GLYPHOSATE RESISTANCE IN KOCHIA (*KOCHIA SCOPARIA*)

by

JASON CHARLES WAITE

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Major Professor
Kassim Al-Khatib

Abstract

Kochia (*Kochia scoparia* (L.) Schrad) is a troublesome weed throughout the western United States and Great Plains. It is an aggressive warm season annual dicot plant that exhibits protogynous flowering and facultative open pollination. The aggressive growth habit and prolific seed production enable kochia to spread and compete well for light, moisture, and nutrients. Kochia is ranked as one of the most problematic weeds in cultivated fields including corn, sorghum, wheat, soybean, and sugarbeet. Kochia has been found to lower yields as well as hinder mechanical harvest. Glyphosate is a nonselective herbicide that is widely used in controlling kochia in no-till cropping systems. With rapid adoption of no-till systems where glyphosate is used for weed burndown treatment before planting and extensive use of glyphosate resistant crops, it is common that glyphosate is frequently applied on the same field during the growing season. In 2007, poor control of kochia was observed in three fields in Western Kansas. Greenhouse experiments were conducted with 10 kochia populations to determine the efficacy of glyphosate on kochia when applied at 10 different rates and at 3 plant heights. Herbicide rates included 0, 0.0625, 0.125, 0.25, 0.50, 1, 1.5, 2, 4 and 6 times a typical use rate of 870 g ae/ha. Resistance to glyphosate was identified in three kochia populations. The glyphosate resistant populations from Ingalls, Norton, and Moscow Kansas were 4.6, 3.3, and 2.8 times more resistant to glyphosate than a susceptible population, respectively, based on the rate required for 50% control. Glyphosate injury symptoms included stunting, and chlorosis, followed by some necrotic tissue but resistant plants generally recovered from injury, or were slow to show symptoms. In general, the level of resistance is greater in more developed plants compared to younger plants. Experiments also were conducted on the different kochia biotypes to evaluate glyphosate absorption and translocation, and any differences in mineral content of the plants that might be detrimental to glyphosate activity. Differences in glyphosate absorption and translocation and kochia mineral content were not sufficient to explain the resistance to glyphosate.

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Introduction

Kochia (*Kochia scoparia* (L.) Schrad) is an invasive broadleaf weed infesting millions of hectares of cropland and natural areas in the United States and Canada. It is an aggressive warm season annual dicot plant that exhibits protogynous flowering and facultative open pollination (Eberlin and Fore 1984). Protogynous flowering allows the stigma to be receptive before pollen is shed from the anthers from the same flower. The aggressive growth habit and prolific seed production enable kochia to spread and compete well for light, moisture, and nutrients (Milchunas et. al. 1992; Stevens 1932; Dwyer and Wolde-Yohannis 1972; Wiese and Vandiver 1970). At plant maturity an abscission zone develops at the base of the stem causing plant disengagement (Zeroni et al. 1978). Therefore, these plants may tumble by the wind and travel long distances spreading seeds across the landscape.

Kochia is a troublesome weed that ranks as one of the most problematic weeds in cultivated fields including corn, sorghum, wheat, soybean, and sugarbeet (Dexter and Luecke 2001). Kochia presence can lower grain yield and quality as well as hinder mechanical harvest (Manthey et al. 1996). For example, kochia competition from 6 plants m^{-1} reduced sunflower yield by 27% (Durgan et al. 1990), whereas competition from 70 plants m^{-1} of row decreased wheat yield by 58% and grain sorghum by 38% (Wicks et al. 1994). Corn grain yields decreased 0.33 kg ha^{-1} for each kg ha^{-1} of kochia biomass produced (Wicks 1993 et al.). In addition, when kochia was allowed to compete with sugarbeet for full season, yield was reduced to 225 kg ha^{-1} compared to 49177 kg ha^{-1} when kochia was removed 4 weeks after sugarbeet emergence (Weatherspoon and Schweizer 1969).

Tillage is an effective practice to control kochia (Ball and Miller 1990; Wilson and Anderson 1981). With rapid adoption of no-till systems, chemical control has become the preferable method to control weeds (Blackshaw 1990: Weatherspoon and Schweizer 1971). Kochia emergence, however, was found to increase almost four fold under no-till compared to tilled systems (Anderson and Nielsen 1996). In addition, in many parts of the western United States, kochia may emerge over an extended period of time from early spring through late July when most crops emerge (Weatherspoon and Schweizer 1969). Kochia frequently emerged after the last postemergence herbicide application making kochia a troublesome plant at crop harvest (Mickelson et al. 2004).

Several soil active herbicides applied before or at planting may effectively control kochia (Thompson et al. 2010). Under dry soil conditions, however, most soil applied herbicides have low efficacy due to lack of moisture for activation (Moyer 1987). In many cropping systems, it is common to control kochia before planting by using postemergence nonselective herbicides such as glyphosate (Donald and Prato 1991). It is critical, however, to apply glyphosate early in the spring before plants become too large and difficult to kill (Schwinghamer 2008). A previous report showed that timely glyphosate application provided up to 99% kochia control (Donald and Prato 1991). Because of high efficacy and the decline in glyphosate cost in recent years, growers are extensively using glyphosate to control kochia prior to crop planting. In addition, repeated glyphosate use in crops has increased due to the widespread use of glyphosate resistant crops. In many instances, glyphosate was sprayed more than once during the growing season. However, reliance on herbicides with the same mode of action for extended periods can contribute to weed shifts and the selection of biotypes with resistance to herbicides (Holt 1992, Knezevic 2007).

Currently there are 19 known weed species with evolved resistance to glyphosate (Heap 2010). Levels of resistance to glyphosate are considered low compared to other mode of action herbicides such as photosystem II (PSII), acetolactate synthase (ALS), or acetyl CoA carboxylase (ACCase) (Feng et al. 2004, Gressel 2002, Owen and Powles 2010). For example, rigid ryegrass resistance to glyphosate is several folds more resistant to glyphosate than a susceptible biotype, whereas the resistance to ALS-inhibiting herbicides is hundreds of folds greater than the susceptible biotypes (Powles et al. 1998). Kochia has been reported to be resistant to triazines (Johnston and Wood 1976), acetolactate inhibiting herbicides (Primiani et al. 1990) and dicamba (Cranston et al. 2001).

It has been found that calcium and magnesium ions can antagonize the efficacy of glyphosate (Mueller et al. 2006). This antagonism is due to the formation of chelates between glyphosate molecules and polyvalent cations Ca^{2+} and Mg^{2+} (Shea and Tupy 1984).

In 2007, poor control of kochia with glyphosate was observed in three fields near Ingalls, Moscow, and Norton, Kansas. These fields were 300 km apart and under different cropping systems. The field in Ingalls was irrigated and in a soybean/corn/wheat rotation whereas the field near Moscow was an irrigated corn/cotton/wheat rotation and the field in Norton was in a soybean/wheat rotation under rainfed conditions. Our preliminary research showed these kochia populations were less susceptible to glyphosate (Waite et al. 2009). The objectives of this research were to determine if kochia populations collected from the Norton, Moscow, and Ingalls sites are resistant to glyphosate and examine possible modes of resistance in these populations.

Materials and Methods

Plant Materials. Kochia seeds were collected from 10 sites from different geographical areas to ensure adequate kochia genetic variability. These populations include; Eden (EDID), Jerome County (JCID), and Minidoka County (MCID), Idaho; Hays (HAKS), Ingalls (INKS), Norton (NTKS), Moscow (MOKS), and Syracuse (SYKS), Kansas; Irrigated Agriculture Research and Extension Center, Washington (IAREC), and Prosser (PRWA), Washington. INKS, NTKS and MOKS were collected from populations suspected to be resistant to glyphosate. These sites have a history of repeated glyphosate use. The resistance levels of the remaining populations were unknown prior to the study but the sites have infrequent use of glyphosate.

Kochia seeds were planted in 50 x 35 x 10 cm flats filled with 11 kg of a soil mix. The soil mixture was 1:1 by volume blend of sand and Morrill loam (fine-loamy, mesic Typic Arguidolls). The soil had 1.0% organic matter and a pH of 7.5. Single kochia seedlings were transplanted into 0.9 L containers. Plants were grown under greenhouse conditions at 28/25 $\pm$ 2 C day/night temperatures and 16/8 h day/night periods. The supplemental photosynthetic photon flux was 80 µmol m^{-2} s^{-1}. Plants were fertilized[1] weekly with a solution containing 0.40 mg L^{-1} nitrogen, 0.34 mg L^{-1} phosphorus, and 0.33 mg L^{-1} potassium.

Dose Response Study. Kochia populations were planted on the same day. Kochia biotypes were treated with glyphosate when the plants were 15 to 20 cm in height. Glyphosate was applied at 0, 0.0625, 0.125, 0.25, 0.50, 1, 1.5, 2, 4 and 6 times a typical use rate of 870 g ae/ha. The glyphosate source for all experiments was Roundup WeatherMax[2]. All treatments included 0.25% (v/v) non-ionic surfactant[3] and 2.0% (w/v) ammonium sulfate[4]. Treatments were applied with a bench-type sprayer[5] calibrated to deliver 187 L ha^{-1} at 138 kPa. Visible injury symptoms were monitored daily and injury ratings were determined at 21 days after treatment (DAT), based on 0 = no injury and 100 = mortality. Plant height and dry weights were determined at 21 DAT.

Stage of Growth Study. Kochia populations were planted on the same day and selected for treatment at application height. Kochia populations from INKS, EDID, IAREC and NTKS sites were selected for this study because INKS and NTKS biotypes expressed highest glyphosate resistance whereas EDID and IAREC were the most susceptible biotypes in the dose response study. Seedlings were treated with glyphosate at 6, 13 and 25 cm growth stages. Glyphosate was applied at 0, 0.0625, 0.125, 0.25, 0.50, 1, 1.5, 2, 4 and 6 times a typical use rate of 870 g ae/ha as described in the dose response study. Visible injury symptoms were monitored daily and injury ratings were determined at 21 DAT and were based on 0= no injury and 100= mortality.

Calcium and Magnesium Content Study. Plants containing elevated levels of calcium and magnesium could be less susceptible to glyphosate due to reduced herbicide activity. A study was designed to determine if kochia plants with resistance to glyphosate have higher calcium or magnesium levels that inactivate glyphosate as suggested by Schuster et al. (2007). Kochia populations were all planted on the same day. Kochia seedlings grown from seed collected from INKS, JCID, IAREC and NTKS sites were grown as described in the growth stages study. Plants were harvested at 6, 13 and 25 cm growth stages as described by Schuster et al. (2007). Plants were dried at 65 C for 96 h, then ground to a fine powder then tissues were digested by using perchloric acid (Gieseking et al. 1935). Calcium and magnesium content was determined with an inductively coupled plasma spectrometer[6].

Glyphosate Absorption and Translocation Study. INKS, EDID, IAREC and NTKS kochia populations were selected for the glyphosate absorption and translocation study because of their diverse response to glyphosate. Uniform 22-cm tall kochia plants were treated with an 870 g ae ha^{-1} glyphosate rate as described in the dose response study and then immediately treated with ^{14}C-glyphosate ([phosphonomethyl-^{14}C]-glyphosate, specific activity 1094 MBq g^{-1}). Two 1-µl droplets containing a total of 1300 Bq ^{14}C-glyphosate were applied to the upper surface of the middle of the leaf at the eighth node from the bottom of the plant. Glyphosate mixture included 0.5% (v/v) crop oil concentrate[7] to help facilitate droplet contact to the leaf surface.

Plants were harvested at 1, 3 and 7 DAT and separated into treated leaf, above treated leaf, below treated leaf and roots. Treated leaves were rinsed with 15 ml of deionized water to remove any unabsorbed glyphosate. The treated leaf was then cut into basal, central and distal thirds. Plant sections were oven dried and oxidized[8] as described by Al-Khatib et al. (1992). The trapped $^{14}CO_2$ and radioactivity in the leaf rinsate were quantified by liquid scintillation spectrometry[9]. Absorption was calculated by comparing the radioactivity recovered in the entire plant with the total amount applied. Herbicide translocation was computed as the amount of radioactivity recovered in a given plant part as a percentage of the total radioactivity in the plant.

Experimental Design and Data Analysis. Dose response and stage of growth experiments were conducted as randomized complete block designs. In the dose response, stage of growth, calcium and magnesium content, and absorption and translocation studies, treatments were replicated 14, 4, 6, and 6 times, respectively, and all experiments were conducted twice. All data were tested for homogeneity of variance, subjected to ANOVA, and pooled when interactions did not occur. Means were separated by Fischer's Protected LSD at <0.05. For the dose response and stage of growth studies, nonlinear regression analysis was used to determine glyphosate rate required to cause 50% visible injury (GR_{50}) (Seefeldt et al. 1995). Resistant index (RI) was calculated by dividing GR_{50} for each population by the GR_{50} for JCID, which was the most glyphosate susceptible population in the dose response study.

Results and Discussion

Dose-response Study. In general, kochia injury increased as the rate of glyphosate increased. Glyphosate injury symptoms were general chlorosis, necrosis and stunting. Symptoms were more severe in glyphosate susceptible populations from JCID, IAREC, MCID, PRWA, EDID, HAKS and SYKS where symptoms appeared 3 DAT and peaked at 7 to 10 DAT. However, similar symptoms were evident the resistant populations. At rates above the use rates, glyphosate symptoms on resistant populations appeared 5 DAT and slowly progressed through the duration of the study. Rates below field use rates caused little to no injury on resistant populations but caused severe injury to susceptible populations. At 21 DAT, glyphosate applied at the use rate caused 4, 10, 12, 68, 69, 77, 78, 79, 82, and 91% injury at NTKS, INKS, MOKS, HAKS, MCID, SYKS, EDID, PRWA, IAREC, and JCID populations, respectively. Injury ratings were estimated from dose response curves to be 82% to 62% at NTKS and INKS populations that survived a 3 X rate of glyphosate treatment whereas susceptible populations were killed (data not shown).

The resistant populations from INKS, NTKS, and MOKS had GR_{50} values of 2.47, 1.80 and 1.52 times the use rate, respectively (Table 1). PRWA, EDID and JCID populations exhibited greater susceptibility to glyphosate with GR_{50} values of 0.69, 0.69 and 0.54, respectively. Three other populations showed an intermediate response. The glyphosate resistant index for INKS, NTKS and MOKS was 4.57, 3.33 and 2.81 respectively suggesting considerable resistance to glyphosate. GR_{50} based on dry weights and plant heights of different kochia populations showed a similar pattern to visible injury (data not shown). These results are in agreement with the observation in the fields where kochia populations showed differential response to glyphosate.

Glyphosate resistant ranking in kochia populations was INKS > NTKS > MOKS > SYKS > HAKS > MCID > PRWA > EDID > IAREC > JCID. Resistance to glyphosate in INKS, NTKS, and MOKS populations is not surprising because these populations were from fields that had a previous history of repeated glyphosate use and producer complaints of poor glyphosate control. Glyphosate is used for a burndown as well as postemergence treatment in glyphosate resistant crops at the INKS, NTKS and MOKS while little glyphosate is used on the Washington and Idaho populations.

Stage of Growth Study. Glyphosate injury symptoms were similar to the dose response study. At 21 DAT, the INKS population was the most glyphosate resistant population at 6, 13 and 25 cm with GR_{50} values of 0.94, 1.81 and 2.62 respectively (Table 2). NTKS was the second most glyphosate resistant at 6, 13 and 25 cm with GR_{50} values of 0.78, 1.48 and 1.80 respectively. IAREC was the most glyphosate susceptible population with GR_{50} values of 0.25 and 0.42 at the 6 and 13 cm growth stages, respectively (Figure 1 vs. Figure 2), while EDID was the most glyphosate susceptible population at the 25 cm height with a GR_{50} value of 0.82 (Figure 3). The increase in glyphosate injury in younger plants agrees with the work of others who have shown that younger plants are more likely to be metabolically active and more susceptible to glyphosate (Chachalis et al. 2001; Devine et al. 1993). The decrease in glyphosate injury at higher rates can be attributed to the ability of more mature plants to tolerate higher glyphosate rates because of morphological and anatomical properties such as a thicker cuticle, dust on leaf surface, spray water quality, spray volume and spray adjuvants, resulting in less herbicide absorption and injury (Peterson 2007; Chachalis et al. 2001; Devine et al. 1993; Post-Biettenmiller 1996; Wanamarta and Penner 1989).

Calcium and Magnesium Content Study. In general, calcium and magnesium contents were similar in all populations within each growth stage. However, slight differences were observed at 6 and 12 cm growth stages (Figure 4). In addition, calcium and magnesium content were lower at the 25 cm growth stage, when plants were least susceptible to glyphosate, compared to 6 cm growth stage where plants were more susceptible to glyphosate. Furthermore, similar calcium and magnesium contents were observed in glyphosate susceptible and resistant populations. These results are in disagreement with reports by Schuster et al. (2007) that showed higher calcium content in more developed common lambsquarters (*Chenopodium album* L.) plants that were relatively more tolerant to glyphosate treatment.

Glyphosate Absorption and Translocation Study. Glyphosate absorption was similar in glyphosate resistant and susceptible kochia (data not shown). At 7 DAT, glyphosate absorption was 62%, 70%, 65%, and 70% at INKS, IAREC, NTKS, and EDID, respectively. These results appear to be different from research that showed lower glyphosate absorption in glyphosate resistant Italian ryegrass that was attributed to leaf cuticle composition (Nandula et al. 2008). In addition, glyphosate translocation within the leaf (data not shown) and to other plant parts was similar in resistant and susceptible kochia populations (Figure 6). Again, these results are different from previous research that showed lower glyphosate translocation in resistant horseweed (*Conyza Canadensis* (L.) Cronq.) (Feng et al. 2004) and rigid ryegrass (Lorraine-Colwill et al. 2001), where altered cellular distribution impaired phloem loading and plastidic import of glyphosate resulting in reduced overall translocation and inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) EPSPS (Feng et al. 2004). However, no difference in glyphosate absorption and translocation was observed between glyphosate resistant and susceptible Palmer amaranth (Culpepper et al. 2006).

Previous research showed that resistance can be generally classified as target-site and non–target-site resistance based on the mechanisms of resistance (Sammons et al. 2007). Target-site resistance is due to a mutation in the herbicide target gene and a change in the binding of the herbicide (Preston et al. 2009). Non–target-site herbicide resistance is more complex and could involve several metabolic sequestration processes of the herbicide molecules (Yuan et al. 2007). Three major mechanisms of glyphosate resistance have been discovered in resistant species including a change in the pattern of glyphosate translocation such that glyphosate accumulates in the leaf tips of resistant plants instead of in the shoot meristem (Preston et al. 2009); amino acid substitutions at Pro 106 within the EPSPS (Preston et al. 2009; Sammons et al. 2007; Busi and Powles, 2009, Baerson et al. 2002 and Ng et al. 2003), and over expression of the EPSPS active site (Westra 2007). With the several resistance mechanisms observed in other species, it is assumed that further research is needed to explore possible glyphosate resistant mechanisms in kochia.

This research clearly showed that kochia biotypes from Ingalls, Norton, and Moscow in Kansas have resistance to glyphosate. Development of glyphosate-resistant kochia may negatively impact several cropping systems since glyphosate is the cornerstone of weed management programs. Integrated weed management programs that include prevention, monitoring, early detection, and integration of weed management practices and different modes of action will likely be the most effective approach to prevent and manage glyphosate resistant weeds.

Source of Materials

[1]Miracle-Gro soluble fertilizer, Scotts Miracle-Gro Products Inc., Consumer Products Division, Port Washington, NY 11050.

[2]Roundup WeatherMax EPA Reg. No. 524-537

[3]Non-ionic surfactant, Preference, Alkylpolyethoxylate and soybean based fatty acids, Winfield Solutions, LLC, 1080 Cty. Rd. F West, Shoreview, MN 55126

[4]Ammonium sulfate, Fisher Chemical, Fair Lawn, New Jersey 07410

[5]Research Track sprayer, De Vries Manufacturing, RR 1 Box 184, Hollandale, MN 56045.

[6]Plasma spectrometer, Agilent 7500 series ICP-MS, Agilent Technologies, 9780 South Meridian Boulevard, Englewood, CO 80112.

[7]Crop oil concentrate, Crop Oil Plus, a mixture of paraffinic oil plus emulsifiers, Land O' Lakes, P.O. Box 64089, St. Paul, MN 55164.

[8]R. J. Harvey Biological Oxidizer, Model OX-600, R. J. Harvey Instrument Co., 123 Patterson St., Hillsdale, NJ 07642.

[9]Tricarb 2100TR Liquid Scintillation Analyzer, Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450.

Table 1. GR_{50} and Resistant Index (RI) for ten kochia populations as affected by glyphosate.

Population	GR_{50}[a]	RI
Ingalls, KS, INKS	2.47	4.57
Norton, KS, NTKS	1.80	3.33
Moscow, KS, MOKS	1.52	2.81
Syracuse, KS, SYKS	0.79	1.46
Hays, KS, HKKS	0.78	1.44
Minidoka County, ID, MCID	0.75	1.38
Prosser, WA, PRWA	0.69	1.28
Eden, ID, EDID	0.67	1.28
Prosser, WA, IAREC	0.67	1.24
Jerome County, ID, JCID	0.54	1

[a]GR_{50} is the glyphosate rate required to cause 50% visible injury. Glyphosate was applied at 0, 0.0625, 0.125, 0.25, 0.50, 1, 1.5, 2, 4 and 6 times a typical use rate of 870 g ae/ha.

Table 2. GR_{50} and RI values for kochia as affected by glyphosate for stage of growth study.

Population	Height at Application (cm)	GR_{50}	RI
NTKS	6	0.78	3.12
EDID	6	0.40	1.6
INKS	6	0.94	3.76
IAREC	6	0.25	1
NTKS	15	1.48	3.52
EDID	15	0.70	1.66
INKS	15	1.81	4.31
IAREC	15	0.42	1
NTKS	25	1.80	2.19
EDID	25	0.82	1
INKS	25	2.62	3.19
IAREC	25	1.04	1.27

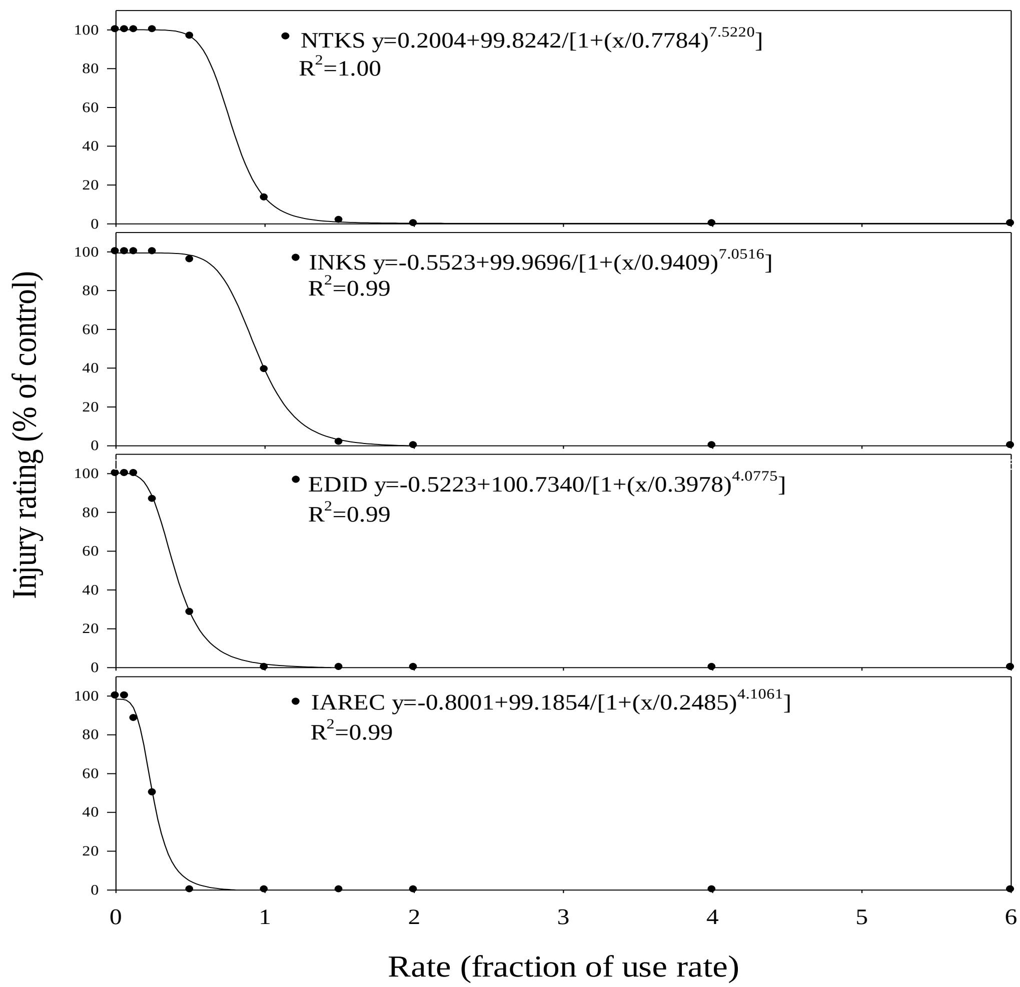

Figure 1. Visible injury ratings 21 DAT with glyphosate applied on 6 cm tall plants of four kochia populations.

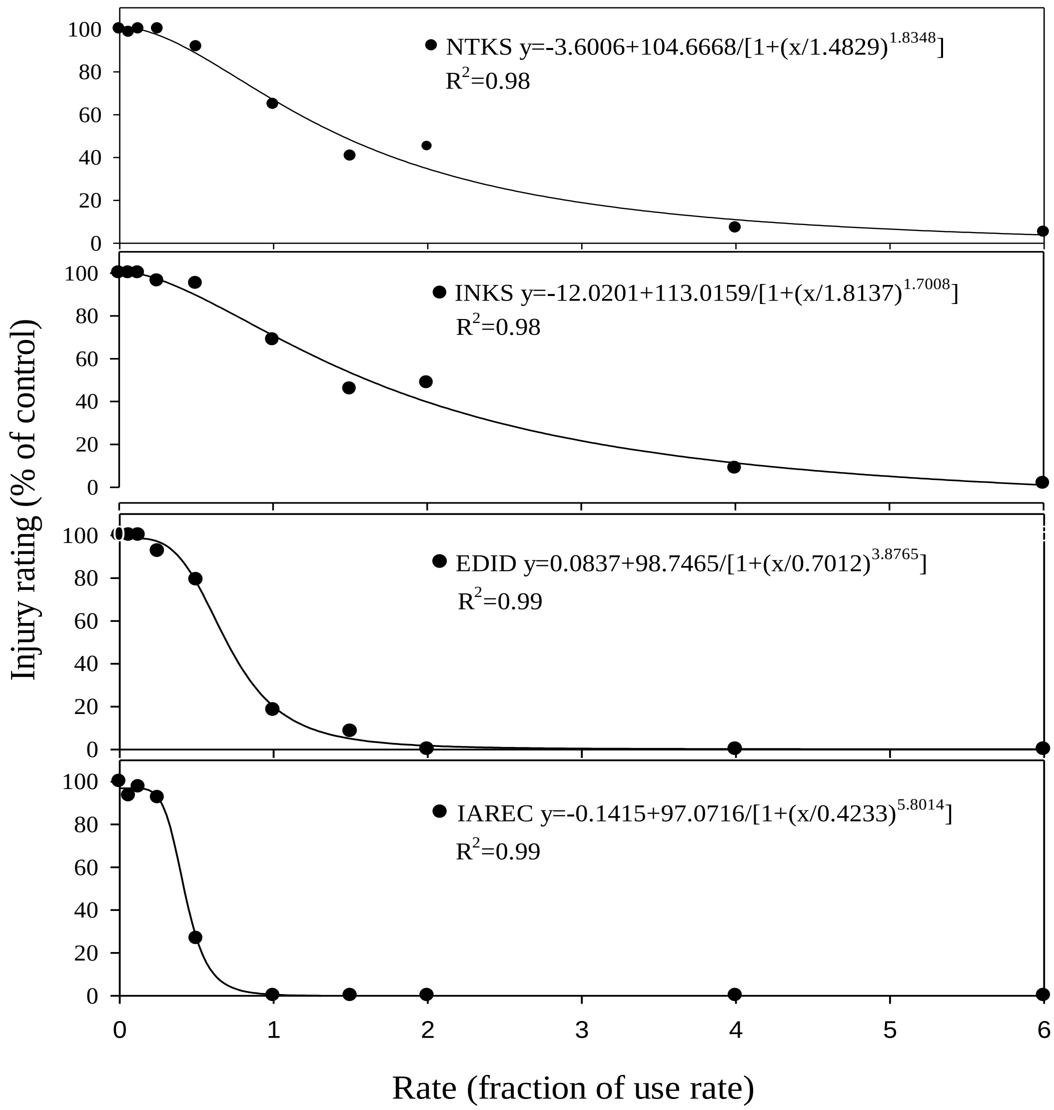

Figure 2. Visible injury ratings 21 DAT with glyphosate applied on 13 cm tall plants of four kochia populations.

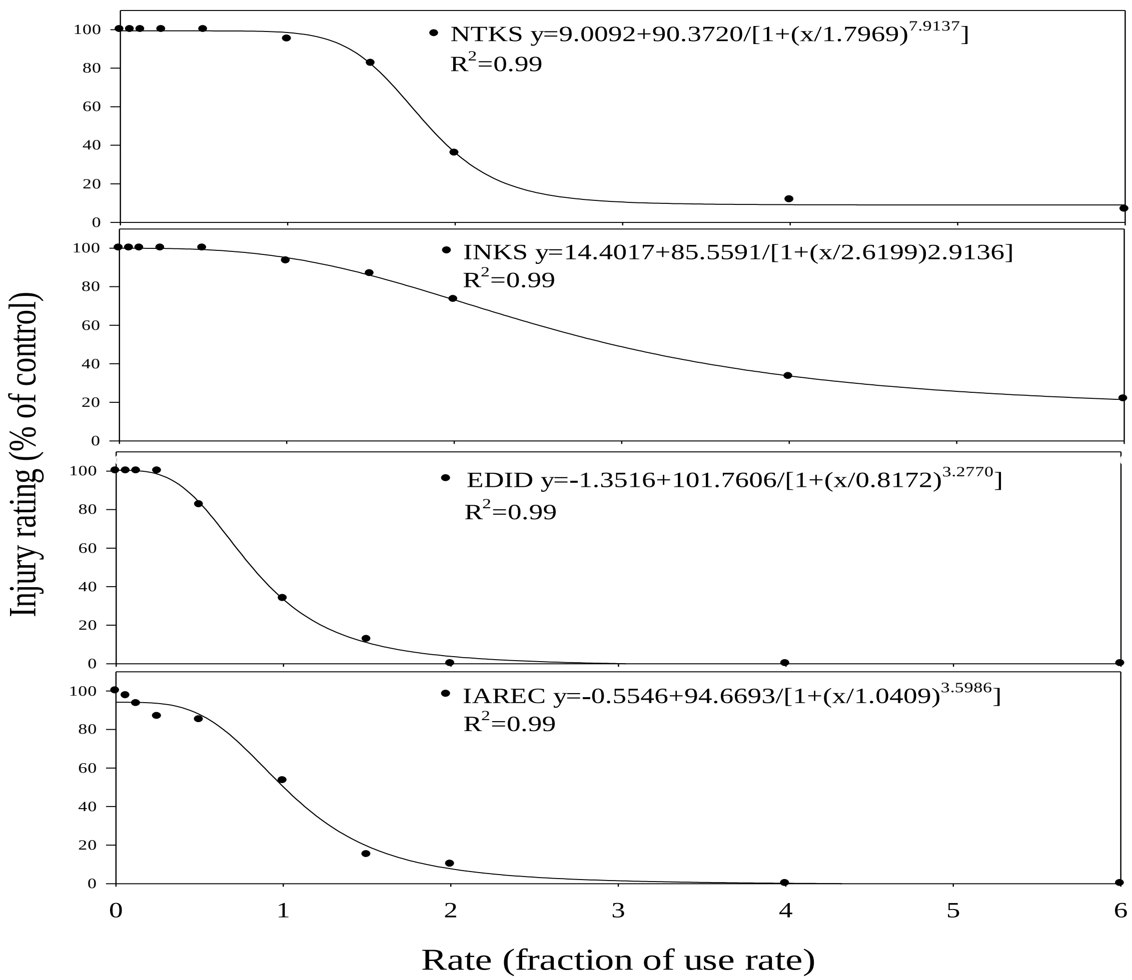

Figure 3. Visible injury ratings 21 DAT with glyphosate applied on 25 cm tall plants of four kochia populations.

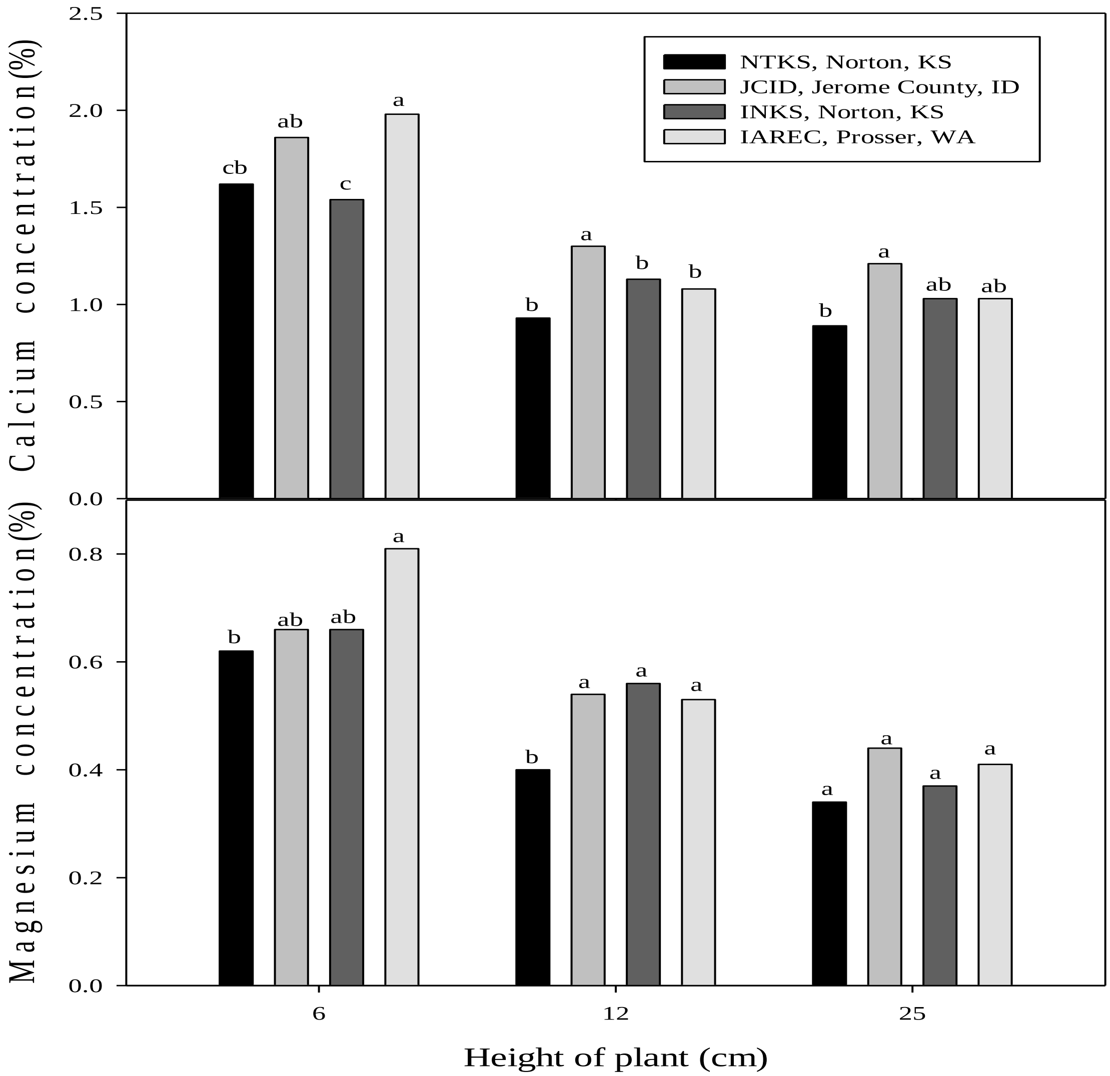

Figure 4. Calcium and magnesium content of four kochia populations at three plant heights.

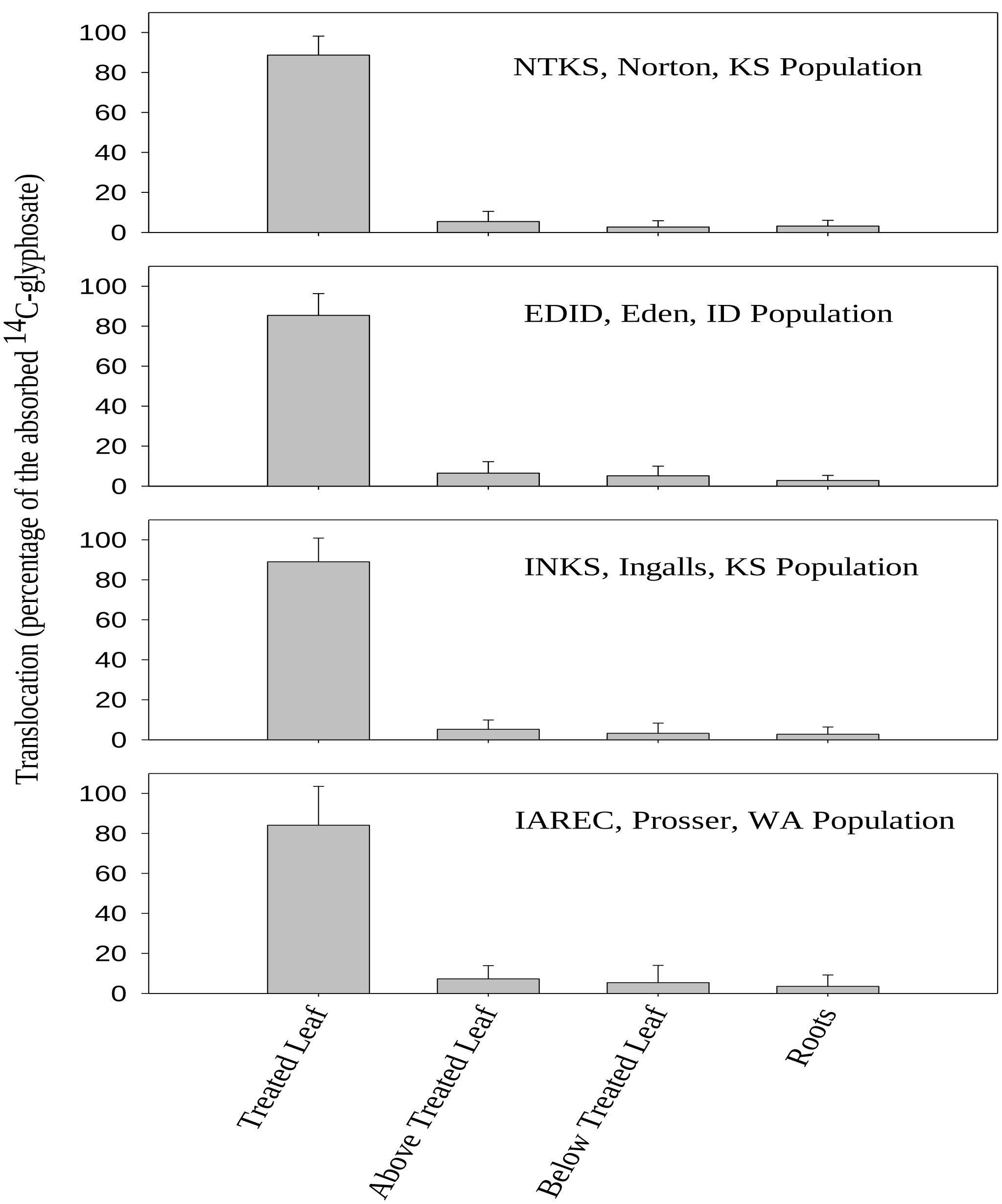

Figure 5. Glyphosate translocation 7 DAT in four kochia populations.

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