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Summary
The rapid development of glyphosate resistance in kochia has increased the use of auxinic herbicides (dicamba and fluroxypyr) in the US Great Plains, including Kansas. Increasing reliance on auxinic herbicides for controlling glyphosate-resistant (GR) kochia may also enhance the evolution of resistance to these herbicide chemistries. The main objectives of this research were to (1) investigate the variation in kochia response to dicamba and fluroxypyr, and (2) characterize the dicamba resistance levels among progeny of kochia accessions collected from western Kansas. Greenhouse experiments were conducted at the Kansas State University Agricultural Research Center near Hays, KS. Discriminate-dose studies with field-use rates of Clarity (dicamba) (16 fl oz/a) and Starane Ultra (fluroxypyr) (9.6 fl oz/a) indicated that progeny from individual kochia plants (accessions) collected near Garden City, KS, had 78 to 100% and 85 to 100% survivors when treated with dicamba and fluroxypyr herbicides, respectively, at 28 days after treatment (DAT). In separate dicamba dose-response experiments, two putative dicamba-resistant (DR) kochia accessions viz., DR-110 and DR-113 collected near Hays, KS, had about 5- and 3-fold resistance to dicamba, respectively, based on fresh weight reduction ($I_{50}$) compared to a dicamba-susceptible (DS) accession. Based on plant dry weight response, the DR-110 and DR-113 accessions showed 9- and 6-fold resistance to dicamba, respectively. These results confirm the co-evolution of cross-resistance to dicamba and fluroxypyr in kochia accessions from Garden City, and moderate to high level resistance to dicamba in the Hays accessions. Growers should adopt stewardship programs for auxinic herbicides and utilize all available weed control tactics to prevent further evolution of auxinic resistance in kochia populations.

Introduction
Kochia (Kochia scoparia L.) is a problematic summer annual broadleaf weed species that has spread across the Great Plains states, including Kansas. Kochia emergence initiates early in the spring (February - March) continuing in flushes through late spring (late May to early June), then slows with occasional plant emergence into summer months. Kochia is a fast growing weed species and produces enormous numbers of seeds (>100,000 seeds/plant) (Kumar and Jha 2015). Kochia manifests the unique way of spreading seeds to long distances, i.e. “tumbling mechanism.” Season-long infestation of kochia at higher densities is known to cause significant crop yield reductions.

The evolution of kochia populations with resistance to herbicides is a tremendous challenge for growers across western Kansas and other US Great Plains states. Currently,
kochia resistant to sulfonylurea (ALS inhibitors), atrazine (photosystem II inhibitors), dicamba (synthetic auxins), and glyphosate has been reported (Heap, 2018). GR kochia was first confirmed in crop production fields in Kansas in 2007 and is now fairly common throughout the central and northern Great Plains. With the increasing spread of GR kochia, growers are relying heavily on preemergence (PRE) and postemergence (POST) dicamba applications. This increasing reliance on dicamba applications for controlling GR kochia may also escalate the risk of widespread evolution of DR kochia. The main objective of this study was to investigate the response of selected kochia accessions from western Kansas to POST dicamba and fluroxypyr herbicides.

**Procedures**

Fully-matured seeds were collected from individual plants that survived a 16 oz/a rate of Clarity (dicamba) herbicide POST application in research plots at the Kansas State University Agricultural Research Center near Hays, KS. These were designated as accessions, i.e. DR-104, DR-107, DR-110, and DR-113. The sampled field was historically under a continuous wheat–sorghum–fallow rotation and had received frequent dicamba applications in glyphosate-based burndown treatments. In addition, seeds of a dicamba-susceptible (DS) kochia accession were collected from pasture land with no previous history of dicamba use, located approximately 2 km from the cultivated field. Similarly, seeds of individual kochia plants surviving two applications of Starane Ultra (fluroxypyr) herbicide at field-use rate (6.4 fl oz/a) were collected from two different corn fields (designated as KS-4 and KS-10) near Garden City, KS. The sampled fields were under a wheat–fallow–wheat rotation for >6 years followed by corn (for KS-4 accession) or a wheat–corn–fallow rotation (for KS-10 accession) with frequent use of dicamba and fluroxypyr herbicides. Kochia seedlings from each accession were grown in a greenhouse at the Kansas State University Agricultural Research Center near Hays, KS. Discriminate-dose experiments with Clarity (16 fl oz/a) and Starane Ultra (9.6 fl oz/a) were conducted using progeny seeds of each selected kochia accession (about 100 seedlings per herbicide). Dose-response experiments were also conducted to further characterize the response of DR-110, DR-113, and DS accessions to dicamba POST applications. Dose-response studies were conducted in a randomized complete block design, with 12 replications (1 plant/pot) and repeated twice. Doses of Clarity (dicamba) herbicide used were 0, 8, 16, 32, 48, 64, and 80 fluid oz/a. Data on visual control (on a scale of 0 to 100; 0 being no injury and 100 being dead plant) were recorded at 14 and 28 days after treatment (DAT), and individual plants were harvested to determine the fresh and shoot dry weight at 28 DAT. Data were analyzed using a three parameter log-logistic model in R software using following equation (Ritz et al., 2015):

\[ y = \frac{d}{1 + \exp \left[ b \left( \log x - \log e \right) \right]} \quad [1] \]

where \( y \) refers to the response variable (fresh or shoot dry weight), \( d \) is the upper limit, \( b \) is the slope of each curve, \( e \) is the herbicide dose required to cause 50% reductions in fresh and shoot dry weight (referred to as \( I_{50} \) or \( GR_{50} \)), and \( x \) is the herbicide dose. Nonlinear regression parameter estimates and standard errors for each accession were determined using the \textit{drc} package in R software. Resistance factor (referred as R/S ratio) to dicamba was estimated by dividing the \( I_{50} \) or \( GR_{50} \) value of each DR accession by the \( I_{50} \) or \( GR_{50} \) value of DS accession.
Results

**Discriminate-Dose Study**

Results from single-dose experiments with dicamba indicated that DR-113, DR-104, DR-107, and DR-110 kochia accessions collected near Hays, KS, had 88, 97, 98, and 100% survivors at 28 DAT, respectively (data not shown). Similarly, progeny seedlings from the two Garden City, KS, accessions had 78 to 100% and 85 to 100% survivors with discriminate doses of Clarity and Starane Ultra herbicides, respectively, at 28 DAT (Table 1, Figure 1). In contrast, seedlings of the DS accession did not survive the discriminate dose of either Clarity or Starane Ultra herbicide at 28 DAT (data not shown).

**Dicamba Dose-Response Study**

Results from dicamba dose-response experiments indicated that about 5- and 3-fold higher dicamba dose was required to obtain 50% fresh weight reduction ($I_{50}$) of DR-110 and DR-113 accessions, respectively, relative to the DS accession (Table 2; Figure 2). Furthermore, about 38 and 24 fl oz/a of Clarity (dicamba) was needed to achieve a 50% dry weight reduction ($GR_{50}$) in DR-110 and DR-113 accessions, respectively. Based on shoot dry weight response ($GR_{50}$ values), the DR-110 and DR-113 accessions showed approximately 9- and 6-fold resistance to dicamba, respectively (Table 2; Figure 2). These results are in agreement with Brachtenbach (2015), who reported at least 8-fold difference among 11 kochia populations in susceptibility to POST dicamba. Thus, both tested accessions in the current study had developed moderate to high level resistance to dicamba herbicide.

**Practical Implications**

These results confirm the evolution of kochia accessions with resistance to auxinic herbicides (dicamba and fluroxypyr) in western Kansas. The continuous and sole reliance on these herbicide chemistries for controlling kochia may further escalate the evolution and spread of auxinic herbicide resistance trait among field populations. Additionally, the rapid adoption of newly-developed dicamba-tolerant crops may further exacerbate the problem of auxinic herbicide resistance in kochia populations. Growers are advised to adopt dicamba and fluroxypyr use stewardship programs and are encouraged to utilize multiple modes of action herbicides in conjunction with other cultural and mechanical approaches to prevent evolution of auxinic-resistant kochia on their production fields. Further studies will focus on characterizing the resistance levels to fluroxypyr in those confirmed kochia accessions. In addition, the response of DR kochia to PRE applications of dicamba will also be investigated under field conditions.
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Table 1. Percent survivors from progeny seeds of individual kochia plants (accessions KS-4 and KS-10) from Garden City, KS, treated with discriminate-doses of Clarity (16 oz/a) or Starane Ultra (9.6 fl oz/a) herbicides at 28 days after treatment

| Kochia plant | % Survivors | % Survivors |
|--------------|-------------|-------------|
|              | Clarity     | Starane Ultra | Clarity | Starane Ultra |
| KS-4A        | 96          | 98          | KS-10A  | 98          | 100         |
| KS-4B        | 80          | 100         | KS-10B  | 87          | 100         |
| KS-4C        | 80          | 85          | KS-10C  | 97          | 94          |
| KS-4D        | 78          | 94          | KS-10D  | 94          | 92          |
| KS-4E        | 98          | 90          | KS-10E  | 100         | 100         |
| KS-4F        | 96          | 89          | KS-10F  | 97          | 100         |
| KS-4G        | 100         | 90          | KS-10G  | 95          | 96          |
| KS-4H        | 83          | 98          | KS-10H  | 98          | 100         |
Table 2. Regression parameter (equation 1) estimates for whole plant dose response of kochia accessions from Hays, KS, treated with Clarity (dicamba) herbicide

| Accession | Regression parameters (±SE) | 95% CI | R/S |
|-----------|----------------------------|--------|-----|
|           | d  | b   | I₅₀ or GR₅₀ |       |     |
| Based on fresh weight |     |     |       |       |     |
| DS        | 40.2 (1.1) | 1.1 (0.1) | 12 | 10–14 | -   |
| DR-110    | 39.4 (1.0) | 1.4 (0.2) | 66 | 58–74 | 5.5 |
| DR-113    | 39.9 (1.1) | 0.9 (0.1) | 38 | 32–44 | 3.1 |
| Based on dry weight |     |     |       |       |     |
| DS        | 10.2 (0.3) | 0.5 (0.01) | 4  | 2–6   | -   |
| DR-110    | 10.0 (0.3) | 1.0 (0.1) | 38 | 31–45 | 9.5 |
| DR-113    | 10.4 (0.2) | 0.8 (0.01) | 24 | 19–29 | 6.0 |

d = upper limit; b = slope of each curve.

*Abbreviations: DS, dicamba susceptible kochia accession from a disturbed area in a pasture near Hays, KS; DR-110 and DR-113, putative dicamba-resistant kochia accessions from research plots in a fallow field near Hays, KS.

*I₀₀ or GR₀₀ is effective dose (fl oz/acre) of Clarity for 50% fresh and shoot dry weight reduction, respectively; *R/S (resistance factor) is the ratio of *I₀₀ or GR₀₀ of a dicamba-resistant to *I₀₀ or GR₀₀ of the DS kochia accession.
Figure 1. Percent survivors from progeny seedlings of individual kochia plants (accession KS-4) from Garden City, KS, treated with discriminate-dose of Starane Ultra (9.6 fl oz/a) herbicide at 28 days after treatment.
Figure 2. Fresh (A) and shoot dry weight (B) response of two putative dicamba-resistant (DR) kochia accessions (DR-110 and DR-113) and one dicamba-susceptible (DS) accession in a whole plant dose–response experiment with Clarity (dicamba) herbicide.