Research Article

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Reduced absorption of glyphosate and decreased translocation of dicamba contribute to poor control of kochia (*Kochia scoparia*) at high temperature

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Abstract

BACKGROUND: Plant growth temperature is one of the important factors that can influence postemergent herbicide efficacy and impact weed control. Control of kochia (*Kochia scoparia*), a major broadleaf weed throughout the North American Great Plains, often is unsatisfactory when either glyphosate or dicamba are applied on hot summer days. We tested effects of plant growth temperature on glyphosate and dicamba phytotoxicity on two Kansas kochia populations (P1 and P2) grown under the following three day/night (d/n) temperature regimes: T1, 17.5/7.5°C; T2, 25/15°C; and T3, 32.5/22.5°C.

RESULTS: Visual injury and above-ground dry biomass data from herbicide dose–response experiments indicated greater susceptibility to both glyphosate and dicamba when kochia was grown under the two cooler temperature regimes, i.e. T1 and T2. At T1, the ED_{50} of P1 and P2 kochia were 39 and 36 g ha^{-1} of glyphosate and 52 and 105 g ha^{-1} of dicamba, respectively. In comparison, at T3 the ED_{50} increased to 173 and 186 g ha^{-1} for glyphosate and 106 and 410 g ha^{-1} for dicamba, respectively, for P1 and P2. We also investigated the physiological basis of decreased glyphosate and dicamba efficacy under elevated temperatures. Kochia absorbed more glyphosate at T1 and T2 compared to T3. Conversely, there was more dicamba translocated towards meristems at T1 and T2, compared to T3.

CONCLUSION: Reduced efficacy of dicamba or glyphosate to control kochia under elevated temperatures can be attributed to decreased absorption and translocation of glyphosate and dicamba, respectively. Therefore, it is recommended to apply glyphosate or dicamba when the temperature is low (e.g. d/n temperature at 25/15°C) and seedlings are small (less than 12 cm) to maximize kochia control.

Keywords: glyphosate; dicamba; growth temperature; kochia

1 INTRODUCTION

Kochia is one of the most troublesome annual C4 broadleaf weeds in croplands in the Great Plains of North America.[1] Kochia can emerge early in spring (early March in Kansas[2]) before most other spring and summer annual weeds and spring-sown crops and can grow rapidly under cool as well as warm temperatures.[1,2] Due to its aggressive growth habit, kochia can cause huge yield loss in grain crops.[1,3] In addition, mature plants of kochia accumulate saponins, alkaloids, oxalates, and nitrates, which are toxic to domestic animals.[4] More than 30 kochia populations across the USA have been reported to have evolved resistance to one or more herbicide modes of action.[5] Yet, herbicide application is still one of the most effective methods to manage kochia in croplands. Weed resistance to herbicide sites of action is evolving at a rapid rate while no new herbicide modes of action have been developed in more than two decades.[6] Thus, more efficient use of existing herbicides is vital to maintain their effectiveness in the future.

Poor control of kochia in western Kansas has been observed numerous times when glyphosate [*N*-(phosphonomethyl)-glycine] or dicamba (3,6-dichloro-*o*-anisic acid) was applied in hot weather (P.W. Stahlman, Personal communication). Incomplete control of kochia can accelerate the evolution of glyphosate or dicamba resistance, since long-term or constant exposure to a low/ineffective concentration of a specific herbicide can significantly contribute to the evolution of resistance in weeds.[7,8]

Several studies have found that the efficacy of commonly used herbicides such as glyphosate, glufosinate, and mesotrione can be affected by temperature. Increased temperature has altered the efficacy of glyphosate on wild oat (*Avena fatua*),[9] liverseed grass (*Urochloa panicoides*),[9] velvetleaf (*Abutilon theophrasti*),[10] and awnless barnyardgrass (*Echinochloa colona*).[11] However, only a few studies have investigated the underlying mechanism of

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altered glyphosate efficacy under different temperature regimes. For instance, Jordan[11] reported glyphosate controlled bermudagrass (*Cynodon dactylon*) better at high than low temperature because more glyphosate was absorbed and translocated out of the treated leaves. Similarly, Coupland[12] found elevated basipetal translocation enhanced glyphosate activity at high temperature in couch grass (*Elymus repens*). However, in quackgrass (*Agropyron repens*), Devine *et al.*[13] concluded altered efficacy of glyphosate at different temperatures was not due to differential absorption or translocation of the herbicide. Similarly, Friesen and Dew[14] reported phytotoxicity of dicamba on tartary buckwheat (*Fagopyrum tataricum*) was not affected when temperature was increased. This study was conducted based on the hypothesis that the temperature can alter absorption and/or translocation of glyphosate or dicamba, thereby affecting kochia control. The objectives of this study were to: (a) evaluate the differential efficacy of glyphosate or dicamba at varying temperatures on kochia control and (b) investigate the mechanisms underlying the differential efficacy of these herbicides on kochia control.

2 MATERIALS AND METHODS

2.1 Plant materials and growth conditions

Kochia seed was collected from field sites in Pratt County[15] (Population 1, P1) and Riley County (Population 2, P2), Kansas, in 2012. Because of the short seed longevity of kochia, 5–10 plants from each population annually were grown together in isolation from other kochia and mature seed bulked and stored in dark at 4°C. Seeds from P1 and P2 produced in 2014 were used to conduct glyphosate and dicamba dose–response experiments in growth chambers under different temperature regimes (described in detail in section 2.1). However, only P1 was used to conduct glyphosate and dicamba absorption and translocation experiments at different temperatures.

In 2015, kochia seed of P1 and P2 were germinated in small trays (25 × 15 × 2.5 cm) filled with commercial potting mixture (Pro-Mix Potting-Mix; Premier Tech Horticulture, Mississauga, Ontario, Canada). Individual seedlings 2–3 cm tall were transplanted into plastic pots (6.5 × 6.5 × 9 cm) in a greenhouse on the campus of Kansas State University in Manhattan. The following greenhouse conditions were maintained: 25/20°C (day/night, d/n) temperatures, 60 ± 10% relative humidity, and 15/9 h day/night photoperiod supplemented with 120 μmol m^{-2} s^{-1} illumination provided with sodium vapor lamps. One week after transplanting, healthy kochia plants (~5 cm tall) were transferred to growth chambers that were maintained at different d/n temperatures: T1: 17.5/7.5°C; T2: 25/15°C; and T3: 32.5/22.5°C. Light in all growth chambers was provided by incandescent and fluorescent bulbs delivering 750 μmol m^{-2} s^{-1} photon flux (15/9 h, d/n) at plant canopy level. Due to the unavailability of settings for constant vapor pressure deficit, all the growth chambers were set to maintain 60 ± 10% relative humidity throughout the experiment. Plants were watered daily.

2.2 Glyphosate and dicamba dose–response experiment

2.2.1 Glyphosate and dicamba treatment

Kochia plants were treated with glyphosate (Roundup WeatherMax; Monsanto Co., St. Louis, MO, USA) at dosages of 0, 26.3, 52.5, 105, 210, 420, 840, and 1680 g ha^{-1} with 2.5% (w/v) ammonium sulfate (AMS) or dicamba (Clarity; BASF Corp., Florham Park, NJ, USA) without AMS at dosages of 0, 17.5, 35, 70, 140, 280, 560, and 1120 g ha^{-1} when the plants were 10–12 cm tall. Herbicides were mixed in water and applied using a bench-type sprayer (Research Track Sprayer; De Vries Manufacturing, Hollandale, MN, USA) equipped with a single moving flat-fan nozzle tip (80015LP TeeJet tip; Spraying Systems Co., Wheaton, IL, USA) delivering 187 L ha^{-1} at 222 kPa in a single pass at 3.21 km h^{-1}. Following treatment, plants were returned to corresponding growth chambers within 30 min after treatment.

2.2.2 Visual injury and biomass measurement

Glyphosate- and dicamba-induced injury was rated based on composite visual estimations of growth inhibition, curling, necrosis, and plant vigor on a scale of 0 (no effect) to 100 (plant death). Visual injury ratings were taken at 1, 2, 3, and 4 weeks after treatment (WAT). At 4 WAT, plant stems were cut at soil level and individual plants were placed in separate paper sacks. After oven drying at 60°C for 72 h, plants were weighed once more to calculate dry biomass.

2.3 Absorption and translocation experiments

Results of the dose–response experiments showed that the two kochia populations, P1 and P2, responded similarly to glyphosate and dicamba at each temperature regime. Therefore, the glyphosate or dicamba absorption and translocation experiments were conducted using only one population, i.e. P1.

Prior to conducting the absorption and translocation experiments, we tested whether absorption or translocation of ^{14}C-glyphosate or ^{14}C-dicamba in kochia would be affected by spraying plants with formulated products of either herbicide before ^{14}C-herbicide treatment using the method described by Perez-Jones *et al.*[16] Briefly, on six 10–12 cm tall kochia seedlings, two newly expanded leaves were marked and wrapped with small pieces of aluminium foil, then the plants were sprayed with formulated product of 840 g ha^{-1} of glyphosate or 560 g ha^{-1} of dicamba using the methods described in section 2.1. After 30 min, when the herbicide droplets dried, the aluminium foil was removed. Likewise, another set of six untreated kochia seedlings of the same size were selected and two newly expanded leaves were marked on these plants as well. On both sets of kochia, the absorption and translocation of ^{14}C-glyphosate or ^{14}C-dicamba were tested under T2 using the method described in detail in sections 3.1 and 3.2. Results (data not shown) showed that neither absorption nor translocation of ^{14}C-dicamba or ^{14}C-glyphosate was affected by spraying the plants with formulated herbicide. Hence, the absorption and translocation experiments using ^{14}C-glyphosate or ^{14}C-dicamba reported here were not sprayed with formulated herbicide.

Additionally, preliminarily testing of ^{14}C-glyphosate or ^{14}C-dicamba translocation in kochia grown at T2 revealed that less than 0.5% of ^{14}C-glyphosate and only 1.3% of ^{14}C-dicamba was translocated to roots at 72 h after treatments (HAT). At the same time, 88–95% and 92–96% of ^{14}C-dicamba and ^{14}C-glyphosate, respectively, was recovered from the aboveground parts of kochia. Hence, the translocation of ^{14}C-glyphosate or ^{14}C-dicamba to plant roots was not measured in subsequent experiments.

2.3.1 Absorption and translocation of glyphosate

One milliliter of ^{14}C-glyphosate working solution with 0.33 kBq μL^{-1} of radioactivity was prepared by mixing 93.6 μL of phosphonomethyl-^{14}C-glyphosate water solution (3.7 kBq μL^{-1}, specific activity: 2.04 kBq μg^{-1}, PerkinElmer, Inc., Boston, MA, USA), 9.2 μL of Roundup Weathermax herbicide (Monsanto Co.), 73.5 μL of ammonium sulfate (AMS) aqueous solution (34%, w/v) and 823.7 μL of water, which was equivalent to 840 g of glyphosate in a carrier volume of 187 L water with 2.5% (w/v) of AMS.

Kochia seedlings (10–12 cm tall) grown under three temperature regimes (as described above) were used. On the upper surface of two newly expanded leaves, 10 μL of ^{14}C-glyphosate working solution (5 μL per leaf) was applied using Wiretrol® (10 μL; Drummond Scientific Co., Broomall, PA, USA). After 30 min, plants were returned to growth chambers. Plants were harvested at 24, 48 and 72 HAT and separated into treated leaf (TL), tissue above the treated leaf (ATL), and tissue below the treated leaf (BTL). Treated leaves were washed twice with 5 mL wash solution [10% (v/v) ethanol aqueous solution with 0.5% of Tween-20] in 20-mL scintillation vials for 1 min. After adding 15 mL Ecolite-(R) (MP Biomedicals, LLC, Santa Ana, CA, USA), radioactivity in leaf rinsate was measured by using liquid scintillation spectrometry (LSS; Tricarb 2100 TR Liquid Scintillation Analyzer, Packard Instrument Co., Meriden, CT, USA). Plant sections were dried at 60°C for 72 h and radioactivity in each plant part was quantified by LSS after combusting for 3 min with a biological oxidizer (OX-501; RJ Harvey Instrument, New York, NY, USA).

2.3.2 *Absorption and translocation of dicamba*

The methods of ^{14}C-dicamba application and sample collection were the same as described above for the ^{14}C-glyphosate experiment, except that the 1 mL of ^{14}C-dicamba working solution was obtained by mixing 29.3 μL of dicamba-(ring-UL-^{14}C) ethanol solution (11.4 kBq μL^{-1}, specific activity: 2.87 kBq μg^{-1}, BASF Corp.), 6.4 μL of Clarity herbicide (BASF Corp.) and 964.3 μL of water, which was equal to 560 g of dicamba in a carrier volume of 187 L.

2.3.3 *Data analysis*

The data from absorption and translocation experiments of both herbicides was converted into percentages for further analysis using the following equations:

$$\text{percentage recovery} = \frac{R_{\text{rinsate}} + R_{\text{ATL}} + R_{\text{TL}} + R_{\text{BTL}}}{R_{\text{applied}}} \times 100 \quad (1)$$

$$\text{percentage absorption} = \frac{R_{\text{applied}} - R_{\text{rinsate}}}{R_{\text{applied}}} \times 100 \quad (2)$$

$$\text{percentage translocation} = \left(100 - \frac{R_{\text{TL}}}{R_{\text{applied}} - R_{\text{rinsate}}}\right) \times 100 \quad (3)$$

$$\text{percentage in ATL} = \frac{R_{\text{ATL}}}{R_{\text{applied}} - R_{\text{rinsate}}} \times 100 \quad (4)$$

$$\text{percentage in TL} = \frac{R_{\text{TL}}}{R_{\text{applied}} - R_{\text{rinsate}}} \times 100 \quad (5)$$

$$\text{percentage in BTL} = \frac{R_{\text{BTL}}}{R_{\text{applied}} - R_{\text{rinsate}}} \times 100 \quad (6)$$

In Eqns 1–6, R_{rinsate} is the radioactivity recovered in leaf rinsate; R_{applied} is total amount of radioactivity applied on the plant; R_{ATL} is the radioactivity recovered in tissue above the treated leaf (ATL); R_{TL} is the radioactivity recovered in the treated leaf (TL); and R_{BTL} is the radioactivity recovered in tissue below the treated leaf (BTL).

2.4 Experimental design and statistical analysis

Split-plot experimental design was used for all experiments. In the glyphosate and dicamba dose–response experiments, temperature and herbicide doses were main and subplots, respectively. In absorption and translocation of ^{14}C-dicamba and ^{14}C-glyphosate experiments, temperature and harvesting time were the main and subplot, respectively. At least four replicates of each dose were included in both studies and all the experiments were repeated twice in time, and the growth chambers were rotated to avoid pseudo-replication.

In the whole-plant dose–response experiments, treatments were arranged in a factorial combination of three levels of growth temperatures (T1, T2, and T3) and different herbicide doses. There was no interaction between experimental runs and treatments; hence, data from the two dose–response experiments were pooled for each population prior to analysis. Using the *drc* package[17] in R (v.3.2.1, R Foundation for Statistical Computing, Vienna, Austria), visual injury and dry biomass were subjected to non-linear regression analysis using four parameter log-logistic model:[18]

$$Y = C + \frac{D - C}{1 + \exp\left\{b\left[\log(x)\right] - \log\left(I_{50}\right)\right\}} \quad (7)$$

In Eqn 7, Y refers to the percentage of control or untreated, C is the lower limit, D is the upper limit, b is the slope, and I_{50} is the dose required for 50% response of plant injury or biomass reduction. This model was used to estimate ED_{50} (effective dose for 50% control of kochia) and GR_{50} (effective dose for 50% biomass reduction) values from the visual injury and dry biomass of kochia, respectively.

For experiments involving absorption and translocation, treatments were arranged in a factorial combination of three levels of growth temperatures (T1, T2, and T3) as main factors, and four levels of measurement time (12, 24, 48, and 72 h) as simple factors. There was no interaction between experimental runs and treatments; hence, data from the two experiments were combined and analyzed by fitting to an asymptotic regression, rectangular hyperbolic or linear model using the method developed by Kniss *et al.*[19] based on *drc*[17] and *qpcR*[20] packages in R program. Furthermore, the bias-corrected Akaike information criteria (AICc) of these three models were compared and the rectangular hyperbolic model [Eqn 8] with the lowest AICc value[19] was chosen for analyzing glyphosate or dicamba absorption data. However, none of these three regression models could be used to analyze glyphosate or dicamba translocation data. Therefore, all translocation data were analyzed using two-way ANOVA ($P < 0.05$) in Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA):

$$\text{absorption} = \frac{A_{\max} \times t}{\left({}^{10}/_{90}\right) \times t_{90} + t} \quad (8)$$

In Eqn 8, $A_{\max}$ is the upper limit (maximum) for absorption of herbicide, t is the time, Absorption is the percentage of absorbed herbicide at time t, and t_{90} refers to the time required to achieve 90% of the maximum absorption.

3 RESULTS

3.1 Dose–response of glyphosate

At 4 WAT, ED_{50} values for glyphosate on P1 kochia at T1 and T2 were 39 and 68 g ha^{-1} [Table 1; Fig. 1(a)], respectively. However, the GR_{50} values for glyphosate at T1 and T2 were 34 and 42 g ha^{-1} for this

Table 1. Glyphosate and dicamba dose–response analysis of kochia visual injury and dry biomass under three different temperatures at 4 weeks after treatment*

				Parameter estimate (dry biomass)†			
Herbicide	Kochia population (site of collection)	Temperature (day/night, °C)	ED_{50} (g · ha^{-1})	GR_{50} (g · ha^{-1})	*b*	*C* (g)	*D* (g)
Glyphosate	P_1 (Pratt County, KS)	17.5/7.5	39 (2.4)^a	34 (5.2)^a	4.34 (1.78)	0.04 (0.01)	0.22 (0.02)^a
		25/15	68 (4.4)^b	42 (11)^a	2.10 (1.18)	0.06 (0.08)	0.95 (0.11)^b
		32.5/22.5	173 (10)^c	171 (55)^b	2.90 (2.28)	0.03 (0.20)	1.27 (0.19)^{bc}
	P_2 (Riley County, KS)	17.5/7.5	36 (2.2)^a	46 (1.2)^a	4.32 (0.55)	0.05 (0.01)	0.55 (0.01)^a
		25/15	68 (6.3)^b	67 (1.8)^b	3.40 (0.22)	0.11 (0.01)	1.24 (0.02)^{ab}
		32.5/22.5	186 (9.8)^c	187 (8.3)^c	3.49 (0.43)	0.08 (0.03)	1.48 (0.23)^b
Dicamba	P_1 (Pratt County, KS)	17.5/7.5	52 (2.4)^a	21 (15)^a	1.96 (3.06)	0.07 (0.06)	0.60 (0.07)^a
		25/15	54 (3.4)^a	26 (16)^a	1.46 (1.94)	0.08 (0.16)	1.28 (0.04)^b
		32.5/22.5	106 (6.5)^b	73 (19)^b	3.99 (5.16)	0.06 (0.26)	1.46 (0.07)^c
	P_2 (Riley County, KS)	17.5/7.5	105 (9.7)^a	46 (15)^a	0.48 (0.08)	0.24 (0.12)	0.59 (0.05)^a
		25/15	167 (34)^a	114 (35)^a	0.68 (0.37)	0.43 (0.11)	0.95 (0.06)^b
		32.5/22.5	410 (36)^b	225 (6.3)^b	2.76 (0.16)	0.01 (0.02)	1.51 (0.02)^c

*Values (mean ± standard error) followed by different letters are significantly ($P < 0.05$) different in each column for each population. ED_{50} values were calculated using visual injury data.
†The four parameters log-logistic model was used for estimation [see Eqn 7, for details].

population [Table 1; Fig. 1(b)]. Differences between T1 and T2 were significant ($P < 0.05$) for ED_{50} but not GR_{50}. However, when d/n temperature was increased to T3, both the ED_{50} and GR_{50} increased significantly ($P < 0.05$) to 173 and 171 g ha^{-1}, respectively in P1 kochia. The results of glyphosate dose–response on P2 kochia population [Table 1; Fig. 1(c and d)] showed similar tendency of growth temperature effects on glyphosate efficacy as described above for P1 kochia. ED_{50} values for glyphosate on P2 were 36, 68 and 176 g ha^{-1} at T1, T2, and T3, respectively, whereas the GR_{50} were estimated as 46, 67, and 187 g ha^{-1}, respectively. Both ED_{50} and GR_{50} of glyphosate on P2 increased significantly as growth temperature increased. When the GR_{50} values were estimated in the four parameters log-logistic model using the raw data of dry biomass, the estimates for other parameters were also generated for glyphosate and listed in Table 1. The estimation of *D* values (the upper limit, which represents the dry biomass accumulation of untreated samples) of P1 and P2 were significantly different at T1 and T3 (Table 1). In general, the untreated kochia plants grown under cooler temperature (T1) produced three times more biomass than at high temperature [T3; Fig. 1(b and d) and Fig. 2(b and d)].

3.2 Dose–response of dicamba

At 4 WAT, both P1 and P2 kochia showed similar response to dicamba when grown at different temperatures. The ED_{50} [Table 1, Fig. 2(a)] of dicamba for P1 kochia was 52, 54, and 106 g ha^{-1} at T1, T2, and T3, respectively. On the basis of dry biomass, GR_{50} [Table 1, Fig. 2(b)] of dicamba for P1 kochia was 21, 26, and 73 g ha^{-1} at T1, T2, and T3, respectively. Likewise, ED_{50} of 105, 167, and 410 g ha^{-1} and GR_{50} of 46, 114 and 225 g ha^{-1} at T1, T2, and T3 [Table 1, Fig. 2(c and d)], respectively, are estimated for P2 kochia. The efficacy of dicamba on both P1 and P2 decreased when temperature was increased from T2 to T3, but not from T1 to T2. Also, estimation of the four parameters for dicamba using raw dry biomass data was also determined and listed in Table 1. The dry biomass accumulation of untreated samples (*D* values) was significantly different among the three temperature regimes, which indicates temperature has siginificant effect on growth of kochia.

3.3 Absorption and translocation of glyphosate

Analysis of the data of ^{14}C-glyphosate absorption/translocation (Table 2) indicates the upper limit of absorption of ^{14}C-glyphosate (A_{max}) as 71, 70, and 41% at T1, T2, and T3, respectively. When the A_{max} at different temperatures was compared, significantly less ^{14}C-glyphosate was absorbed by kochia at T3 than at T1 or T2. Similarly, analysis of the data by regression model also suggest the time required to achieve 90% of the maximum absorption (t_{90}, Table 2) as 188, 144, and 313 h for T1, T2, and T3, respectively, but the comparison of t_{90} at different temperatures showed the time differences were not significant among the three temperature regimes. Interestingly, regardless of the amount of ^{14}C-glyphosate absorbed, there was no significant difference in the percent of ^{14}C-glyphosate translocated (Fig. 3b) either to ATL or BTL of kochia grown under any of the temperature regimes tested (Fig. s 3d to 3e). Overall, absorption of ^{14}C-glyphosate was significantly reduced when kochia was grown under T3 (Fig. 3a). However, translocation of ^{14}C-glyphosate in kochia appeared not to be influenced by alterations in temperature (Fig. 3b). Therefore, reduced absorption of glyphosate may contribute to the lack of control of kochia grown under high temperature.

3.4 Absorption and translocation of dicamba

Similar to absorption of glyphosate, the upper limit of dicamba absorption (A_{max}) and time required to achieve 90% of the maximum absorption (t_{90}) were generated using regression analysis, and the results are listed in Table 2. The data suggest A_{max} of 99, 98, and 100%, and t_{90} of 57, 36, and 48 h for T1, T2, and T3, respectively. However, in contrast to glyphosate, the data of the A_{max} and t_{90} of dicamba was not significantly affected by temperature. While absorption of dicamba increased with time, translocation out of the TL also increased [Fig. 4(b)], regardless of temperature. Translocation of ^{14}C-dicamba at 12 and 72 HAT increased from 26 to 47% and 20 to 58% at T1 and T2, respectively [Fig. 4(b)]. In

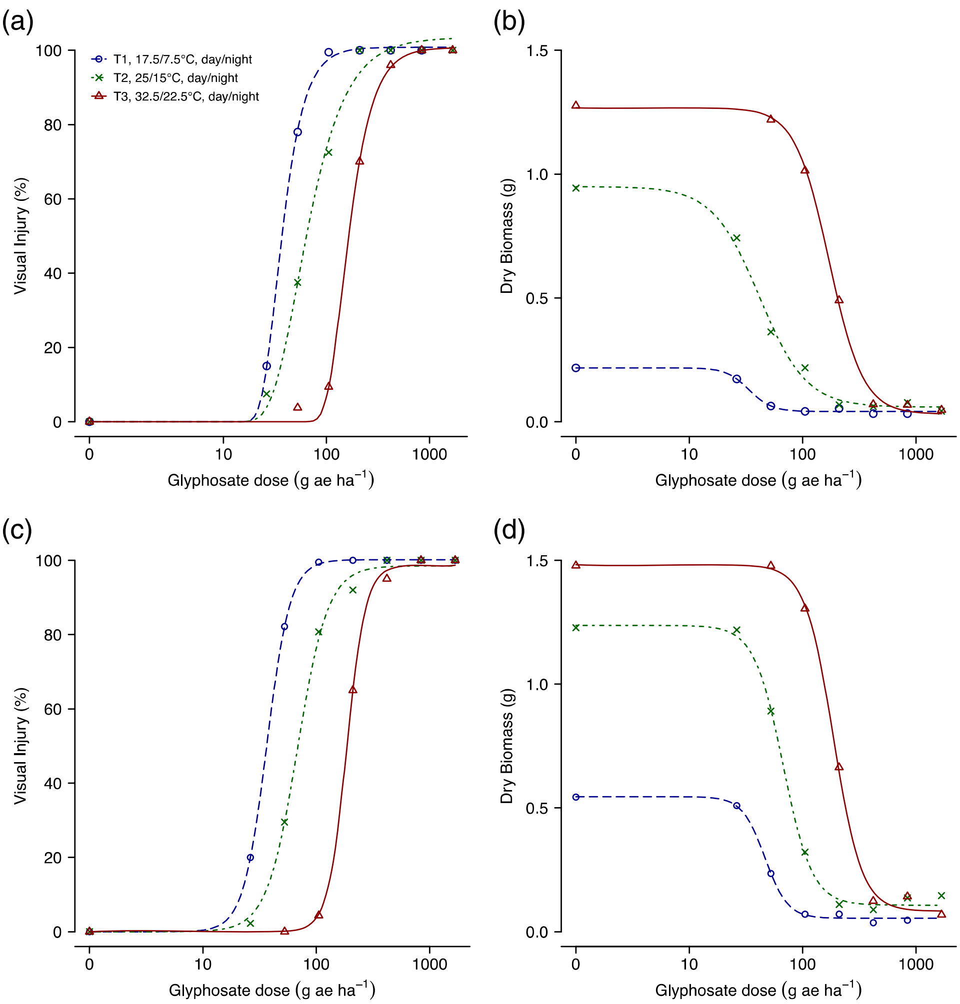

Figure 1. Whole-plant glyphosate dose–response of kochia at different temperatures as measured by (a) visual injury (P1), (b) dry biomass (P1), (c) visual injury (P2), and (d) dry biomass (P2) at 4 WAT.

contrast, at 72 HAT translocation of ^{14}C-dicamba increased from only 6.9% to 21% in kochia grown at T3. This means 20–30% more ^{14}C-dicamba was retained in the TL [Fig. 4(c)] of kochia grown at T3, than in kochia grown at T1 or T2. More importantly, at 12 HAT, 16.5% and 16% of ^{14}C-dicamba was translocated to ATL at T1 and T2, respectively, but only 3.2% moved towards meristems in kochia grown at T3 [Fig. 4(d)]. Conversely, there was no difference ($P > 0.05$) in the amount of ^{14}C-dicamba translocated to BTL [Fig. 4(e)] in kochia grown at any of the temperature regimes tested. Thus, the poor control of kochia grown under high temperature may be attributed to decreased translocation of dicamba to above treated leaves.

4 DISCUSSION

In western Kansas, kochia emerges early- to mid-March and continues into April[2] when d/n temperatures are normally about 17.5/7.5°C.[21] Thereafter, kochia emergence slows down but some seeds can still emerge throughout the growing season. After the major flush of emergence in March to April, kochia starts to grow and accumulates biomass when the d/n temperatures increase to 25/15°C.[21] Post-application of glyphosate or dicamba to control kochia is normally done in mid- to late-June after crop emergence or in July for post-wheat harvest applications when the d/n temperatures are soaring to 32.5/22.5°C or higher. This validates selection of these three d/n temperature regimes in this study.

In the dose–response experiments, we found the efficacy of glyphosate decreased significantly when the d/n temperatures were increased from 25/15°C to 32.5/22.5°C. Similar results were observed for GR_{50} of glyphosate for P2 kochia [Fig. 1(d)], except the GR_{50} of glyphosate at T1 and T2 on P1 kochia were not significantly different whereas the ED_{50} of glyphosate on P1 kochia, and both the ED_{50} and GR_{50} of glyphosate on P2 kochia were significantly different. These results clearly indicate that plant growth temperature had substantial impact on the efficacy of glyphosate in controlling kochia. Additionally, the nonsignificant estimation of *C* values (data not shown) in the four parameter log-logistic model indicates that kochia (both P1 and P2) accumulated different amounts of dry biomass at all temperatures tested in response to the high rates (lethal rates or higher) of glyphosate or dicamba. This difference in biomass accumulation within each population can be attributed to the inherent genetic variability, which is expected among field populations of kochia. In contrast, in response to any rate of glyphosate or dicamba applied, (except for P1 at T1), the estimation of dry biomass accumulation of untreated samples (*D* values) of both P1 and P2 (Table 2) was significant at all temperature regimes. Specifically, there was significantly higher (about two times) biomass accumulation at T3

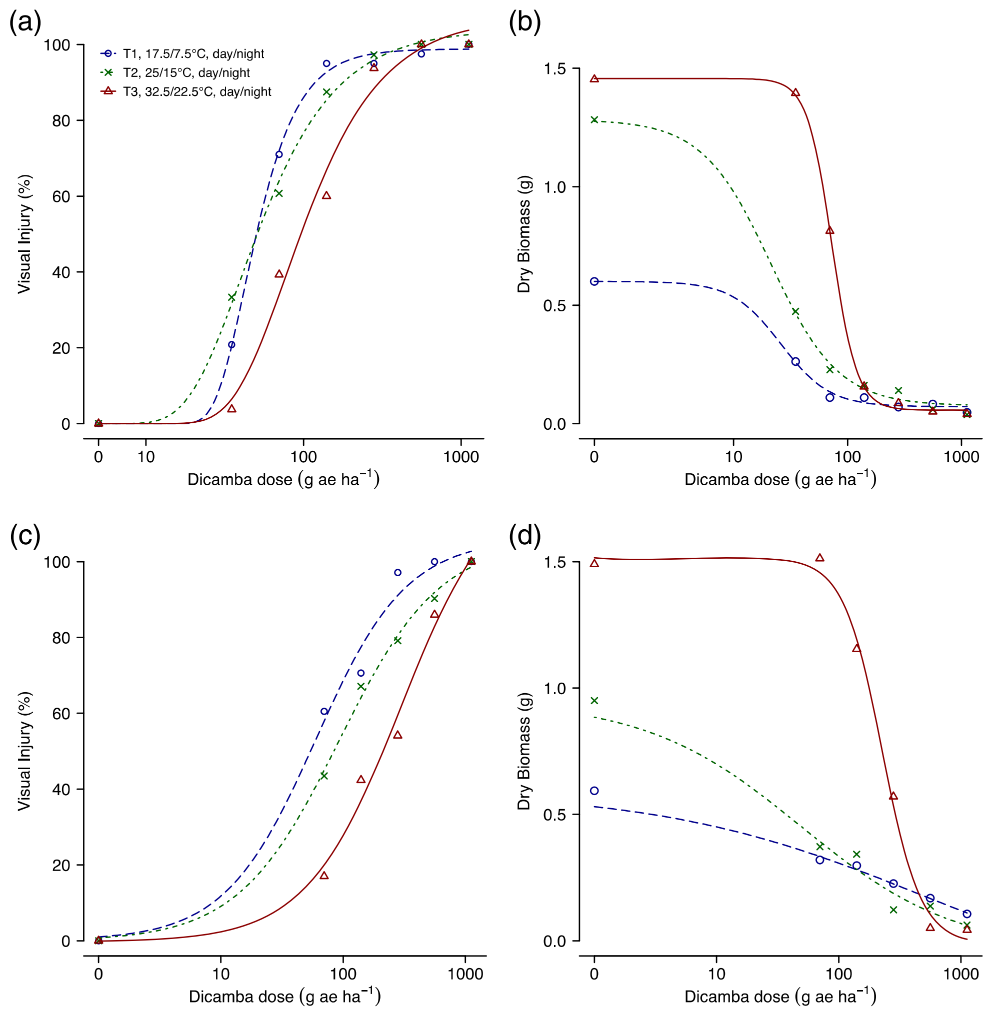

Figure 2. Whole-plant dicamba dose–response of kochia at different temperatures as measured by (a) visual injury (P1), (b) dry biomass (P1), (c) visual injury (P2), and (d) dry biomass (P2) at 4 WAT.

than at T1, for P1 and P2 kochia [Fig. 1(b and d) and Fig. 2(b and d)], which clearly suggests that kochia growth was substantially affected by temperature. The difference in biomass accumuling of kochia at different temperatures may influence the absorption or translocation of herbicides. In general, larger plants are more tolerant to herbicides than the smaller plants. The decreased efficacy of dicamba or glyphosate on kochia grown under high temepratures, possibly because of dilution effect that caused by rapid growth and high biomass accumulation.[22]

It is known that even with the addition of surfactants, relatively low amounts of applied glyphosate is absorbed by leaves[23] compared to other systemic herbicides such as dicamba. Our data also show less than 60% of glyphosate absorbed by kochia at 72 HAT [Fig. 3(a)]. More importantly, plants typically develop thick, lipophilic cuticles to prevent water loss at high temperature.[24,25] Therefore, when grown under high temperatures (T3) kochia may develop thicker cuticle, which may have contributed to reduced absorption of glyphosate even when the herbicide was formulated with surfactants.[26] As we observed in our glyphosate dose–response experiments, efficacy of glyphosate was decreased at high temperatures, which is highly interrelated with our absorption and translocation data. We conclude the decreased efficacy of glyphosate on kochia at high growth temperature was due to decreased absorption of this herbicide.

In the dicamba experiment, GR_{50} and ED_{50} dosages for P2 kochia plants were three and four times higher, respectively, compared to GR_{50} and ED_{50} dosages for P1 kochia plants (Table 1), indicating greater tolerance to dicamba in P2 kochia. Yet, the increase in d/n temperature from 25/15°C to 32.5/22.5°C reduced the efficacy of dicamba on both P1 and P2 kochia. Based on the dose–response results it is evident that efficacy of dicamba on kochia control did not differ when plants were grown under temperature regimes of 17.5/7.5°C or 25/15°C; however, efficacy was significantly decreased when they were exposed to 32.5/22.5°C. In the physiological mechanism study, no difference was found in the amount of ^{14}C-dicamba absorbed by kochia grown at the temperatures tested in this experiment. However, less ^{14}C-dicamba was translocated to ATL in kochia grown at T3 than at T1 or T2, while the amount of ^{14}C-dicamba translocated to BTL was not affected by temperature. Reduced dicamba efficacy on kochia grown at T3 compared to T1 or T2 (Fig. 2), likely was because of reduced translocation of dicamba to actively growing meristems at T3 [Fig. 4(b)]. Dicamba is a systematic herbicide and must be translocated to the meristems[27] to obtain satisfactory weed control. Therefore, the lack of kochia control with dicamba treatment at high temperature (i.e. T3) can be attributed to reduced translocation of this herbicide.

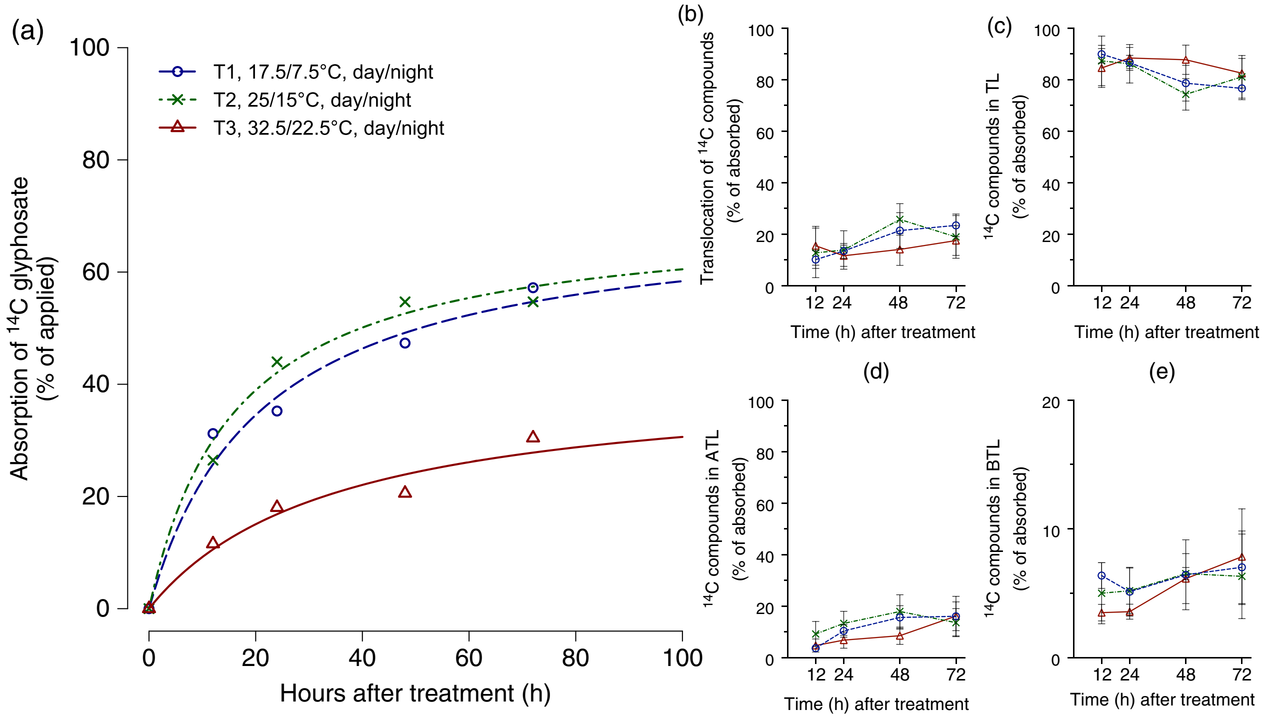

Figure 3. (a) ^{14}C-glyphosate absorption, (b) translocation, (c) retained in treated leaf, (d) translocation to above treated-leaf, and (e) below treated-leaf at three different temperatures. (*P-value < 0.05, **P-value < 0.01, ***P-value < 0.001, which indicate the levels of significance within each time points at different temperatures; error bars represent standard error).

Table 2. Regression parameter estimates of glyphosate absorption in kochia at different temperatures using rectangular hyperbolic model *

Herbicide	Temperature (day/night, °C)	Parameter estimate A_{max}	Parameter estimate t_{90}
Glyphosate	17.5/7.5	70.58 (5.77)^a	188.03 (44.23)^a
	25/15	70.22 (4.32)^a	144.55 (29.79)^a
	32.5/22.5	41.28 (9.05)^b	313.67 (155.89)^a
Dicamba	17.5/7.5	98.66 (3.38)^a	57.19 (11.73)^a
	25/15	97.78 (3.13)^a	35.62 (8.86)^a
	32.5/22.5	100.0 (3.05)^a	47.92 (9.46)^a

*See Eqn 8 for the equation of the rectangular hyperbolic model. Values with different superscript letters are significantly ($P < 0.05$) different in each column for each herbicide.

Dicamba absorbed into plant cells can be trapped in phospholipid vesicles due to a hydrophobic interaction between the non-polar portion of dicamba molecule and the hydrocarbons present in the phospholipid vesicles.[28] Since dicamba is predominantly translocated via symplast,[29] it is prone to becoming trapped in phospholipid vesicles. It is also known that increased temperature can enhance the strength of hydrophobic interactions of organic molecules.[30] Therefore, in this study, when dicamba was applied on kochia grown under higher temperature, though the absorption of dicamba was not affected [Fig. 4(a)], it is possible that dicamba may have attached to phospholipid vesicles in leaf cells, resulting in lack of movement of this molecule from the site of absorption. Additional study is needed to test this hypothesis.

Furthermore, dicamba is volatile and increased temperature can also accelerate the volatilization of dicamba, regardless of the type of dicamba formulation used.[31] Under field conditions, vapor or spray drift of dicamba can cause severe crop damage on soybean,[3] tomatoes,[32] and corn,[33] especially on hot days. Therefore, applying dicamba during periods of high temperature not only reduces kochia control but also increases the risk of off-target crop injury. Dicamba is an auxinic herbicide and sensitive plants show severe injury symptoms (e.g. epinasty, meristem inhibition, etc.) when treated or exposed to low doses[34] of off-target drift. However, dicamba kills susceptible plants slowly. Some of the plants treated with higher than field recommended doses of dicamba in this experiment, although injured severely, still had green tissues at 4 WAT. As a result, it is easy to underestimate dicamba injury symptoms. This can explain the variation in values obtained for ED_{50} when compared to GR_{50} at each temperature regime.

5 CONCLUSION

Although the mechanisms responsible for the reduced efficacy of dicamba or glyphosate may differ, our results clearly show that kochia is less sensitive to both these herbicides when grown under higher temperatures, especially at 32.5°C. This research provides evidence to support the anecdotal observations made in the field regarding reduced efficacy of herbicides such as dicamba or glyphosate at high temperature. Therefore, to maximize efficacy of glyphosate and dicamba on kochia and minimize the chances of losing these effective tools for controlling kochia, it will be critical to take action and apply glyphosate or dicamba early in the season after the main flush of kochia emergence when the temperature is low (e.g. day/night temperature at 25/15°C, or even lower) and the kochia seedlings are small (less than 12 cm).

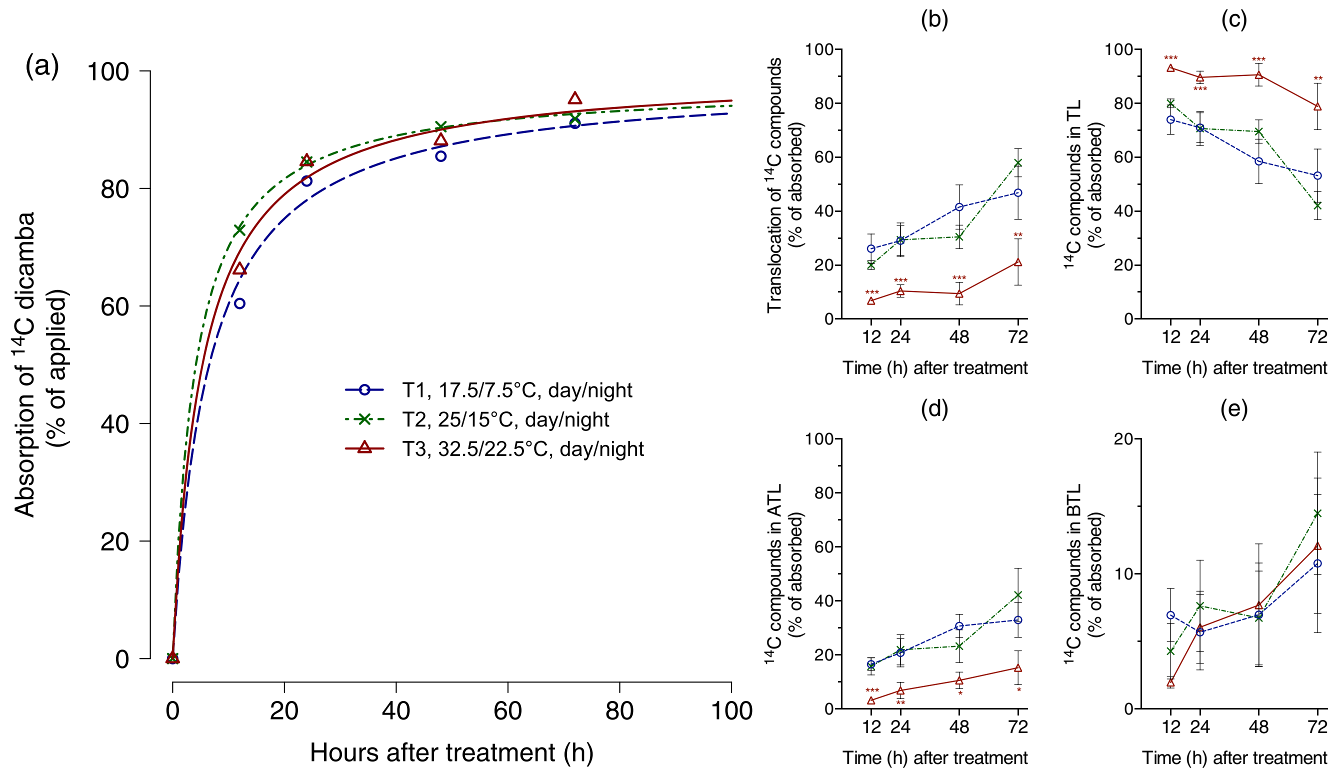

Figure 4. (a) ^{14}C-dicamba absorption, (b) translocation, (c) retained in treated leaf, (d) translocation to above treated-leaf, and (e) below treated-leaf at three different temperatures. (***P*-value < 0.01, ****P*-value < 0.001, which indicate the levels of significance within each time points at different temperatures; error bars represent standard error).

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