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Response of Nebraska Kochia (*Kochia scoparia*) Accessions to Dicamba

Roberto J. Crespo, Mark L. Bernards, Gustavo M. Sbatella, Greg R. Kruger, Don J. Lee, and Robert G. Wilson*

Kochia is a troublesome weed in the western Great Plains and many accessions have evolved resistance to one or more herbicides. Dicamba-resistant soybean is being developed to provide an additional herbicide mechanism of action for POST weed control in soybean. The objective of this study was to evaluate variation in response to dicamba among kochia accessions collected from across Nebraska. Kochia plants were grown in a greenhouse and treated when they were 8 to 12 cm tall. A discriminating experiment with a single dose of 420 g ae ha^{-1} of dicamba was conducted on 67 accessions collected in Nebraska in 2010. Visual injury estimates were recorded at 21 d after treatment (DAT) and accessions were ranked from least to most susceptible. Four accessions representing two of the most and least susceptible accessions from this screening were subjected to dose-response experiments using dicamba. At 28 DAT, visible injury estimates were made and plants were harvested to determine dry weight. An 18-fold difference in dicamba dose was necessary to achieve 90% injury (I_{90}) between the least (accession 11) and most susceptible accessions. Approximately 3,500 g ha^{-1} of dicamba was required in accession 11 to reach a 50% dry weight reduction (GR_{50}). There was less than twofold variation among the three more susceptible accessions for both the I_{90} and GR_{90} parameters, suggesting that most kochia accessions will be similarly susceptible to dicamba. At 110 DAT, accession 11 had plants that survived doses of 35,840 g ha^{-1}, and produced seed at doses of 17,420 g ha^{-1}. The identification of one resistant accession among the 67 accessions screened, and the fact that dicamba doses greater than 560 g ha^{-1} were required to achieve GR_{80} for all accessions suggest that repeated use of dicamba for weed control in fields where kochia is present may quickly result in the evolution of dicamba-resistant kochia populations.

Nomenclature: Dicamba; kochia, *Kochia scoparia* (L.) Schrad. KCHSC; soybean, *Glycine max* L. Merr. GLYMX.

Key words: Dicamba-resistant kochia, dose-response, herbicide resistance, injury, risk assessment.

Kochia scoparia es una maleza problemática en el oeste de las Grandes Planicies y muchas accesiones han evolucionado resistencia a uno o más herbicidas. Se está desarrollando soya resistente a dicamba para proveer un mecanismo de acción adicional para el control de malezas POST en soya. El objetivo de este estudio fue evaluar la variación en la respuesta a dicamba entre accesiones de *K. scoparia* colectada a lo largo de Nebraska. Plantas de *K. scoparia* fueron crecidas en un invernadero y tratadas cuando tuvieron 8 a 12 cm de altura. Se realizó un experimento de discriminación con una sola dosis de 420 g ae ha^{-1} de dicamba con 67 accesiones colectadas en Nebraska en 2010. Estimaciones visuales de daño se realizaron 21 días después del tratamiento (DAT) y las accesiones fueron ordenadas de menor a mayor susceptibilidad. Cuatro accesiones representando dos de las accesiones más y menos susceptibles en la evaluación fueron sometidos a experimentos de respuesta a dosis usando dicamba. A 28 DAT, se realizaron las estimaciones visuales de daño y las plantas fueron cosechadas para determinar su peso seco. Una diferencia de 18 veces en la dosis de dicamba fue necesaria para alcanzar 90% de daño (I_{90}) entre la accesión menos susceptible (accesión 11) y las más susceptibles. Se necesitó aproximadamente 3,500 g ha^{-1} de dicamba para reducir en 50% el peso seco (GR_{50}) de la accesión 11. Hubo una variación de menos de dos veces en los valores de los parámetros I_{90} y GR_{90} entre las tres accesiones más susceptibles, lo que sugiere que la mayoría de las accesiones de *K. scoparia* serán similarmente susceptibles a dicamba. A 110 DAT, la accesión 11 tenía plantas que sobrevivieron la dosis de 35,840 g ha^{-1}, y produjeron semillas a dosis de 17,420 g ha^{-1}. La identificación de una accesión resistente entre 67 accesiones evaluadas, y el hecho de que las dosis de dicamba mayores a 560 g ha^{-1} fueron necesarias para alcanzar GR_{80} para todas las accesiones sugiere que el uso repetido de dicamba para el control de malezas en campos donde *K. scoparia* está presente podría resultar rápidamente en la evolución de poblaciones de esta maleza resistentes a dicamba.

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Kochia is a common broadleaf weed in field crops, rangeland, and waste areas (Stubbendieck et al. 2003). Although kochia is primarily problematic in the semiarid regions of the Great Plains and the western United States and Canada, it is also found in most of the eastern United States (Eberlein and Fore 1984; Forcella 1985; USDA–NRCS 2011). Kochia is highly competitive, emerges early in the season, and is drought-tolerant (Durgan et al. 1990; Pafford and Wiese 1964; Schwinghamer and Van Acker 2008). Kochia flowers are wind-pollinated, facilitating cross-pollination among plants and resulting in a high level of genetic variation both between and within kochia populations (Mengistu and Messersmith 2002). Genetic variability has facilitated the evolution of kochia populations with resistance to one or more herbicides with distinct mechanisms of action. Accessions of kochia have evolved resistance to acetolactate synthase– and photosystem II–inhibiting herbicides (Foes et al. 1999; Primiani et al. 1990). Recently, glyphosate-resistant populations of kochia have been confirmed in several U.S. states (Heap 2013). Because kochia seed does not remain viable for extended periods of time in the soil (Schwinghamer and Van Acker 2008), favorable traits for fitness can quickly become predominant in a population. In addition, dispersal of seed from resistant plants that tumble across the landscape following senescence allows resistant alleles to spread widely. Thus, herbicide-resistant kochia rapidly becomes common in accessions where the same herbicide mechanism of action is used repeatedly.

Dicamba has been effective and economical for controlling kochia prior to planting in soybean or after planting in wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.). Although resistance to synthetic auxin herbicides is less common than to other herbicide families (Gustafson 2008; Heap 2013), kochia accessions from several states have been reported to exhibit reduced sensitivity to synthetic auxin herbicides. In 1997, Miller et al. (1997) and Manthey et al. (1997) reported that kochia accessions growing in Nebraska, Montana, and North Dakota were not controlled by the lowest recommended dicamba dose of 70 g ha^{-1}. Subsequent studies using inbred dicamba-resistant and -susceptible kochia biotypes have reported that the resistant biotypes were four- to fivefold more resistant than inbred susceptible biotypes (Cranston et al. 2001; Dyer et al. 2000). Preston et al. (2009) developed inbreds highly resistant or susceptible to dicamba over seven generations, and then demonstrated an resistant : susceptible (R : S) ratio of 30-fold between the biotypes. The resistant inbred was developed from an accession originally collected from Henry, Scotts Bluff County, Nebraska. This high R : S ratio may be explained by genetic variability among accessions that was intensified by the inbreeding process.

New transgenic technologies conferring herbicide resistance to dicamba, 2,4-D, and hydroxyphenyl-pyruvate dioxygenase inhibiting herbicides are being developed to complement glyphosate-resistance traits in corn, soybean, and cotton (*Gossypium hirsutum* L.) (Hinz et al. 2011; Johnson et al. 2010; Peterson et al. 2009; Seifert-Higgins 2010; Simpson et al. 2009). Although soybean is sensitive to dicamba, transgenic soybean can tolerate dicamba rates of 2,800 g ha^{-1}, far greater than the maximum standard use rate of 560 g ha^{-1} (Behrens et al. 2007; Herman et al. 2005). The commercialization of dicamba-resistant soybean seems likely to result in an increased use of dicamba, particularly in areas where broadleaf weeds such as kochia have evolved resistance to glyphosate. Of particular concern is the scenario in which dicamba is the only effective herbicide used to control glyphosate-resistant weeds. This will intensify selection pressure for weed species or accessions that are already tolerant to or that may become resistant to dicamba.

A survey was conducted by the University of Nebraska–Lincoln among weed scientists, agronomists, and farmers to determine which weed species would be most likely to evolve dicamba resistance after the commercial release of dicamba-resistant soybean. Thirty-two percent of respondents rated kochia as having a high likelihood of evolving resistance to dicamba, and 52% rated kochia as having medium risk (Crespo et al. 2012). Quantifying baseline resistance response levels of a given weed to a herbicide, prior to the widespread release of that herbicide or herbicide-resistance trait, will enable scientists to assess the degree of variability in response across multiple accessions and monitor changes in response to the herbicide over time (Burgos et al. 2013). Additionally, herbicide dose-response baseline studies may be useful for product registration as a way to anticipate the weed response to a given pesticide before it becomes commercially

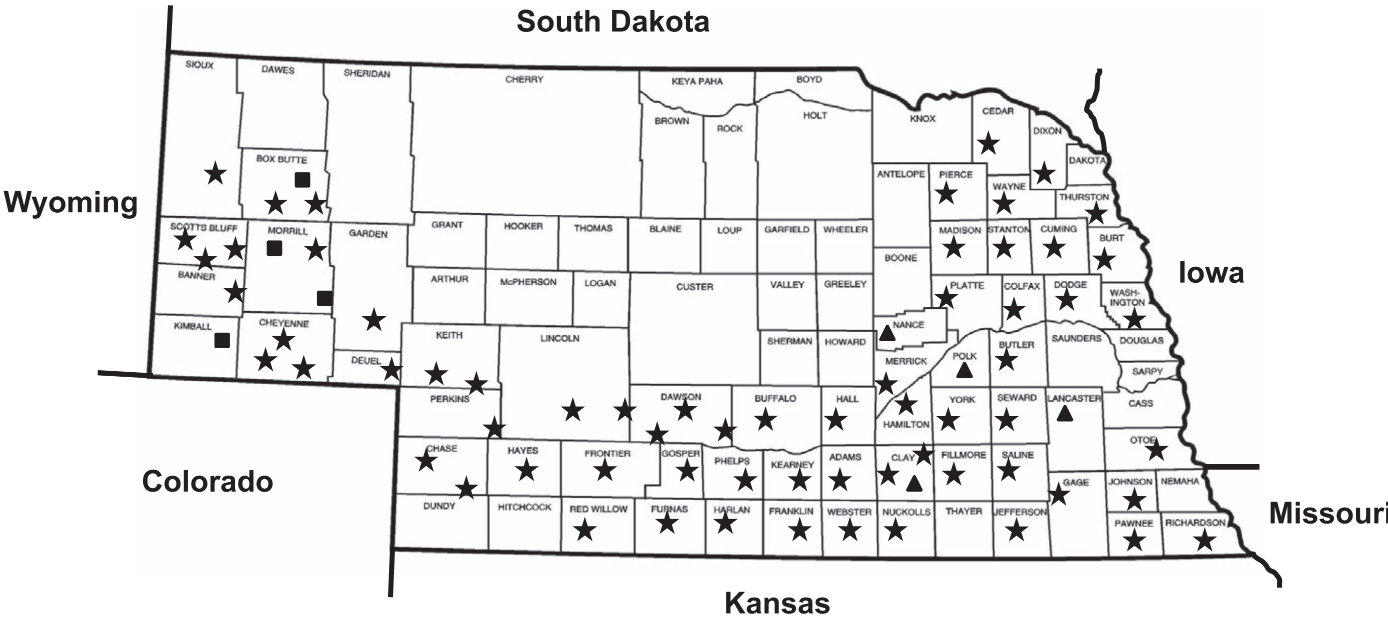

Figure 1. Dentification of areas in Nebraska where kochia accessions were collected in 2009 (triangles) and 2010 (stars). Locations of the four kochia accessions collected in 2010 and used in the dicamba dose-response study are marked by squares.

available. The objectives of this study were to (1) evaluate the variation in response to dicamba of kochia accessions collected across Nebraska during 2009 and 2010 and (2) evaluate kochia growth and seed production as affected by dicamba dose.

Materials and Methods

Accession Sampling. Seeds from kochia accessions growing in Nebraska were collected in the fall of 2009 and 2010. In 2009, four kochia accessions (80, 81, 91, and 100) were collected in southeast Nebraska (Figure 1). In 2010, 67 kochia accessions were collected from 57 counties in Nebraska (Figure 1). A route was mapped through Nebraska counties with significant crop acreage (primarily wheat and corn in western Nebraska, and corn and soybean in the remainder of the state) and when a field with kochia was observed, a sample was taken from the field. Each kochia sample was a composite of 40 or more plants and was considered an accession. Kochia samples were air-dried and then seed was cleaned and stored at 4 C.

Plant Growth and Dicamba Application. Kochia plants from collected seed were grown in greenhouses located at the University of Nebraska-Lincoln in Lincoln, NE. Kochia seed was planted in potting mix (BM1® Growing Mix, Berger Peat Moss Ltd, Saint-Modeste, Quebec, Canada) in 10- by 10- by 12.5-cm black plastic pots. Prior to herbicide treatment, seedlings were thinned to one plant per pot. Supplemental lighting (400 µE m^{-2} s^{-1}) in the greenhouse provided a 15-h photoperiod. The day and night temperatures were 24 $\pm$ 2 C and 19 $\pm$ 3 C, respectively.

In the experiments described below, dicamba (diglycolamine salt of 3,6-dichloro-2-methoxybenzoic acid formulated as Clarity® SL at 480 g L^{-1}, Herbicide, BASF Corporation, NC) was applied when kochia plants were 8 to 12 cm tall. Herbicide treatments were prepared with distilled water and applied in 190 L ha^{-1} carrier volume at a spray pressure of 207 kPa using a TP8001E flat-fan nozzle tip (Spraying Systems Co., North Avenue, Wheaton, IL 60187) in a research chamber sprayer (DeVries Mfg. Corp., Hollandale, MN 56045).

Dicamba Discriminating-Dose Experiment. Kochia accessions collected in 2010 were treated with 560 g ha^{-1} dicamba (Beckie et al. 2000). Twenty-one DAT visible estimates of injury were recorded on a scale of 0 (no injury) to 100 (dead plants) for seven plants (seven replications) of each kochia accession. Shoots of those plants were then cut at the soil surface and dried for 48 h in a forced air dryer at 65 C, after which dry weight biomass was measured. Average estimates of visible injury and

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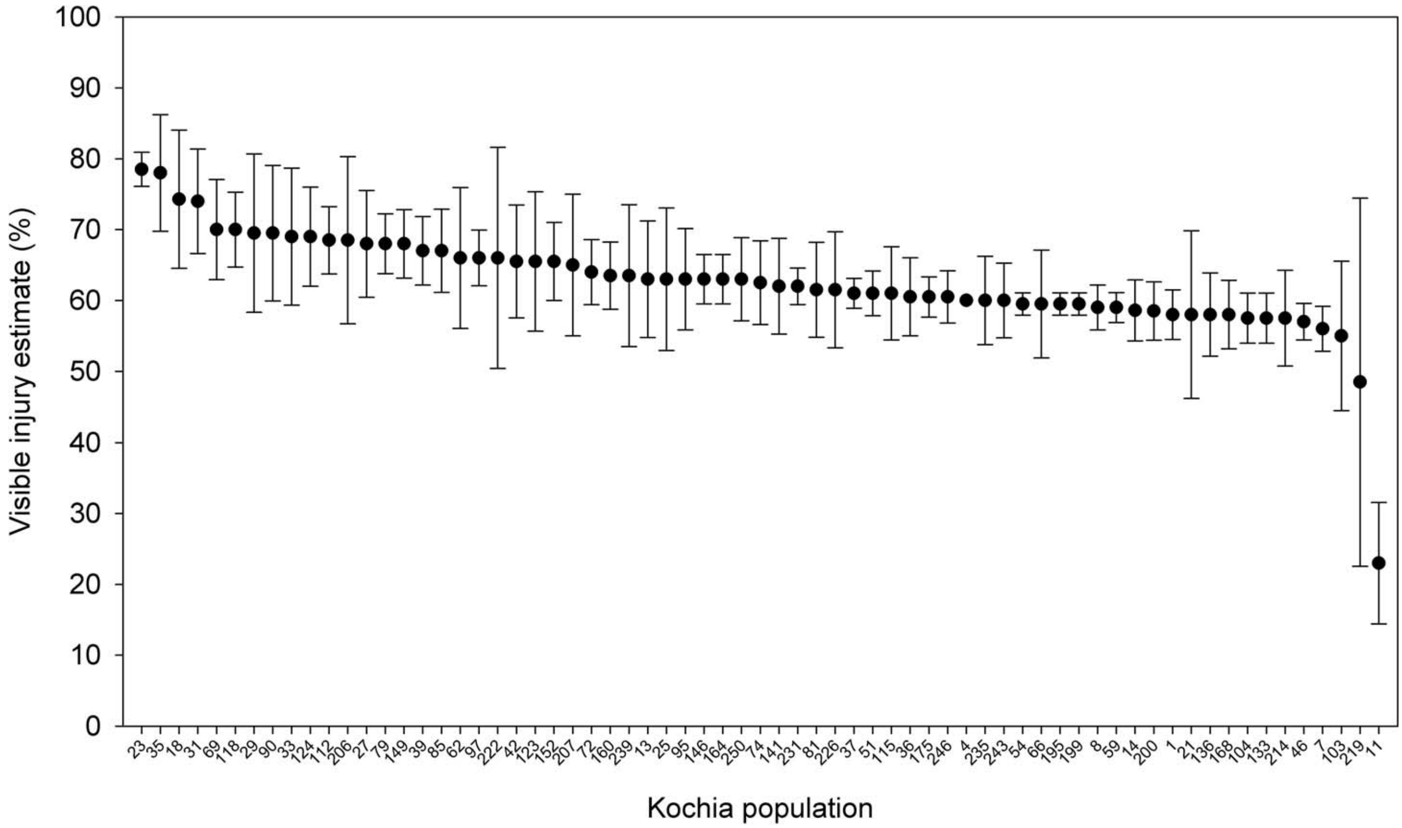

Figure 2. Injury estimates at 21 d after treatment of 67 Nebraska kochia accessions treated with 560 g ae ha^{-1} dicamba. Data represent the mean of seven replications and the standard error of the data.

standard error for each kochia accession were used to select less and more susceptible accessions for further dose-response testing. Accessions 7 and 11 collected in Morrill and Box Butte counties, respectively, showed less susceptibility to dicamba, while accessions 23 and 35, collected in Kimball and Morrill counties, respectively, showed greater susceptibility.

Dicamba Dose-Response Experiment. Dicamba dose-response experiments were conducted on the four accessions collected in 2009 and the four kochia accessions (7, 11, 23, and 35) selected from the discriminating-dose experiment. Experiments were arranged using a randomized complete block design with five replications. Each dose-response experiment was conducted twice (repeated in time). The 11 dicamba doses applied to the 2009-collection kochia accessions were: 0, 17, 35, 70, 105, 140, 420, 560, 1,120, 2,240, and 4,480 g ha^{-1} of dicamba. Because accession 11 displayed less than 25% injury to dicamba at 560 g ha^{-1} in the discriminating experiment (Figure 2), higher dicamba doses were selected for the 2010-collection kochia accessions: 0, 35, 70, 140, 280, 560, 1,120, 2,240, 4,480, 8,960, 17,920, and 35,840 g ha^{-1}. Because dicamba can volatilize, plants treated with the three highest dicamba doses were isolated on separate tables from plants treated with the lower doses to minimize the risk of cross-treatment contamination. At 3 or 4 DAT, plants were rerandomized. Injury estimates were made at 7, 14, 21, and 28 DAT for the eight accessions. At 28 DAT, plant shoots were harvested, dried for 48 h at 65 C, and weighed.

Kochia Growth and Seed Production. In a companion study to the dose-response experiment, three replications (i.e., individual plants) of accessions 7, 11, 23, and 35 were treated with one of 12 dicamba doses (0, 35, 70, 140, 280, 560, 1,120, 2,240, 4,480, 8,960, 17,920, and 35,840 g ha^{-1}) and grown for 110 DAT to evaluate long-term survival, plant growth, and seed production as affected by dicamba dose. Injury estimates were made at 28, 56, 84, and 110 DAT. Plant survival at

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110 DAT at a specific dicamba dose was expressed as a percentage from the six treated plants (three replications and two experimental runs). At 110 DAT, plants were harvested, dried at 65 C for 48 h, and weighed. Seed was individually threshed, cleaned, and weighed for each plant. Two 100-seed weight samples were collected for each plant. The number of seeds per plant was calculated using the whole-seed weight per plant and the average weight of the 100 seed samples specific to that plant.

Statistical Analysis. Visible estimates of injury and dry weight at 28 DAT for the dose-response component were analyzed using a nonlinear regression model with the *drc* 1.2 version package in R 2.3.0 (R statistical software, R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org) (Knezevic et al. 2007). Dose-response models were constructed using a four-parameter log-logistic equation (Equation 1) (Seefeldt et al. 1995; Streibig et al. 1993):

$$y = c + \{d - c/1 + \exp[b(\log x - \log e)]\} \quad [1]$$

where y is the response based on the visible injury estimate or dry weight, c is the lower limit, d is the upper limit, x is the dicamba dose, e is the dicamba dose giving a 50% response (GR_{50} or I_{50}) between the upper and lower limit and is also the inflection point, and b is the slope of the line at the inflection point. The dicamba dose required to cause 50, 80, and 90% reduction in dry weight (GR) and injury estimates (I) at 28 DAT were calculated. One of our objectives was to measure the relative variation among accessions. We estimated this by dividing the I or GR values of less-susceptible accessions by the I or GR values of the most-susceptible accession (Beckie et al. 2000).

A four-parameter log-logistic equation (Equation 1) was used to describe the dry weight (g $plant^{-1}$) and seed production data at 110 DAT (Seefeldt et al. 1995; Streibig et al. 1993). In this analysis, y is the response (e.g., dry weight or seed production), c is the lower limit, d is the upper limit, x is the dicamba dose, e is the dicamba dose resulting in a response 50% between the upper and lower limit, and b is the slope of the line at the inflection point. Two-way ANOVA for dry weight at 110 DAT and the number of seed per treatment for each accession was performed using SigmaPlot 11.0® (Systat Software, Inc., IL). Means were separated using an LSD procedure with $\alpha = 0.05$.

Results and Discussion

Dicamba Discriminating-Dose Experiment. Injury estimates at 21 DAT for the 67 kochia accessions collected in 2010 treated with a dicamba dose of 560 g ha^{-1} ranged from 23% for the least-susceptible accession (11) to 78% for the most-susceptible accession (23) (Figure 2). Accession 11 was collected within an irrigated corn field in Box Butte County (west-central Nebraska) whereas accession 23 was collected along the edge of an irrigated corn field in Kimball County (southwestern Nebraska) (Figure 1). Wheat, corn, dry bean (*Phaseolus vulgaris* L.), and sugarbeet (*Beta vulgaris* L.) are common in crop rotations for this region, and dicamba is often used to manage weeds in wheat and occasionally in corn. In western Nebraska, dicamba is also commonly used to manage weeds in eco-fallow.

The majority of the accessions collected (60 of 67) responded similarly to dicamba, with injury estimates ranging between 50 and 70% at 21 DAT (Figure 2). Only five accessions showed more than 70% injury and two accessions showed less than 50% injury (48 and 23%) (Figure 2). Injury estimates observed in this experiment were similar to those observed by Howatt (1999). Howatt (1999) screened approximately 50 Colorado kochia accessions with dicamba doses between 70 and 560 g ha^{-1} and found that although most accessions were susceptible to dicamba, five accessions had reduced dicamba sensitivity (70 to 90% visible injury) at 560 g ha^{-1}. The degree of visible injury observed in Howatt's (1999) study may have been greater than that observed in our study because he applied dicamba to kochia plants 3 to 7 cm in height, smaller than the 8- to 12-cm heights at which our plants were treated.

Dicamba Dose-Response Experiment. For the four accessions collected in 2009, the dicamba dose calculated to achieve I_{90} for accessions ranged from 2,664 g ha^{-1} for the most-susceptible accession (81) to 5,134 g ha^{-1} for the least-susceptible accession (91), representing a 1.9-fold variation in response (Table 1; Figure 3). The variation for the I_{50} dose was less, only 1.2-fold, among the least and most susceptible accessions (Table 1). In contrast, there was greater variation among the four 2010 accessions selected from the discriminating-dose experiment. The dicamba dose calculated to achieve

Table 1. Injury estimate regression parameters, dicamba doses necessary to achieve I_{50}, I_{80} and I_{90} values, and standard errors at 28 d after treatment for eight Nebraska kochia accessions collected in 2009 and 2010.

Year	Accession	Regression parameters[a]			
		b	I_{50}[b] ± SE	I_{80} ± SE	I_{90} ± SE
			g ae ha^{-1}		
2009	80	−0.91	455 ± 73	2,072 ± 562	5,030 ± 1,876
2009	81	−1.14	388 ± 46	1,309 ± 249	2,664 ± 695
2009	91	−0.84	370 ± 51	1,946 ± 443	5,134 ± 1,618
2009	100	−0.90	438 ± 49	2,033 ± 384	4,987 ± 1,311
	R : S		1.2	1.6	1.9
2010	7	−0.87	270 ± 30	1,320 ± 240	3,350 ± 870
2010	11	−0.88	5,120 ± 620	24,600 ± 5,130	61,580 ± 17,910
2010	23	−0.84	270 ± 40	1,410 ± 300	3,700 ± 1,110
2010	35	−0.84	300 ± 30	1,560 ± 220	4,120 ± 820
	R : S		19.0	18.6	18.4

[a] Regression parameters were estimated using a four-parameter log-logistic equation, $y = c + \{d - c/1 + \exp[b(\log x - \log e)]\}$, where c represents the lower limit (0 = no injury), d represents the upper limit (100 = plant death), b represents the slope of the line at the inflection point, and *e* represents the herbicide dose necessary to provide 50% injury (I_{50}).

[b] Abbreviations: I_{50}, 50% injury reduction; I_{80}, 80% injury reduction; I_{90}, 90% injury reduction; R : S, resistant : susceptible ratio between the least and most susceptible accession for each parameter.

90% injury ranged from 3,350 g ha^{-1} for the most-susceptible accession (7) to 61,580 g ha^{-1} for the least-susceptible accession (11), representing a 18.4-fold variation in response (Table 1; Figure 4). However, with the exception of accession 11, there was little variation in response to dicamba dose (less than 2.0-fold) among the other three accessions collected in 2010 for the I_{50} and I_{90} estimates (Table 1), similar to what was observed with the accessions collected in 2009. The dicamba dose required to control these kochia accessions was greater than what may have been expected (Bernards et al. 2011; Howatt 1999). Typical dicamba use rates in cereal grains and corn range from 70 to 280 g ha^{-1}. In this study, that recommended rate was inadequate to achieve I_{50} in most of the accessions when dicamba was applied to plants 8 to 12 cm in height. In these greenhouse studies, the lack of a competitive crop to shade kochia after it had been treated and the absence of typical insects and diseases that attack herbicide-weakened plants likely contributed to the "less-than-expected" control at recommended use rates.

Calculated R : S ratios for dry weight were similar to those calculated for injury estimates for the 2009 accessions: 1.8-fold for the GR_{90} (Table 2), compared to an I_{90} R : S ratio of 1.9-fold (Table 1, Figure 5). The variation in the GR_{90} R : S ratio among the 2010 accessions was 7.4-fold (Table 2, Figure 6), less than 18.4-fold difference of the I_{90} ratio (Table 1). In contrast to the injury estimate results, the calculated dicamba dose necessary to achieve GR_{50} for the four 2009 accessions was less than 110 g ha^{-1}, and was within the range of

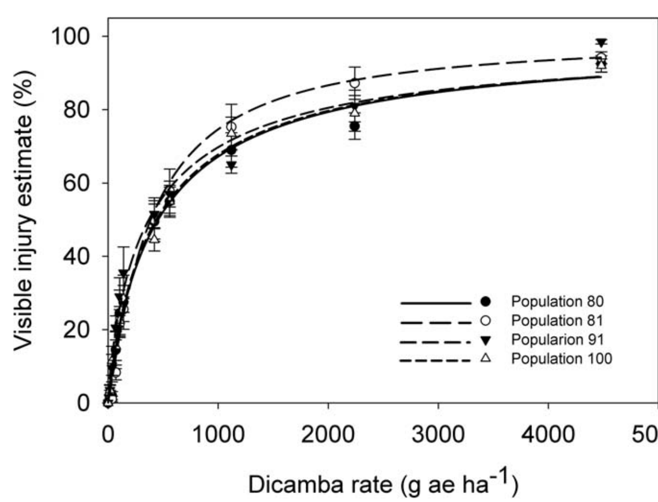

Figure 3. Injury response to dicamba dose on four kochia accessions collected from southeastern Nebraska in 2009 at 28 d after treatment. Regression lines were fit to the data using a four-parameter log-logistic equation, $y = c + \{d - c/1 + \exp[b(\log x - \log e)]\}$, where c represents the lower limit (0 = no injury), d represents the upper limit (100 = plant death), b represents the slope of the line at the inflection point, and *e* represents the herbicide dose necessary to provide 50% injury. Regression parameters are given in Table 1.

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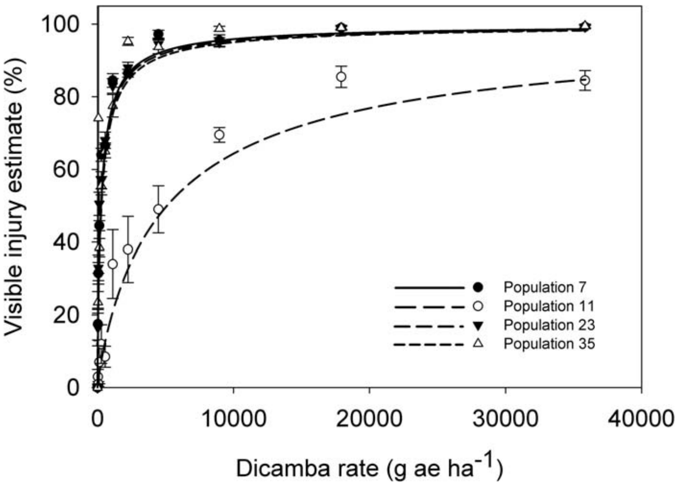

Figure 4. Injury response to dicamba dose at 28 d after treatment for four kochia accessions collected in Nebraska in 2010. Regression lines were fit to the data using a four-parameter log-logistic equation, $y = c + \{d - c/1 + \exp [b (\log x - \log e)]\}$, where c represents the lower limit (0 = no injury), d represents the upper limit (100 = plant death), b represents the slope of the line at the inflection point, and *e* represents the herbicide dose necessary to provide 50% injury. Regression parameters are given in Table 1.

normal use rates. However, for the 2010 accession, the calculated rates necessary to achieve GR_{50} were 300 g ha^{-1} or more, greater than the typical maximum use rate of 280 g ha^{-1}, for the four accessions (Table 2).

With the exception of accession 11, there was little variation in response to dicamba dose among the accessions evaluated. Somewhat surprisingly, accession 7 was ranked as one of the least-susceptible accessions in the discriminating dose study with an injury rating of 57%, but when it was subjected to a range of doses and a regression equation was fit to the data, the susceptibility of accession 7 was not different from that of accessions 23 and 35, both of which appeared more susceptible (78%) in the preliminary screening (Figure 2; Tables 1 and 2). Although it is impractical to do full-dose responses on hundreds or even dozens of accessions, these results demonstrate the importance of caution when attempting to infer differences between accessions unless the variation is large, or the number of individuals that can be evaluated from each accession is also very large.

We observed that accessions differed in the visible symptoms they expressed after being treated with dicamba. For example, some accessions showed severe epinasty, resulting in higher injury estimates, yet still accumulated biomass, particularly in calloused and swollen stem tissue. In contrast, other accessions displayed limited epinasty and callous tissue formation, but were stunted and exhibited significant dry weight reduction compared to nontreated controls. These phenomena may explain the reduction in R : S ratios between injury

Table 2. Dry weight reduction regression parameters, dicamba doses necessary to achieve GR_{50}, GR_{80} and GR_{90}, and standard errors (SE) at 28 DAT for eight Nebraska kochia accessions collected in 2009 and 2010.

		Regression parameters[a]					
Year	Accession	c	d	b	GR_{50}[b] ± SE	GR_{80} ±SE	GR_{90} ± SE
						g ae ha^{-1}	
2009	80	0.4	6.9	0.82	105 ± 39	571 ± 342	1,535 ± 1,338
2009	81	0.4	9.1	0.74	88 ± 38	571 ± 369	1,706 ± 1,656
2009	91	0.5	4.9	0.61	77 ± 16	736 ± 224	2,759 ± 1,278
2009	100	0.7	8.3	0.77	102 ± 35	618 ± 320	1,769 ± 1,344
	R : S[b]				1.4	1.3	1.8
2010	7	0.4	2.7	0.70	310 ± 50	2,290 ± 590	7,300 ± 2,740
2010	11	0.8	2.4	0.80	3,490 ± 1,080	19,640 ± 10,730	53,960 ± 43,730
2010	23	0.5	2.5	0.76	600 ± 70	3,710 ± 650	10,750 ± 2,670
2010	35	0.5	1.8	0.77	670 ± 100	4,010 ± 1,050	11,440 ± 4,190
	R : S[b]				11.3	8.6	7.4

[a] Regression parameters were estimated using a four-parameter log-logistic equation, $y = c + \{d - c/1 + \exp [b (\log x - \log e)]\}$, where, where c represents the lower limit (minimum dry weight for each accession), d represents the upper limit (maximum dry weight for each accession), b represents the slope of the line at the inflection point, and *e* represents the herbicide dose necessary to provide 50% reduction in dry matter (GR_{50}).

[b] Abbreviations: GR_{50}, 50% dry weight reduction; GR_{80}, 80% growth reduction; GR_{90}, 90% dry weight reduction; R : S, resistant : susceptible ratio between the least and most susceptible accession for each parameter.

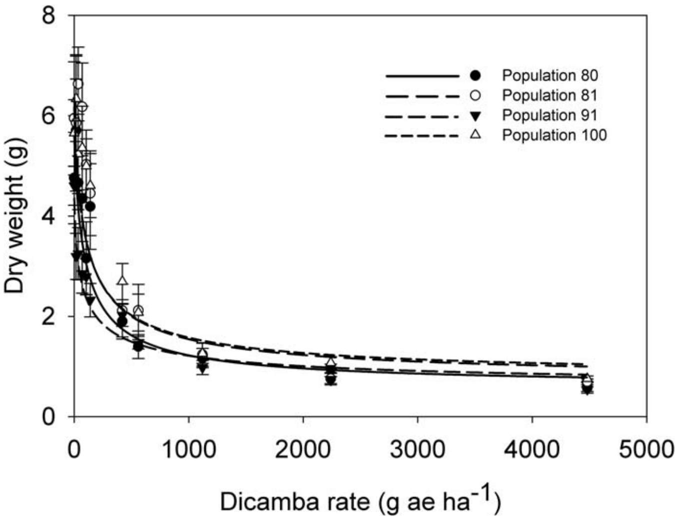

Figure 5. Dry weight response to dicamba dose of four kochia accessions collected from southeast Nebraska in 2009 at 28 d after treatment. Regression lines were fit to dry weight data using a four-parameter log-logistic equation, y = c + {d − c/1 + exp [b (log *x* − log *e*)]}, where c represents the lower limit (minimum dry weight for each accession), d represents the upper limit (maximum dry weight for each accession), b represents the slope of the line at the inflection point, and *e* represents the herbicide dose necessary to provide 50% reduction in dry matter. Regression parameters are given in Table 2.

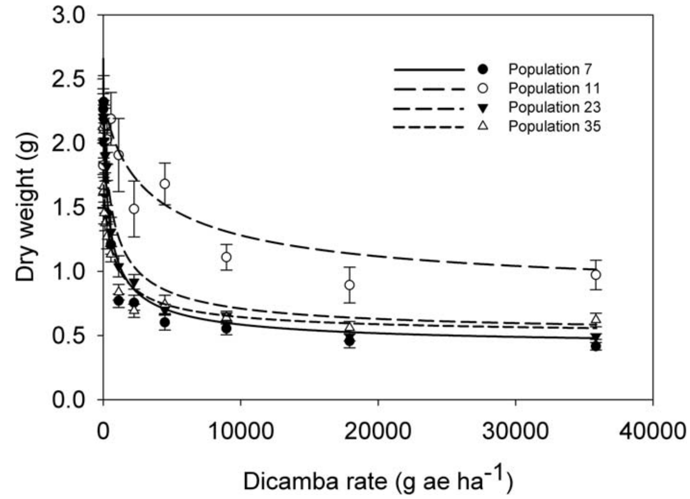

Figure 6. Dry weight response of four kochia accessions collected in Nebraska in 2010 to dicamba dose at 28 d after treatment. Regression lines were fit to data using a four parameter log-logistic equation, y = c + {d − c/1 + exp [b (log *x* − log *e*)]}, where c represents the lower limit (minimum dry weight for each accession), d represents the upper limit (maximum dry weight for each accession), b represents the slope of the line at the inflection point, and e represents the herbicide dose necessary to provide 50% reduction in dry matter. Regression parameters are given in Table 2.

estimates and dry weight for accessions 7 and 11. The growth of accession 11 was reduced by dicamba application, but plants showed relatively little epinasty compared to other accessions.

Growth and Seed Production. In the companion study to the dose-response study, three replications of the four 2010 accessions were grown until 110 DAT. At a dicamba dose of 1,120 g ha^{-1}, survival of accessions 7, 23, and 35 was 17%, compared to 100% for accession 11. Even at a dicamba dose of 35,840 g ha^{-1}, 64 times the maximum labeled rate of 560 g ha^{-1}, accession 11 had a survival rate of 17% (Table 3). Plants from accession 11 treated with dicamba showed less-severe epinastic symptoms and no chlorosis or necrosis compared to the other three accessions. New growth on the more-susceptible accessions continued to show epinasty of leaves and stem long after treatment, but accession 11 showed only mild, transitory epinastic symptoms, and remained upright (data not shown). Average dry weight biomass of the accessions decreased as dicamba dose was increased (Table 4; Figure 7). Somewhat surprisingly, dry weight of accession 11 declined at the lowest dose applied (Table 4), whereas two of the susceptible accessions did not show an immediate decrease in plant dry weight. Seed production, estimated as the average seed number per plant based on a 100-seed weight, also decreased as dicamba dose increased (Table 5).

Table 3. Plant survival at 110 d after treatment among four kochia accessions treated with 12 dicamba doses. Data represent the percentage of survival based on three replications and two experimental runs for each kochia accession.

	Accession			
Dicamba dose	7	11	23	35
g ae ha^{-1}	Plant survival (%)			
0	100	100	100	100
35	100	100	100	100
70	100	100	100	83
140	100	100	100	67
280	100	100	83	0
560	50	100	17	0
1,120	17	100	17	17
2,240	0	67	0	0
4,480	0	100	0	0
8,960	0	83	0	0
17,920	0	67	0	0
35,840	0	17	0	0

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Table 4. Average dry weight biomass at 110 d after treatment (DAT) among four kochia accessions treated with 12 dicamba dose. Data represent the average of three replications and two experimental runs for each kochia accession. A weight of 0 g plant^{-1} represents plants that died prior to 110 DAT.

	Accession			
Dicamba dose	7[a]	11	23	35
g ae ha^{-1}	g plant^{-1}			
0	19.0 a	12.7 a	13.6 a	11.3 a
35	18.9 a	9.1 b	9.8 b	10.6 a
70	13.8 b	8.5 b	11.3 ab	9.5 ab
140	8.3 bc	7.9 bc	14.9 a	4.8 b
280	10.9 b	4.1 d	11.5 ab	0.0 c
560	3.2 cd	7.7 bc	0.2 c	0.0 c
1,120	1.6 d	5.9 cd	0.2 c	0.2 c
2,240	0.0 d	7.4 bc	0.0 c	0.0 c
4,480	0.0 d	4.2 d	0.0 c	0.0 c
8,960	0.0 d	2.0 e	0.0 c	0.0 c
17,920	0.0 d	0.9 f	0.0 c	0.0 c
35,840	0.0 d	0.2 g	0.0 c	0.0 c

[a] Means followed by the same letter within the same column are not significantly different ($P < 0.05$).

In contrast to the immediate reduction in plant dry weight for accession 11, statistically equal numbers of seed were produced by plants treated with up to 560 g ha^{-1} (with the exception of the 280 g ha^{-1}

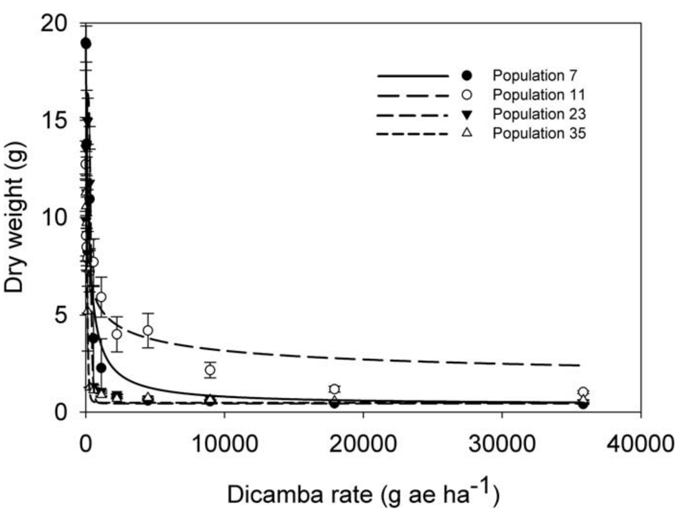

Figure 7. Dry weight of four kochia accessions collected in Nebraska in 2010 as affected by dicamba dose at 110 d after treatment. Regression lines were fit to data using a four-parameter log-logistic equation, $y = c + \{d - c/1 + \exp[b(\log x - \log e)]\}$, where c represents the lower limit (minimum dry weight for each accession), d represents the upper limit (maximum dry weight for each accession), b represents the slope of the line at the inflection point, and *e* represents the herbicide dose necessary to provide 50% reduction in dry matter.

Table 5. Seed production at 110 d after treatment among four kochia accessions treated with 12 dicamba doses. Data represent the average of two experimental runs and three replications per run.

	Accession			
Dicamba dose	7[a]	11	23	35
g ae ha^{-1}	No. of seed plant^{-1b}			
0	5,197 a	2,702 a	2,509 a	2,337 a
35	3,654 ab	3,447 a	2,179 a	1,073 b
70	2,413 b	2,184 a	1,927 a	1,554 ab
140	2,006 bc	2,002 a	1,997 a	738 b
280	1,859 c	367 c	534 b	0 c
560	157 d	1,505 ab	0 c	0 c
1,120	223 d	664 c	0 c	0 c
2,240	0 d	678 bc	0 c	0 c
4,480	0 d	113 d	0 c	0 c
8,960	0 d	0 e	0 c	0 c
17,920	0 d	17 e	0 c	0 c
35,840	0 d	0 e	0 c	0 c

[a] Means followed by the same letter within the same column are not significantly different ($P < 0.05$).

[b] The number of seeds per plant was calculated using the whole seed weight per plant and the average weight of two 100-seed samples specific to that plant.

dose, which was highly effective on the accession—perhaps a result of heterogeneity within the accession). Not all plants that survived to 110 DAT produced seed, including accessions 11, 23, and 35 (Tables 3 and 5). The maximum dicamba dose at which seed production was observed ranged from 140 g ha^{-1} (accession 35) to 17,920 g ha^{-1} (accession 11) (Table 5).

The field where kochia accession 11 was collected had been in irrigated continuous corn for the previous 10 yr, and dicamba had frequently been applied in tank-mixtures with glyphosate or atrazine to 2.5-cm-tall kochia. Dicamba or 2,4-D had been applied regularly to control broadleaf weeds growing in wheat stubble in the pivot corners. Thus, accession 11 had already been subjected to extensive selection pressure with dicamba. Based on the history of selection pressure, the relatively high GR_{50} (3,450 g ha^{-1}), and the large I_{50} R : S ratio (17- to 19-fold) compared to all other accessions evaluated lead us to conclude that accession 11 is resistant to dicamba.

Our objective in conducting this research was not to identify a dicamba-resistant biotype of kochia. Nevertheless, the identification of a dicamba-resistant biotype among a random collection of 71

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accessions may not be surprising given previous reports of resistant biotypes in western Nebraska (Cranston et al. 2001; Dyer et al. 2001; Howatt 1999; Miller et al. 1997; Preston et al. 2009). However, what is notable about accession 11 is that it was a heterogeneous accession selected by repeated applications of dicamba in the field but which still exhibited a high level of resistance (17- to 19-fold) relative to accessions that were only marginally susceptibility to dicamba (I_{50} of 270 g ha^{-1} or greater). In most other reports of dicamba-resistant kochia, the biotypes evaluated have been "inbred" populations selected over two or more generations in the greenhouse to insure phenotypic uniformity, although the potential for inbreeding depression was noted as a possibility (Cranston et al. 2001). The susceptible inbred accessions in these studies typically required a much lower dose to achieve I_{50} or GR_{50} values than we observed in the heterogeneous susceptible accessions we evaluated. For example, Cranston et al. (2001) treated inbred resistant and susceptible kochia biotypes that were 3 to 5 cm tall, yet reported only a 4.6-fold difference in the I_{50} dose, 31 and 143 g ha^{-1}, between the most and least susceptible accessions, respectively. Nandula and Manthey (2002) treated inbred resistant and susceptible accessions from North Dakota when plants were 1.3 to 2.5 cm tall. They reported a 5- to 10-fold difference between the least and most susceptible accessions, but only 10 g ha^{-1} dicamba was required to reach 50% injury in the most susceptible accession, whereas a dicamba dose of 140 g ha^{-1} caused between 68 and 93% injury among the remaining evaluated accessions (Nandula and Manthey 2002). The only example in the literature where the magnitude of resistance was greater than what we report is from Preston et al. (2009) who reported 30-fold difference between dicamba-resistant and -susceptible kochia biotypes. The resistant biotype Preston et al. (2009) used was an inbred biotype that had been selected over seven generations, and was compared to a highly sensitive inbred biotype. When 11-cm-tall plants were treated, the resistant biotype required 1,331 g ha^{-1} of dicamba whereas the susceptible biotype required only 45 g ha^{-1} to reach GR_{50}. The doses required to reach GR_{50} of the inbred accessions were considerably less than those required for the heterogeneous accessions used in our study (310 to 3,450 g ha^{-1}). In essence, we happened upon a field where many years of selection pressure had resulted in an accession allowed to cross naturally that evolved very high levels of resistance similar to that observed with seven generations of selection in the greenhouse (although it still showed signs of some heterogeneity in response to dicamba [see Table 4]).

Although dicamba resistance of kochia from western Nebraska has been reported for many years, it may not yet be widespread despite the plant's potential to disperse seed over a large area through the movement of tumbleweeds. The most-susceptible accessions were collected in counties adjacent to the county where the resistant accession was collected. In addition, all three samples taken from Scotts Bluff County were susceptible, and Scotts Bluff County was where the resistant accession reported by Preston et al. (2009) was collected. Based on cropping practices and history, we might have expected less susceptibility to dicamba in the west than in the east, where dicamba is not used as frequently because there is less wheat in the rotation. But the heterogeneous accessions in western Nebraska were not different in their susceptibility when compared with heterogeneous accessions from eastern Nebraska, with the exception of accession 11. This suggests that most kochia accessions should be similarly susceptible to dicamba, if its use were to increase dramatically in coming years.

Dicamba-resistant soybean is being developed in part to help manage glyphosate-resistant broadleaf weeds. In order to preserve the efficacy of dicamba-resistant crop technology and to effectively control kochia accessions, it will be crucial that growers carefully monitor the response of kochia accessions in their fields and that they strictly follow recommended management practices to prevent resistance evolving to dicamba (Norsworthy et al. 2012). First, dicamba must not be the only effective herbicide used to control kochia accessions already resistant to triazine, glyphosate, or acetolactate synthase–inhibiting herbicides. If that dicamba-only approach is used, it is highly likely that more accessions like accession 11 will be selected. A second recommendation is to use the correct rate at the correct weed stage and size. In our study, we treated 8- to 12-cm-tall kochia plants. This has become a common size at which to treat many weeds in glyphosate-resistant crops, but at this size the susceptible heterogeneous accessions we collected were not controlled adequately at current recommended use rates of 70 to 280 g ha^{-1}. In studies in which kochia was treated when it was less than 5 cm tall, dicamba rates necessary to achieve GR_{50} or I_{50} were less than 280 g ha^{-1} (Cranston et al. 2001; Nandula and Manthey 2002). Third, growers who do use dicamba should carefully monitor their fields for plants similar to those in accession 11 that are stunted by dicamba but that do not show epinasty. They should remove these plants before they produce seed.

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