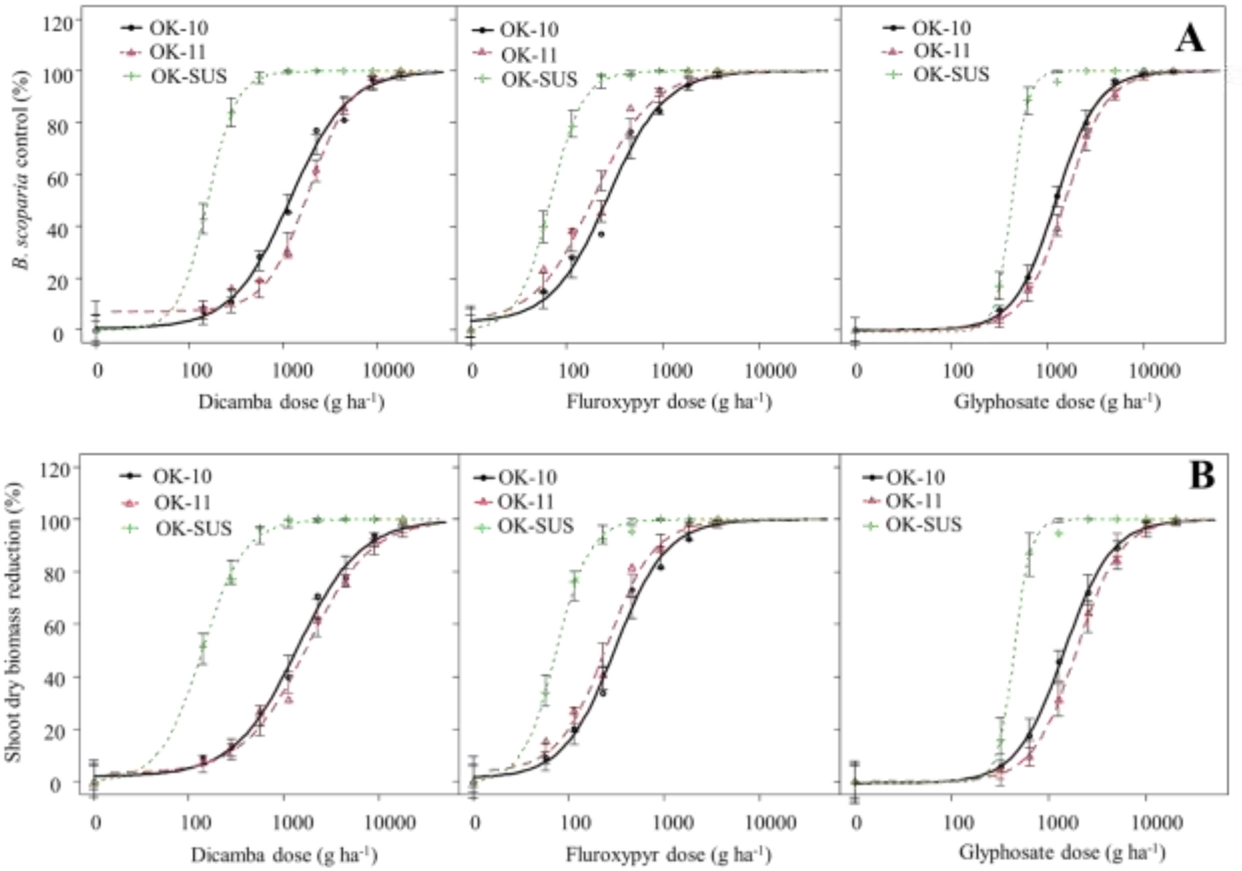

Figure 4. Percent control (A) and shoot dry biomass reduction (B) response of *Bassia scoparia* populations to various doses of dicamba, fluroxypyr, and glyphosate. OK-10 and OK-11 were multiple herbicide-resistant *B. scoparia* populations and OK-SUS was the susceptible population collected from western Oklahoma

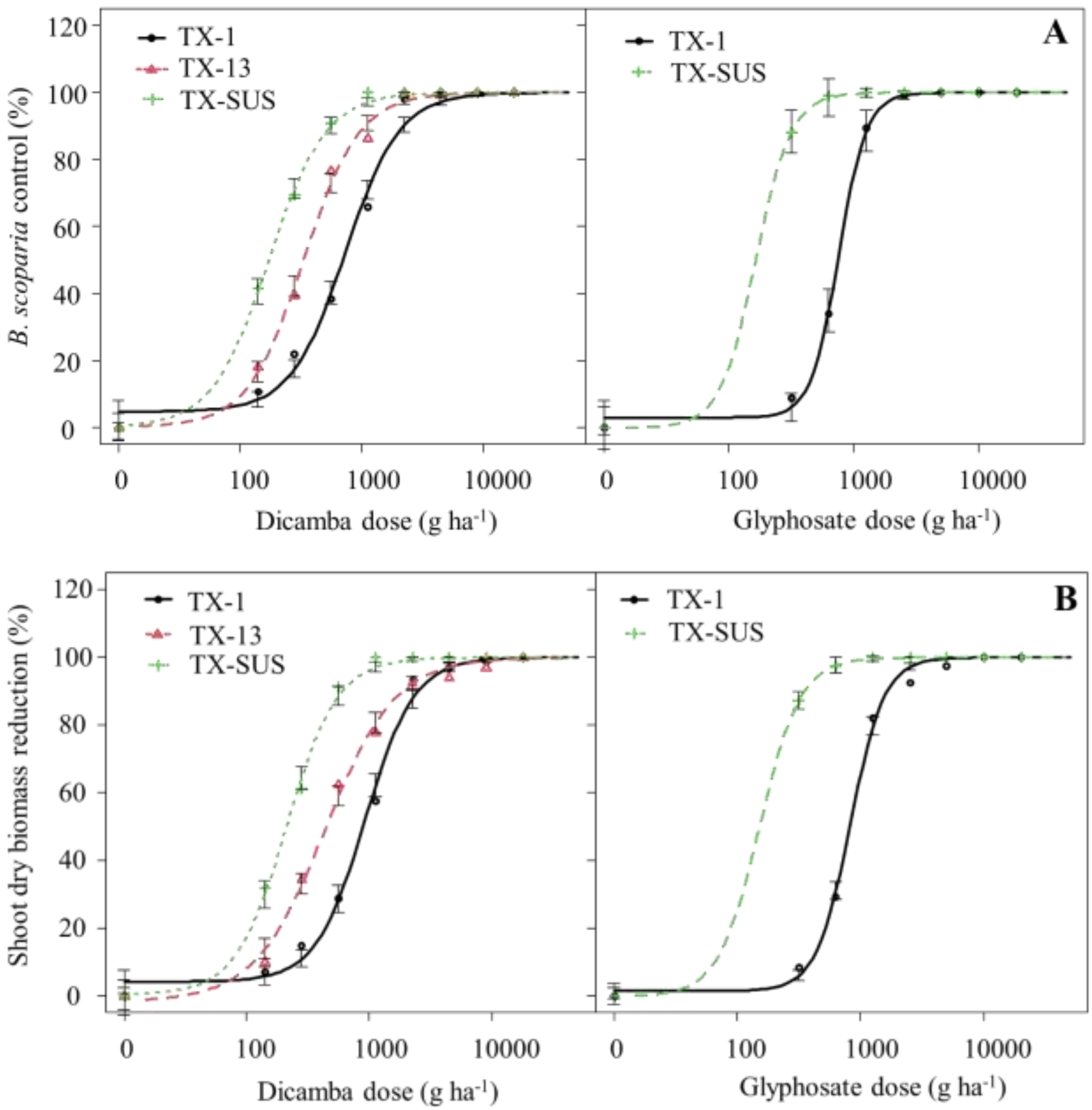

Figure 5. Percent control (A) and shoot dry biomass reduction (B) response of *Bassia scoparia* populations to various doses of dicamba and glyphosate. TX-1 and TX-13 were multiple herbicide-resistant *B. scoparia* populations and TX-SUS was the susceptible population collected from Texas High Plains