

Growth Characterization of Kochia (*Kochia scoparia*) with Substitutions at Pro₁₉₇ or Trp₅₇₄ Conferring Resistance to Acetolactate Synthase–Inhibiting Herbicides

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Over 90% of Canadian kochia populations are resistant to acetolactate synthase (ALS)–inhibiting herbicides. We questioned whether the target site–based resistance could affect plant growth and competitiveness. Homozygous F₂ herbicide-resistant (HR) kochia plants with an amino acid substitution at Trp₅₇₄ (sources: Alberta [AB], Saskatchewan [SK], and Manitoba [MB]), or Pro₁₉₇ (MB, AB with two populations) were grown in replacement series with homozygous F₂ herbicide-susceptible (HS) plants from the corresponding heterogeneous population (total: six populations). In pure stands, growth of HR plants from AB and SK was similar to that of HS plants, regardless of mutation; conversely, MB2-HR plants (Trp₅₇₄Leu) developed more slowly and were taller than MB2-HS plants. Final dry weight of HR plants in pure stands was similar across all six populations, whereas that for HS plants in pure stands and HR–HS plants in mixed stands (50–50%) varied with population. Results for AB and SK populations suggest little impact of either ALS mutation on kochia growth, whereas those for MB lines would suggest an unidentified factor (or factors) affecting the HS, HR, or both biotypes. The variable response within and between lines, and across HS biotypes highlights the importance of including populations of various origins and multiple susceptible controls in HR biotype studies.

Nomenclature: *Kochia*, *Kochia scoparia* (L.) Schrad. KCHSC.

Key words: Acetohydroxyacid synthase, AHAS, ALS, cost of herbicide resistance, Pro₁₉₇ mutation, Trp₅₇₄ mutation.

Kochia is an annual, dicotyledonous, diploid plant species from the Chenopodiaceae family (Friesen et al. 2009). It is a weed of economic importance, particularly in the Canadian Prairies and American Northern Great Plains. By the early 2000s, kochia was ranking as the 10th most abundant weed of prairie field crops in Canada (Leeson et al. 2005). *Kochia* is an agricultural weed of particular concern because of its competitiveness and capacity to evolve herbicide resistance (Friesen et al. 2009). To date, resistance to herbicides from four site–of–action groups, including glyphosate (Beckie et al. 2013; Wiersma et al. 2011), has been reported for kochia populations, mainly in North America but also in Europe (Heap 2012). Outcrossing allows for gene flow and maintenance of high genetic diversity in kochia, whereas its tumbleweed habit contributes to seed dispersal (up to 30,000 seeds plant⁻¹) and thus the spread of herbicide resistance traits over vast tracks of land (Friesen et al. 2009; Guttieri et al. 1998; Mengistu and Messersmith 2002; Stallings et al. 1995).

Over 90% of kochia populations from the Canadian Prairie provinces (MB, SK, AB) are resistant to ALS (acetohydroxyacid synthase, AHAS)–inhibiting herbicides (Beckie et al. 2011; Warwick et al. 2008). ALS, the first enzyme in the biosynthesis pathway of the branched-chain amino acids (BCAA)—leucine, isoleucine, and valine—is encoded by the nuclear ALS gene. ALS resistance in kochia is due to various single-point target site mutations, generally present singly, but also in combination in any given individual (e.g., in kochia:

Pro₁₉₇ + Trp₅₇₄ or Pro₁₉₇ + Asp₃₇₆; Warwick et al. 2008). The Trp₅₇₄Leu substitution was the most frequent (70%) in Canadian kochia populations (Beckie et al. 2011; Warwick et al. 2008). This substitution provides a high level of resistance to various classes of ALS-inhibiting herbicides (imidazolinones, sulfonylureas [SUs], triazolopyrimidines, pyrimidinylthiobenzoates, sulfonylaminocarbonyltriazolinones) (Beckie and Tardif 2012; Tranel and Wright 2002). Mutations at Pro₁₉₇ (16% of HR kochia populations) were associated with nine amino acid substitutions and generally conferred a lower level of herbicide resistance to fewer ALS-inhibiting herbicides (SUs mainly) than mutations at Trp₅₇₄ (Beckie and Tardif 2012; Tranel and Wright 2002; Warwick et al. 2008).

The impact of the various mutations conferring resistance to ALS-inhibiting herbicides on ALS enzyme function, plant growth, and fitness appears to vary across plant species and point mutations (Duggleby et al. 2003; Li et al. 2012; Tranel and Wright 2002; Vila-Aiub et al. 2009; Yu et al. 2012). The Trp₅₇₄Leu substitution may (common cocklebur [*Xanthium strumarium* L.]; Bernasconi et al. 1995) or may not [*Arabidopsis thaliana* (L.) Heynh.; Chang and Duggleby 1998; rigid ryegrass (*Lolium rigidum* Gaudin); Yu et al. 2010] affect enzyme activity. The Trp₅₇₄Leu substitution had no effect on the relative growth rate of homozygous lines of rigid ryegrass plants (Yu et al. 2010) or that of wild radish (*Raphanus raphanistrum* L.) (Li et al. 2012). Conversely, the Trp₅₇₄Leu substitution had major effects on growth of Powell amaranth (*Amaranthus powellii* S. Wats.): resistant plants showed abnormal morphological and structural patterns, grew slowly, produced less biomass (–67%) and leaf area (–58%), and were much less competitive than susceptible plants (Tardif et al. 2006).

The Pro₁₉₇His substitution in isonuclear lines of lettuce (*Lactuca sativa* L. cv. Bibb) reduced enzyme activity and resulted in altered regulation of BCAA synthesis (Eberlein et al. 1999). Similarly, the Pro₁₉₇Thr substitution in prickly lettuce (*Lactuca serriola* L.) resulted in less sensitivity to feedback inhibition and a lower K_m (Michaelis-Menten

DOI: 10.1614/WS-D-12-00116.1

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Table 1. Origin (nearest town) of kochia populations and substitution associated with acetolactate synthase herbicide resistance.

Population	Origin	Coordinates (latitude, longitude)	Substitution
AB56	Wheatland, Alberta, Canada	51°4'N, 112°56'W	Pro ₁₉₇ Gln
AB75	Wheatland, Alberta, Canada	51°4'N, 112°56'W	Pro ₁₉₇ Thr
AB82	Lethbridge, Alberta, Canada	49°45'N, 112°45'W	Trp ₅₇₄ Leu
MB2	St.-Jean, Manitoba, Canada	49°15'N, 97°20'W	Trp ₅₇₄ Leu
MB6	Victoria, Manitoba, Canada	49°39'N, 98°53'W	Pro ₁₉₇ Gln
SK4	Fish Creek, Saskatchewan, Canada	52°36'N, 106°0'W	Trp ₅₇₄ Leu

constant: substrate concentration at which the reaction rate has reached half its maximum value) for pyruvate but extractable ALS activity similar to that of HS plants (Preston et al. 2006). Substitutions at Pro₁₉₇ with Ala, Arg, Gln, and Ser in homozygous rigid ryegrass lines had higher extractable ALS activity and little if any change in ALS kinetics (Yu et al. 2010). Of the latter lines, only plants with the Pro₁₉₇Arg substitution had lower (approximately 5%) relative growth rates compared to susceptible plants or plants with another resistance mutation (Pro₁₉₇Ser, Trp₅₇₄Leu). In contrast, rice (*Oryza sativa* L.) cultivars with different ALS-inhibiting herbicide resistance mutations (Ala₁₂₂Thr, Gly₆₅₄Glu, Ser₆₅₃-Asp) germinated faster than the susceptible cultivar at lower but not at higher temperatures, and at various rates depending on mutation (Goulart et al. 2012). Resistant eastern black nightshade (*Solanum ptychanthum* Dunal) had higher germination and vegetative growth than susceptible plants under cool alternating temperature regimes but the Ala₂₀₅Val mutation came at a cost under optimal growth condition (Ashigh and Tardif 2011).

ALS-inhibiting HR and HS kochia would appear to have comparable responses at the enzyme and plant level. The ALS activity from chlorsulfuron (SU herbicide)-resistant kochia biotypes (mutation undetermined) was similar to that of the susceptible biotypes (Friesen et al. 1993; Saari et al. 1990) as were growth patterns under noncompetitive greenhouse conditions (Christoffoleti et al. 1997). Similarly, there was little to no fitness or growth penalty in HR kochia biotypes (Pro₁₉₇Thr, Pro₁₉₇Arg) compared to HS biotypes under competitive and noncompetitive greenhouse conditions (Thompson et al. 1994b). Similar to HR rice biotypes described above, these HR kochia biotypes germinated faster than HS biotypes at low temperatures (Thompson et al. 1994a), likely as a consequence of elevated seed levels of BCAA due to reduced feedback inhibition in HR biotypes (Dyer et al. 1993).

Attributing the cost of resistance to a resistance gene or mutation without resorting to isogenic lines is difficult (Roux et al. 2004). However, differences due to genetic background and environment can be minimized by using lines from the same population. The objective of this study was to characterize growth of ALS-inhibitor HR and HS kochia biotypes, with each homozygous HR F₂ line compared to an homozygous HS F₂ line from within the same population, and determine whether growth patterns would vary according to mutations providing different levels and patterns of resistance (Pro₁₉₇ or Trp₅₇₄). As indicated above, ALS HR and HS kochia biotypes would appear to have comparable responses at the enzyme and plant level, although early studies do not always specify mutation type or control for genetic background. Therefore, the null hypothesis tested in this study is that growth of HR biotypes would be similar to that of HS biotypes, regardless of mutation or substitution.

Materials and Methods

Source of Materials. Kochia seeds from the second filial generation (F₂) were obtained from a greenhouse seed increase of HR and HS individuals from populations heterogeneous for resistance and having HR individuals homozygous for one of the ALS-inhibiting herbicide-resistance target site mutations. Molecular characterization of these HR and HS genotypes is described in Beckie et al. (2011). Seed from HR individuals (codes correspond to population-individual as described in table 1 in Beckie et al. [2011]) with a mutation at Trp₅₇₄ (Leu₅₇₄ substitution for all accessions) originated from Alberta (AB82-4), Saskatchewan (SK4-17), and Manitoba (MB2-9), whereas seed with a mutation at Pro₁₉₇ originated from two fields on the same farm in AB (AB56-19: Gln₁₉₇; AB75-10: Thr₁₉₇), and one in Manitoba (MB6-16: Gln₁₉₇) (Table 1). F₂ seed from homozygous susceptible plants from each heterogeneous population (AB82-9, SK4-7, MB2-5, AB56-15, AB75-15, MB6-6, respectively) was obtained from the same greenhouse seed increase (see below). These HS lines served as a corresponding control for each HR line.

Kochia seeds obtained from selfing 12 individuals (six HR and six HS) (described in Beckie et al. 2011) were planted in 10-cm square pots with 60 pots per line. Seedlings were thinned to two plants per pot and each line isolated, using 12 different greenhouses. Two pollen bags were placed over the plants before flowering to limit pollen flow between greenhouses and for easy seed capture. Seed of all 60 pots were cleaned and bulked separately for each line.

Replacement Series Experiment. Greenhouse experiments were conducted according to a replacement series design, with three HS : HR ratios, including pots with pure stands of HS kochia plants (4 HS : 0 HR), pure stands of HR plants (0 HS : 4 HR), or a 50–50% mixed stand of HS–HR plants (2 HS : 2 HR). The total of four kochia plants per pot was equivalent to 82 plants m⁻², a density likely to be observed in field crops (Leeson et al. 2005). There were three replicates of each treatment, and pots were completely randomized on the bench. Experiments were conducted under a 16-h photoperiod (20/17C), with 220 μmol m⁻² s⁻¹ supplemental lighting.

The first run of the experiment (hereafter referred to as experiment 1) included HR and HS biotypes from all six populations (AB82, SK4, MB2, AB56, AB75, MB6) (Table 1), three HS–HR ratio treatments, and three replicates, for a total of 54 experimental units. Kochia was sown in 16-L pots (27 cm diam, 30 cm ht), filled with soilless growth media (sphagnum peat moss, medium-grade vermiculite, ground calcium carbonate, and sand screened to 1 mm particle size), to which 10 g L⁻¹ of controlled-release fertilizer with micronutrients (Osmocote PLUSTM; 15–9–12 The Scotts Company LLC, Marysville, OH) was added. Approximately

10 seeds of the appropriate accession were sprinkled into each of four quadrants of the pot surface. A thin layer (approximately 5 mm) of soilless mixture was then spread over the seeds and lightly packed. The first emerged seedling in each quadrant was kept and all other seedlings removed as they emerged, for a total of four HS or four HR plants per pot in pure-stand treatments, and two HS + two HR plants per pot in the mixed-stand treatment. Each pot was watered to saturation, and as needed thereafter. Overall, 80% of the retained seedlings had emerged by 3 d after seeding (DAS), regardless of treatment.

Nondestructive growth measurements were taken for each plant at approximately weekly intervals and included the following: height of main stem (seven measurements between 29 and 73 DAS), stem diameter at the cotyledon level measured with digital calipers (six measurements between 37 and 74 DAS), and growth stage according to the BBCH scale (Hess et al. 1997) (eight measurements between 16 and 72 DAS). At 169 DAS, plants were measured for final height, cut at soil level, dried at 55 C for 1 wk, and weighed. Seeds were separated from the vegetative material and weighed. The 1,000-seed weights were also obtained.

Results of experiment 1 generally validated our hypothesis of no difference between HS and HR for accessions from AB and SK, but not for those from MB. To further confirm results for MB accessions, the experiment was run a second time: experiment 2 was set up as experiment 1 but only included MB2 (Trp₅₇₄Leu) and MB6 (Pro₁₉₇Gln) accessions with their respective HS counterparts, for a total of 18 pots. All seed had germinated by 3 DAS. Height of main stem, stem diameter, and growth stage were measured as described above at regular intervals, 10 times between 10 and 102 DAS. Plants were harvested at 102 DAS, dried at 55 C for 3 d, and weighed. The experiment was terminated prior to seed production.

Statistical Analysis. Growth variables (growth stage, height, and stem diameter) were regressed against DAS using a beta growth function model (Yin et al. 2003) as follows:

$$\text{Response} = w_{\max} \left(1 + \frac{DAS_e - DAS}{DAS_e - DAS_m} \right) \left(\frac{DAS}{DAS_e} \right)^{\frac{DAS_e}{DAS_e - DAS_m}} \quad [1]$$

where w_{\max} represents the maximum predicted response, and DAS_m and DAS_e respectively represent the DAS at which the inflexion point (c_m) and maximum response occur. The inflexion point corresponded to point of concavity change where growth rate is maximum, and was estimated as follows:

$$c_m = \frac{DAS_e - DAS_m}{DAS_e (DAS_e - DAS_m)} \left(\frac{DAS_m}{DAS_e} \right)^{\frac{DAS_m}{DAS_e - DAS_m}} w_{\max} \quad [2]$$

Nonlinear regression model coefficients were estimated with the NLMIXED procedure of SAS (SAS Institute, Inc. 2004). Contrasts were used to statistically compare coefficient estimates among the 12 treatment combinations of six populations and two biotypes (HS and HR). Some modifications had to be applied to experiment 2 data in order to fit the beta function nonlinear regression. For BBCH data, regression was limited to $DAS \leq 55$ for MB2 accessions, and to $DAS \leq 76$ for MB6 accessions. Results beyond these time limits were represented on the graphs as means rather than regression predictions. For stem diameter, data were scaled up by a factor of 10 for the regression and

results were back-transformed. Regression on stem diameter data was limited to $DAS \leq 66$ for MB6-HS. Stem diameter results beyond this time limit were represented by means rather than regression predictions. No modifications were required for plant height.

Data at final harvest for experiment 1 height, plant and seed dry weight, and reproductive effort (seed dry weight/plant dry weight), and experiment 2 plant weight were analyzed with the MIXED procedure of SAS (SAS Institute, Inc. 2004). The analysis was conducted with pure-stand data only, and then with pure- and mixed-stand data. The effects of population and biotype were considered to be fixed. A separate residual variance was estimated for each population; corrected Akaike's information model fit criterion (AIC_C) confirmed the benefit of heterogeneous residual variances. A similar analysis was also done for relative yield (RY), which was calculated for each biotype as follows:

$$RY_{HS} \text{ or } RY_{HR} = \frac{\text{dry weight in 50-50\% mixture}}{\text{dry weight in pure stand (100\%)}} \quad [3]$$

RY would approximate 0.5 if plants produced the same amount of biomass, regardless of being grown in mixture or pure stand. A biotype would be considered more competitive if $RY > 0.5$. The relative yield total (RYT) was calculated as the sum of $RY_{HS} + RY_{HR}$ for any given mixed treatment, to verify the assumption that both biotypes were making similar demands on the same limiting resources ($RYT \approx 1.0$) (Harper 1977). To understand the nature of population by biotype interactions, contrasts were used to test whether statistically significant differences occurred among biotypes for each population and among populations for each biotype. For all analyses, statistical tests were considered significant at $P \leq 0.05$.

Results

Kochia Growth in Pure Stands. When various growth curve parameters were compared for growth stage, height, and stem diameter, differences between the 12 kochia accessions (two biotypes by six populations) were observed for growth rate at inflexion point and maximum value reached during the initial 72-d period in experiment 1 (Table 2). On average, the maximum growth rate at inflexion point and maximum values for height and stem diameter for HR biotypes were 13 to 20% greater than that for HS biotypes (Table 2). The reverse was observed for growth stage, with HR biotypes developing somewhat more slowly (3 to 8%) than HS biotypes (Table 2). These differences were mainly due to values observed in the MB2 population in which the HS biotype had a much smaller stature (height and stem diameter) and was developing faster (growth stage: BBCH scale) than its HR counterpart and other HR accessions (Table 2; Figure 1). Additionally, time to reach the inflexion point on the growth curve was 45 d for MB2-HS compared to 63 d for MB2-HR and between 54 and 64 d for all other accessions (data not shown).

When MB populations were grown again in the greenhouse (experiment 2), MB6 plants were shorter and had a smaller stem diameter than MB2 plants, regardless of biotype (Figure 1; Table 3). Models for BBCH data converged but large standard error (SE) values indicated that certain estimates were not reliable. For example, the maximum BBCH estimate for MB2-HR was half that of MB2-HS but

Table 2. ANOVA results (A, B) from the NLMIXED analysis and mean estimates of maximum parameter values (C) and maximum growth rate (D) (with SE values and contrast P values for HR-HS comparisons) for the effects of population and biotype on nonlinear growth curve parameters calculated for growth stage, height and stem diameter during the 1 to 72 d after seeding (DAS) growth period (experiment 1) for Alberta (AB56, AB75, AB82), Saskatchewan (SK4) and Manitoba (MB2, MB6) populations.^a

Growth curve parameters	Growth stage BBCH scale			Height			Stem diameter		
	df	F	P	df	F	P	df	F	P
A) Among treatment levels (12 levels: 2 populations by 2 biotypes)									
Maximum value	11, 284	7.2	< 0.001	11, 215	7.6	< 0.001	11, 213	16.5	< 0.001
Time to reach inflexion point	11, 284	1.1	0.344	11, 215	1.7	0.084	11, 213	0.7	0.740
Time to reach max value	11, 284	0.6	0.820	11, 215	0.4	0.942	11, 213	0.3	0.984
Max growth rate (at inflexion point)	11, 284	2.9	0.001	11, 215	4.4	< 0.001	11, 213	4.2	< 0.001
B) Contrast: HS vs. HR									
Maximum value	6, 284	8.5	< 0.001	6, 215	0.8	< 0.001	6, 213	6.9	< 0.001
Time to reach inflexion point	6, 284	0.5	0.842	6, 215	0.9	0.084	6, 213	0.7	0.740
Time to reach max value	6, 284	0.3	0.944	6, 215	0.1	0.942	6, 213	0.2	0.984
Max growth rate (at inflexion point)	6, 284	4.0	0.001	6, 215	1.8	< 0.001	6, 213	2.4	< 0.001
Population: substitution									
	HS	HR	P	HS	HR	P	HS	HR	P
--- BBCH scale unit ---									
C) Maximum parameter value: mean estimate (SE) and P value for biotype comparison									
AB56: Pro ₁₉₇ Gln	60.1 (2.6)	53.2 (4.7)	0.195	77.5 (11.8)	103.7 (41.8)	0.547	8.7 (0.9)	9.5 (0.5)	0.454
AB75: Pro ₁₉₇ Thr	50.5 (1.5)	56.9 (1.4)	0.002	81.2 (34.3)	77.5 (7.7)	0.918	8.4 (1.5)	9.2 (5.2)	0.891
MB6: Pro ₁₉₇ Gln	57.0 (2.1)	54.2 (1.4)	0.280	71.5 (9.2)	98.3 (12.0)	0.077	7.6 (1.2)	9.8 (1.0)	0.147
AB82: Trp ₅₇₄ Leu	69.2 (19.5)	61.5 (5.4)	0.704	91.9 (12.4)	89.8 (14.2)	0.912	9.6 (2.5)	9.0 (0.5)	0.819
SK4: Trp ₅₇₄ Leu	61.7 (6.3)	76.7 (56.9)	0.794	70.7 (7.2)	81.4 (14.6)	0.511	8.6 (1.0)	8.9 (1.3)	0.878
MB2: Trp ₅₇₄ Leu	65.7 (1.3)	51.1 (2.0)	< 0.001	41.9 (2.3)	62.3 (26.9)	0.451	4.1 (0.2)	6.6 (0.3)	< 0.001
Mean	60.7	58.9		72.5	85.5		7.8	8.8	
Percent change: HR vs. HS									
			-3%		+18%			+13%	
Population: Substitution									
	HS	HR	P	HS	HR	P	HS	HR	P
--- BBCH scale unit day ⁻¹ ---									
D) Growth rate at inflexion point : treatment mean (SE) and P value for biotype comparison									
AB56: Pro ₁₉₇ Gln	1.32 (0.09)	1.19 (0.39)	0.744	1.75 (0.19)	2.20 (0.21)	0.123	0.17 (0.02)	0.21 (0.02)	0.260
AB75: Pro ₁₉₇ Thr	1.13 (0.05)	1.26 (0.05)	0.064	2.01 (0.23)	1.86 (0.20)	0.614	0.17 (0.02)	0.1 (0.03)	0.281
MB6: Pro ₁₉₇ Gln	1.15 (0.07)	1.24 (0.05)	0.306	1.91 (0.23)	2.47 (0.21)	0.078	0.15 (0.02)	0.20 (0.02)	0.118
AB82: Trp ₅₇₄ Leu	1.07 (0.10)	1.10 (0.10)	0.853	2.32 (0.22)	2.42 (0.24)	0.744	0.16 (0.02)	0.22 (0.02)	0.119
SK4: Trp ₅₇₄ Leu	1.17 (0.08)	0.99 (0.84)	0.827	1.70 (0.20)	2.14 (0.23)	0.156	0.17 (0.02)	0.17 (0.02)	0.895
MB2: Trp ₅₇₄ Leu	1.50 (0.07)	1.04 (0.08)	< 0.001	1.06 (0.17)	1.52 (0.21)	0.092	0.09 (0.01)	0.16 (0.02)	0.009
Mean	1.2	1.1		1.8	2.1		0.15	0.18	
% Change: HR vs. HS									
			-8%		+17%			+20%	

^a Abbreviations: HR, herbicide resistant; HS, herbicide susceptible; BBCH.

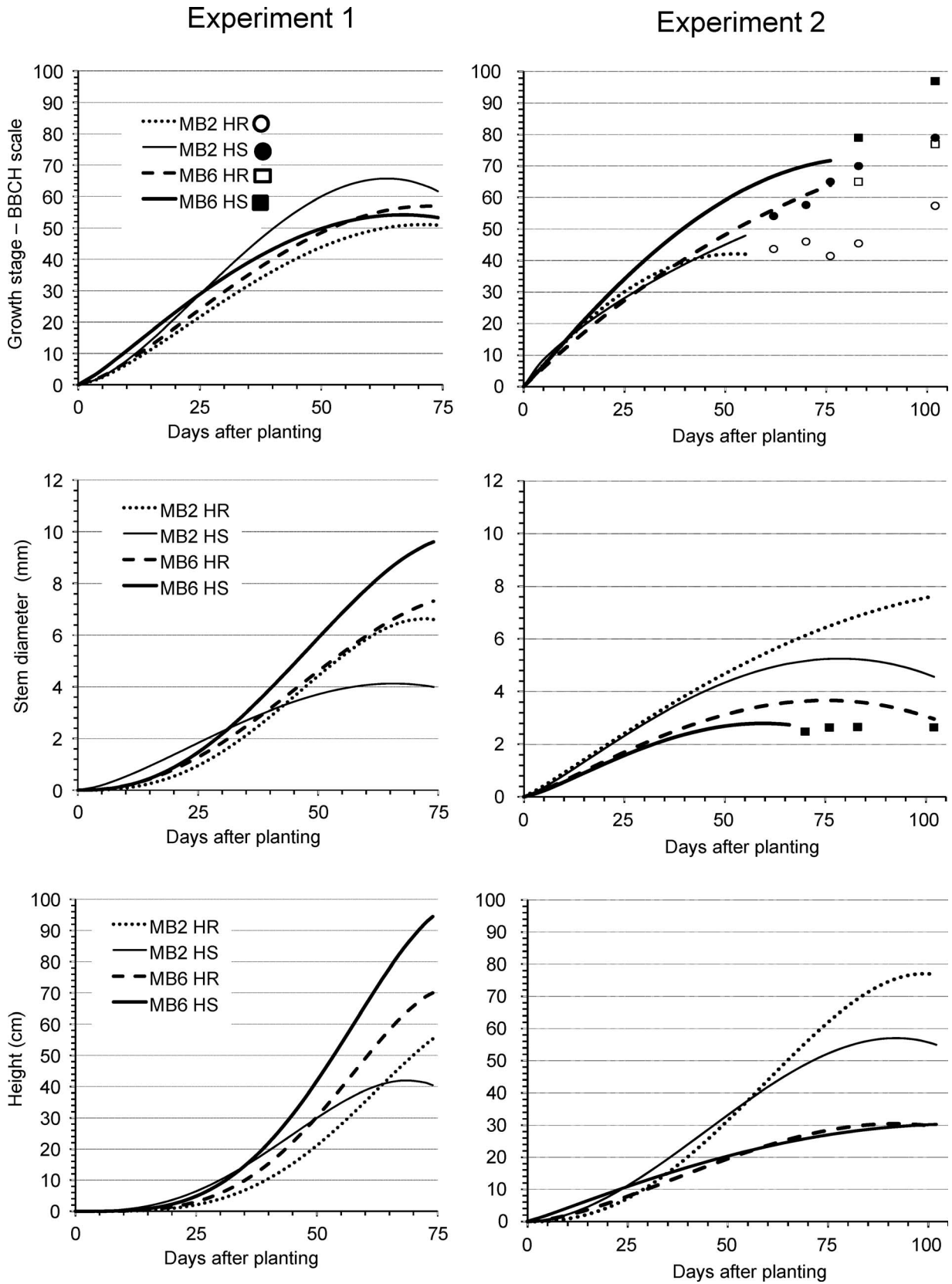


Figure 1. Predicted growth curves for growth stage development (key BBCH stages: 20, side shoots; 60, first flowers; 70, fruit developing; 80, fruit ripening; 90, senescing), stem diameter, and plant height according to beta function nonlinear regression equations for acetolactate synthase-inhibiting herbicide-resistant (dashed lines: MB2-HR, MB6-HR) and -susceptible (solid lines: MB2-HS, MB6-HS) kochia plants from two Manitoba populations.

Table 3. ANOVA results (A, B) from the NLMIXED analysis and mean estimates of maximum parameter values (C) and maximum growth rate (D) (with SE values and contrast P values for HR–HS comparisons) for the effects of population and biotype on nonlinear growth curve parameters calculated for growth stage, height and stem diameter during the 1 to 102 d after seeding growth period (experiment 2) for Manitoba (MB2, MB6) populations.^a

	Growth stage BBCH scale			Height			Stem diameter		
	df	F	P	df	F	P	df	F	P
A) Among treatment levels (4 levels: 2 populations by 2 biotypes)									
Maximum value	3, 24	34.4	< 0.001	3, 29	467.4	< 0.001	3, 25	136.5	< 0.001
Time to reach inflexion point	3, 24	0.1	0.953	3, 29	20.9	< 0.001	3, 25	0	0.999
Time to reach max value	3, 24	3.6	0.029	3, 29	3.0	0.046	3, 25	5.6	0.004
Max growth rate (at inflexion point)	1, 24	0	0.977	3, 29	114.2	< 0.001	3, 25	33.4	< 0.001
B) Contrast: HS vs. HR									
Maximum value	2, 24	0.1	0.936	2, 29	109.0	< 0.001	2, 25	29.6	< 0.001
Time to reach inflexion point	2, 24	0.1	0.909	2, 29	22.8	< 0.001	2, 25	0	0.997
Time to reach max value	2, 24	0.3	0.731	2, 29	4.3	0.023	2, 25	5.9	0.008
Max growth rate (at inflexion point)	—	—	—	2, 29	22.4	< 0.001	2, 25	0.1	0.910
Population: Substitution	HS	HR	P	HS	HR	P	HS	HR	P
	—BBCH scale unit—			—cm—			—mm—		
C) Maximum parameter value: mean estimate (SE) and P value for biotype comparison									
MB6: Pro ₁₉₇ Gln	72.6 (2.7)	77.0 (18.6)	0.815	30.4 (1.9)	30.4 (0.8)	0.984	2.8 (1.3)	3.7 (0.6)	< 0.001
MB2: Trp ₅₇₄ Leu	87.6 (163.7)	42.2 (1.3)	0.784	57.1 (0.8)	77.0 (1.1)	< 0.001	5.2 (0.8)	8.0 (5.7)	< 0.001
Population: Substitution	HS	HR	P	HS	HR	P	HS	HR	P
	—BBCH scale unit day ⁻¹ —			—cm day ⁻¹ —			—mm day ⁻¹ —		
D) Growth rate at inflexion point: treatment mean (SE) and P value for biotype comparison									
MB6: Pro ₁₉₇ Gln	1.4 (0.1)	—	—	0.5 (< 0.1)	0.48 (< 0.1)	0.569	0.70 (0.09)	0.73 (0.02)	0.667
MB2: Trp ₅₇₄ Leu	—	1.4 (0.3)	—	0.92 (< 0.1)	1.26 (< 0.1)	< 0.001	1.01 (0.03)	1.0 (< 0.01)	0.975

^a Abbreviations: HR, herbicide resistant; HS, herbicide susceptible.

this difference was not significant due to the large SE for the latter treatment (Table 3). Maximum height, height accumulation rate at inflexion point, and stem diameter estimates for MB2-HR were 33, 37, and 54% greater, respectively, than values for MB2-HS (Table 3). Stem diameter estimate for MB6-HR was also 32% greater than that for MB6-HS (Table 3). The difference between MB2-HS and MB2-HR increased over time, with lower height and smaller stem diameter values at 102 DAS for MB2-HS than MB2-HR (Figure 1).

Pure vs. Mixed Kochia Stands at Harvest. Final plant height, reproductive effort, and 1,000-seed weight varied mainly according to population, regardless of biotype and biotype ratio (pure stands: HS₁₀₀ and HR₁₀₀; mixed stands: HS₅₀–HR₅₀) (Table 4). Final height averaged 64 cm in MB2 compared to 105 cm for the other five populations (P < 0.001). Final height for MB2-HS₁₀₀ and MB2-HR₅₀ was approximately half that for MB2-HR₁₀₀ and MB2-HS₅₀ (P = 0.036). Reproductive effort (seed weight/total plant weight) was low for all populations ranging from 0.14 for MB2 to a low of 0.03 for MB6. Plants from the MB6 population also had the lowest 1,000-seed weight at 0.389 g compared to 0.561 g for the other five populations. Seed weight varied mainly with population, but also with biotype

ratio (Table 4). There were no clear trends in these variations, with AB56-HS₁₀₀, AB75-HR₁₀₀, SK4-HS₅₀, and SK4-HR₅₀ producing between 8.0 and 10.8 g seed compared to 0.2 g for MB6-HR₅₀ (lowest value). These inconclusive results concerning seed production and the very low reproductive effort values were attributed to the indeterminate growth habit under greenhouse conditions that appeared to delay maturity, and the difficulty in collecting the mature seed from very large plants.

Final aboveground plant dry weight at 169 DAS varied with population and biotype ratio (Table 4). All HR₁₀₀ accessions had similar plant dry weight (P = 0.247; range of back-transformed estimates: 48.8 to 90.2 g; Figure 2), whereas dry weight for HS₁₀₀, HS₅₀, and HR₅₀ varied with population (P < 0.001, P = 0.030, P < 0.001, respectively). Plant dry weight (back-transformed estimates) ranged for HS₁₀₀ from 12.5 g (MB2) to 73.6 g (AB75); for HS₅₀, between 45.5 g (AB56) and 110.6 g (MB6); and for HR₅₀, between 13.9 g (MB2) and 81.8 g (AB75) (Figure 2). Dry weight for MB2 and MB6 HR₁₀₀ plants was greater than that for HS₁₀₀ plants, whereas dry weight of HR₅₀ plants was smaller than that for HS₅₀ plants (P < 0.001) (Figure 2). Conversely, for AB56 lines, dry weight for HR₁₀₀ plants was smaller than that for HS₁₀₀ plants, whereas dry weight for HR₅₀ plants was greater than that for HS₅₀ plants (P = 0.04).

Table 4. ANOVA results for the effects of population, biotype (HR₁₀₀, HR₅₀, HS₁₀₀, HS₅₀), and population by biotype interaction on final plant growth variables measured at 169 d after seeding (experiment 1).^a

Factor	df	Plant height		Plant dry weight		Seed weight		Reproductive effort		1,000-Seed weight	
		F	P	F	P	F	P	F	P	F	P
Population	5, 48	5.75	< 0.001	7.91	< 0.001	7.04	< 0.001	7.15	< 0.001	6.42	< 0.001
Biotype	3, 48	1.02	0.392	5.31	0.003	1.25	0.303	0.79	0.503	0.43	0.732
Population by biotype	15, 48	0.86	0.611	4.99	< 0.001	2.58	0.007	0.94	0.526	1.41	0.181

^a Abbreviations: HR, herbicide resistant; HS, herbicide susceptible.

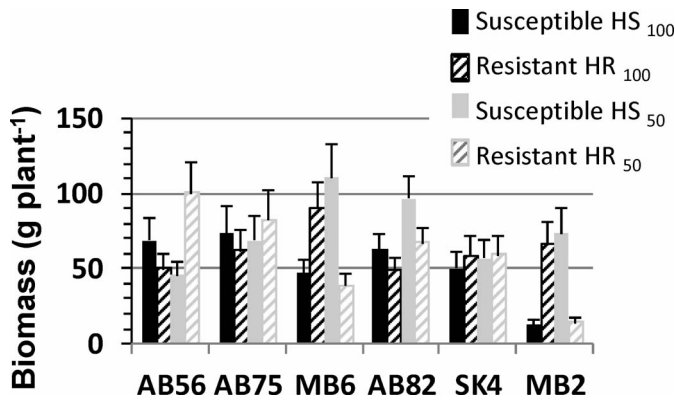


Figure 2. Effects of population and biotype ratio on final plant dry weight (back-transformed means) measured at 169 d after seeding in experiment 1 (error bars correspond to SE values).

When considered on a yield basis (dry weight per unit area), HR and HS biotypes from populations AB75, AB82, and SK4 had similar RY, resulting in RYT values averaging 1.2 (Figure 3; Table 5). In AB56, yield of HR₅₀ plants was equal to that of HR₁₀₀, indicating that when in mixture with HS, two HR plants were producing as much biomass as the four HR plants in a pure stand. Conversely, in MB2 and MB6, HS₅₀ plants were producing as much (MB6) and even three times (MB2) the biomass produced by plants in pure stands (HS₁₀₀) (Figure 3; Table 5). However, no such differences in relative yield were observed for MB2 and MB6 biomass harvested at 102 DAS in experiment 2 (Figure 3; Table 5). Although the HR₅₀ relative yield was three times that of HS₅₀ in MB6, this difference was not significant (Table 5). Overall, MB6 dry weight was below 30 g pot⁻¹ for pure and mixed stand treatments, which is less than half to a fifth of that in MB2 treatments (Figure 3).

Discussion

Growth of kochia plants from AB and SK with substitutions at Pro₁₉₇ (lines AB56, AB75) or Trp₅₇₄ (lines AB82, SK4) conferring resistance to ALS-inhibiting herbicides was similar to that of HS plants, whether in pure or mixed stands (with the exception of AB56-HR being more competitive than AB56-HS in mixed stands) under greenhouse conditions. Similar results had been obtained under greenhouse conditions for ALS-resistant kochia accessions from Kansas (Pro₁₉₇Thr) and North Dakota (Pro₁₉₇Arg) (Thompson et al. 1994b). The lack of effect of the Trp₅₇₄Leu substitution on HR kochia accessions from AB and SK was similar to that observed for rigid ryegrass (Yu et al. 2010) and wild radish (Li et al. 2012) accessions but contrary to the severe negative pleiotropic effects observed for HR Powell amaranth (Tardif et al. 2006). In similar replacement series studies, no competitive differences between HR and HS biotypes were reported for ALS-resistant redroot pigweed (*Amaranthus retroflexus* L.) (Pro₁₉₇Leu) under field conditions (Sibony and Rubin 2003), and under greenhouse conditions for greater beggarticks (*Bidens subalternans* DC.) (Trp₅₇₄Leu) (Lamego et al. 2011) and eastern black nightshade (Ala₂₀₅Val) (Ashigh and Tardif 2009). Overall, the AB and SK results would validate our hypothesis and support the general consensus of little to no effect of ALS substitutions at Pro₁₉₇ or Trp₅₇₄ on kochia plant growth.

However, biotype effects on the MB lines differed from other lines, and also across experiments. MB2 and MB6 HS plants were more competitive than HR plants when grown in mixture in the first but not the second experiment. Differences in assessment dates between experiments could partly explain differences in values and competitiveness. Assessment of aboveground dry weight at 102 DAS in experiment 2 produced considerably lower biomass values than those measured at 169 DAS in experiment 1, particularly for MB6. However, growth parameters also varied somewhat between experiments, in spite of a common seed source and similar environmental conditions. Photoperiod may possibly have affected kochia growth in spite of supplemental lighting, given that experiment 1 was run in spring and experiment 2 in autumn.

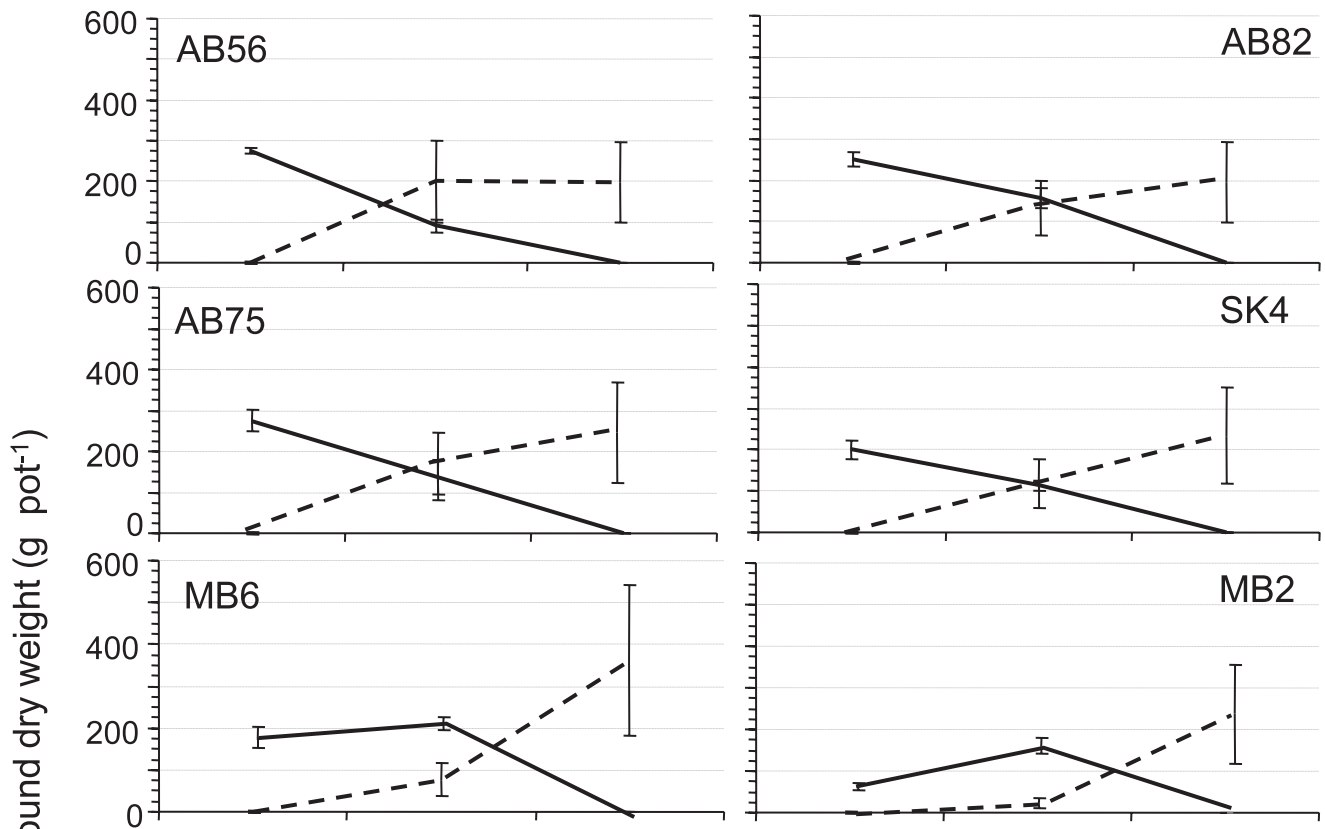
Growth of MB-HR plants was generally comparable to that of HR plants from other populations, whereas that of MB-HS plants was reduced compared to that of other HS accessions, such that the magnitude of the biotype difference for MB accessions was exacerbated by the poor HS growth. Similar to our study, a kochia HS biotype from North Dakota had a different growth form and matured earlier than other HS and HR biotypes when grown in competition with a spring cereal (Thompson et al. 1994b). Also, when F₃ kochia homozygous for resistance to chlorsulfuron and HS kochia were grown in the field in competition with barley (*Hordeum vulgare* L.), the HS kochia was shorter than barley throughout the season whereas HR plants grew taller than barley from flowering onward; the HS biotype also flowered earlier than the HR biotype (Stallings et al. 1995). Whether accumulation of BCAA could affect expression of flowering genes (FT, TFL1: Hanzawa et al. 2005) in HR kochia is unknown. Moreover, in a study of pleiotropic effects of various ALS gene mutations in wild radish, two HS lines had each the highest and lowest rate of vegetative biomass loss under increasing wheat (*Triticum aestivum* L.) density compared to five HR accessions (Li et al. 2012).

We questioned whether variability in MB accessions could be attributable to local adaptation, i.e., having a greater fitness in their original environment than elsewhere (Friesen and von Wettberg 2010; Hereford 2009). MB accessions originated from a region with more diversified cropping systems compared to AB and SK accessions. Kochia accessions included in this study were examined for potential resistance to herbicides with sites of action other than ALS inhibition (Heap 2012), and were found to be susceptible to the herbicides tested, with the exception of MB2 lines (Beckie et al. 2012). MB2-HR and MB2-HS responded similarly to bromoxynil, fluroxypyr, and glyphosate, but MB2-HR plants were 80, 60, and 50% more sensitive than HS plants to pyrasulfotole and mesotrione (hydroxyphenylpyruvate dioxygenase [HPPD] inhibitors), and to carfentrazone (protoporphyrinogen oxidase [PPO] inhibitor), respectively. This suggested negative cross-resistance to PPO and HPPD inhibitors in some but not all HR kochia populations with the Trp₅₇₄ mutation. The differences in sensitivity between MB2-HS and MB2-HR to the three herbicides were attributed to a greater sensitivity of the HR biotype relative to other HR biotypes, but also a tendency (although not significant and not consistent among herbicides) for greater tolerance of the HS biotype (relative to other HS biotypes), again suggesting the singularity of the MB2-HS biotype.

Substitution at Pro197

Substitution at Trp 574

Experiment 1 – 169 DAS



Experiment 2 – 102 DAS

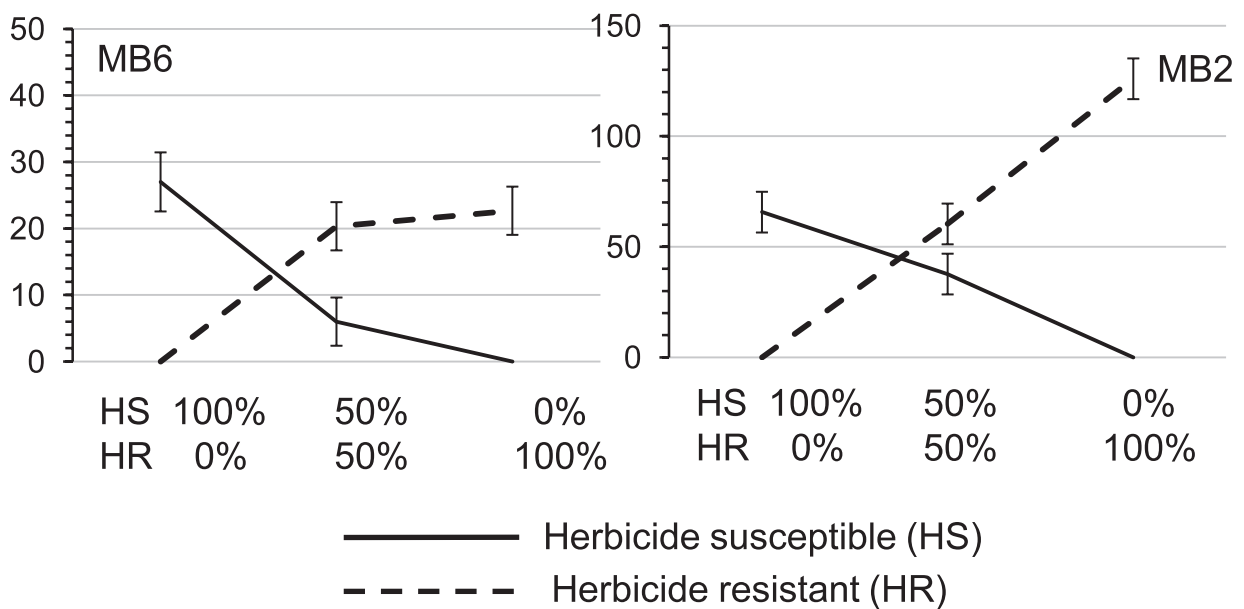


Figure 3. Replacement series diagram for aboveground dry weight (untransformed original data) for acetolactate synthase-inhibiting herbicide-resistant (dashed lines) and -susceptible (solid lines) kochia plants measured for six populations (Alberta: AB56, AB75, AB82; Saskatchewan: SK4; Manitoba: MB2, MB6) at 169 d after seeding in experiment 1, and two Manitoba populations (Manitoba: MB2, MB6) measured at 102 DAS in experiment 2 (error bars correspond to SE values).

Table 5. Relative yield (RY = dry weight in mixed stands/ dry weight in pure stands) for HS and HR biotypes and relative yield total (RYT = RY_{HS} + RY_{HR}) measured at 169 DAS for six populations (experiment 1), and at 102 DAS for Manitoba populations (experiment 2); LSD values are reported for those instances where significant differences were observed.^a

Population: substitution	RY		LSD _{0.05}	RYT
	RY _{HS} (HS ₅₀ /HS ₁₀₀)	RY _{HR} (HR ₅₀ /HR ₁₀₀)		
Experiment 1: 168 DAS				
AB56: Pro ₁₉₇ Gln	0.3	1.0	0.5	1.3
AB75: Pro ₁₉₇ Thr	0.5	0.6	—	1.1
MB6: Pro ₁₉₇ Gln	1.2	0.2	0.4	1.4
AB82: Trp ₅₇₄ Leu	0.6	0.7	—	1.3
SK4: Trp ₅₇₄ Leu	0.6	0.5	—	1.1
MB2: Trp ₅₇₄ Leu	2.9	0.1	1.6	3.0
Experiment 2: 102 DAS				
MB6: Pro ₁₉₇ Gln	0.3	0.9	—	1.2
MB2: Trp ₅₇₄ Leu	0.6	0.5	—	1.1

^a Abbreviations: RY, relative yield; HR, herbicide resistant; HS, herbicide susceptible; RYT, relative yield total; DAS, days after seeding.

High within-population levels of genetic variability (90% of molecular variance) have been reported for kochia (Mengistu and Messersmith 2002), and therefore inferences of population differences in our study were limited by the fact that each of the 12 F₂ homozygous lines was derived from a single parent, which may or may not represent the whole population. Various factors probably contributed to some extent to the differences between kochia populations, particularly those from MB. The latter could have been segregating for other characteristics unrelated to ALS mutations and may account for some of the inconsistent growth and seed production responses observed across accessions and between experiments in this study.

Although assessment of biotype differences was enhanced by selection of homozygous HR and HS lines from the same population at each location, our results highlight the variable growth responses within individual lines, and thus suggest that such experiments should include a greater number of lines from each population. However, the unfeasibility of obtaining such lines in many cases lends support to individual-based modeling as an alternative approach to investigating costs and consequences of herbicide resistance (Renton 2012). Additionally, our study, like that of Li et al. (2012), demonstrates the importance of using multiple sources of material in comparative growth studies of HR and HS biotypes, particularly with respect to the inclusion of HS control populations with similar genetic backgrounds when isogenic lines are not available (Poston et al. 2002; Vila-Aiub et al. 2011). Cousens et al. (1997) warned of the probability of an incorrect conclusion inferred from single-population comparisons of HR and HS biotypes. Our conclusions concerning effects of ALS mutations on kochia growth could have varied greatly, depending on which of the six populations would have been considered in a single-population study, or if only one control line had been included.

By highlighting the variable response within lines and between accessions derived from populations from different geographical origin, and particularly across control HS biotypes, our work sets cautionary guidelines for the conduct and interpretation of HR biotype growth and fitness studies. Nevertheless, the nature and magnitude of the differences

observed between HR and HS biotypes would suggest little impact of the ALS substitutions at Trp₅₇₄ and Pro₁₉₇ on kochia growth across the Canadian Prairie provinces. Lack of significant growth or fitness penalties in HR biotypes favors their evolution, spread, and competitiveness in input-intensive cropping systems.

Literature Cited

- Ashigh, J. and F. J. Tardif. 2009. An amino acid substitution at position 205 of acetohydroxyacid synthase reduces fitness under optimal light in resistant populations of *Solanum ptychanthum*. *Weed Res.* 49:479–489.
- Ashigh, J. and F. J. Tardif. 2011. Water and temperature stress impact fitness of acetohydroxyacid synthase-inhibiting herbicide-resistant populations of eastern black nightshade (*Solanum ptychanthum*). *Weed Sci.* 59:341–348.
- Beckie, H. J., R. E. Blackshaw, R. Low, L. M. Hall, C. A. Sauder, S. Martin, R. N. Brandt, and S. W. Shirriff. 2013. Glyphosate- and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. *Weed Sci.* DOI: 10.1614/WS-D-12-00116.1
- Beckie, H. J., E. N. Johnson, and A. Légère. 2012. Negative cross-resistance of acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) to protoporphyrinogen oxidase and hydroxyphenylpyruvate dioxygenase-inhibiting herbicides. *Weed Technol.* 26:570–574.
- Beckie, H. J. and F. J. Tardif. 2012. Herbicide cross resistance in weeds. *Crop Prot.* 35:15–28.
- Beckie, H. J., S. I. Warwick, C. A. Sauder, C. Lozinski, and S. Shirriff. 2011. Occurrence and molecular characterization of acetolactate synthase (ALS) inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. *Weed Technol.* 25:170–175.
- Bernasconi, P., A. R. Woodworth, B. A. Rosen, M. V. Subramanian, and D. L. Siehl. 1995. A naturally occurring point mutation confers broad range tolerance to herbicides that target acetolactate synthase. *Biol. Chem.* 270:17381–17385.
- Chang, A. K. and R. G. Duggleby. 1998. Herbicide-resistant forms of *Arabidopsis thaliana* acetohydroxyacid synthase: characterization of the catalytic properties and sensitivity to inhibitors of four defined mutants. *Biochem. J.* 333:765–777.
- Christoffoleti, P. J., P. Westra, and F. Moore III. 1997. Growth analysis of sulfonyl-resistant and -susceptible kochia (*Kochia scoparia*). *Weed Sci.* 45:691–695.
- Cousens, R. D., G. S. Gill, and E. J. Speijers. 1997. Comment: number of sample populations required to determine the effects of herbicide resistance on plant growth and fitness. *Weed Res.* 37:1–4.
- Duggleby, R. G., S. S. Pang, H. Yu, and L. W. Guddat. 2003. Systematic characterization of mutations in yeast acetohydroxyacid synthase. *Eur. Biochem.* 270:2895–2904.
- Dyer, W. E., P. W. Chee, and P. K. Fay. 1993. Rapid germination of sulfonyl-resistant *Kochia scoparia* L. accessions is associated with elevated seed level of branched chain amino acid. *Weed Sci.* 41:18–22.
- Eberlein, C. V., M. J. Guttieri, P. H. Berger, J. K. Fellman, C. A. Mallory-Smith, D. C. Thill, R. J. Baerg, and W. R. Belknap. 1999. Physiological consequences of mutation for ALS-inhibitor resistance. *Weed Sci.* 47:383–392.
- Friesen, L. F., H. J. Beckie, S. I. Warwick, and R. C. Van Acker. 2009. The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. *Can. J. Plant Sci.* 89:141–167.
- Friesen, L. F., I. N. Morrison, A. Rashid, and M. D. Devine. 1993. Response of a chlorsulfuron-resistant biotype of *Kochia scoparia* to sulfonyl urea and alternative herbicides. *Weed Sci.* 41:100–106.
- Friesen, M. L. and E. J. von Wettberg. 2010. Adapting genomics to study evolution and ecology of agricultural systems. *Curr. Opin. Plant Biol.* 13:119–125.
- Goullart, I.C.G.R., F. O. Matzenbacher, and A. Merotto Jr. 2012. Differential germination pattern of rice cultivars resistant to imidazolinone herbicides carrying different acetolactate synthase gene mutations. *Weed Res.* 52: 224–232.
- Guttieri, M. J., C. V. Eberlein, and E. J. Souza. 1998. Inbreeding coefficients of field populations of *Kochia scoparia* using chlorsulfuron resistance as a phenotypic marker. *Weed Sci.* 46:521–525.
- Hanzawa, Y., T. Money, and D. Bradley. 2005. A single amino acid converts a repressor to an activator of flowering. *Proc. Natl. Acad. Sci. U. S. A.* 102:7748–7753.
- Harper, J. L. 1977. *Population Biology of Plants*. London, UK: Academic. 892 p.
- Heap, I. M. 2012. *International Survey of Herbicide Resistant Weeds*. <http://www.weedscience.org>. Accessed: August 2012.

- Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *Am. Nat.* 173:579–588.
- Hess, M., G. Barralis, H. Bleiholder, L. Buhr, Th. Eggers, H. Hack, and R. Stauss. 1997. Use of extended BBCH scale—general for the descriptions of the growth stage of mono- and dicotyledonous weed species. *Weed Res.* 37:433–441.
- Lamego, F. P., R. A. Vidal, and N. R. Burgos. 2011. Competitiveness of ALS inhibitor resistant and susceptible biotypes of greater beggarticks (*Bidens subalternans*). *Planta Daninha* 29:457–464.
- Leeson, J. Y., A. G. Thomas, L. M. Hall, C. A. Brenzil, T. Andrews, K. R. Brown, and R. C. Van Acker. 2005. Prairie Weed Surveys of Cereal, Oilseed and Pulse Crops from the 1970s to the 2000s. Saskatoon, Saskatchewan, Canada: Agriculture and Agri-Food Canada Weed Survey Series Publ. 05-1. 395 p.
- Li, M., Q. Yu, H. Han, M. Vila-Aiub, and S. B. Powles. 2012. ALS herbicide resistance in *Raphanus raphanistrum*: evaluation of pleiotropic effects on vegetative growth and ALS activity. *Pest Manag. Sci.* DOI: 10.1002/ps.3419
- Mengistu, L. W. and C. G. Messerimith. 2002. Genetic diversity of kochia. *Weed Sci.* 50:498–503.
- Poston, D. H., H. P. Wilson, and T. E. Hines. 2002. Growth and development of imidazolinone-resistant and -susceptible smooth pigweed biotypes. *Weed Sci.* 50:485–493.
- Preston, C., L. M. Stone, M. A. Rieger, and J. Baker. 2006. Multiple effects of a naturally occurring proline to threonine substitution within acetolactate synthase in two herbicide-resistant populations of *Lactuca serriola*. *Pestic. Biochem. Physiol.* 84:227–235.
- Renton, M. 2013. Shifting focus from the population to the individual as a way forward in understanding, predicting and managing the complexities of resistance to pesticides. *Pest Manag. Sci.* 69:171–175.
- Roux, F., J. Gasquez, and X. Reboud. 2004. The dominance of the herbicide resistance cost in several *Arabidopsis thaliana* mutant lines. *Genetics* 166: 449–460.
- Saari, L., J. C. Cotterman, and M. M. Primiani. 1990. Mechanism of sulfonyl urea herbicide resistance in the broadleaf weed, *Kochia scoparia*. *Plant Physiol.* 93:55–61.
- SAS Institute, Inc. 2004. SAS/STAT 9.1 User's Guide. Cary, NC: SAS Institutetetra. 5121 p.
- Sibony, M. and B. Rubin. 2003. The ecological fitness of ALS-resistant *Amaranthus retroflexus* and multiple-resistant *Amaranthus blitoides*. *Weed Res.* 43:40–47.
- Stallings, G. P., D. C. Thill, C. A. Mallory-Smith, and B. Shafii. 1995. Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). *Weed Sci.* 43:95–102.
- Tardif, F. J., I. Rejcan, and M. Costea. 2006. A mutation in the herbicide target site acetohydroxyacid synthase produces morphological and structural alterations and reduces fitness in *Amaranthus powellii*. *New Phytol.* 169:251–264.
- Thompson, C. R., D. C. Thill, and B. Shafii. 1994a. Germination characteristics of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). *Weed Sci.* 42:50–56.
- Thompson, C. R., D. C. Thill, and B. Shafii. 1994b. Growth and competitiveness of sulfonylurea-resistant and -susceptible kochia (*Kochia scoparia*). *Weed Sci.* 42:172–179.
- Tranel, P. J. and T. R. Wright. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? *Weed Sci.* 50:700–712.
- Vila-Aiub, M. M., P. Neve, and S. B. Powles. 2009. Fitness costs associated with evolved herbicide resistance alleles in plants. *New Phytol.* 184:751–767.
- Vila-Aiub, M. M., P. Neve, and F. Roux. 2011. A unified approach to the estimation and interpretation of resistance costs in plants. *Heredity* 107:386–394.
- Warwick, S. I., R. Xu, C. Sauder, and H. J. Beckie. 2008. Acetolactate synthase target-site mutations and single nucleotide polymorphism genotyping in ALS-resistant kochia (*Kochia scoparia*). *Weed Sci.* 56:797–806.
- Wiersma, A., P. Westra, J. E. Leach, and C. Preston. 2011. Response Patterns of Suspected Glyphosate Resistant Kochia Accessions. WSSA Abstracts, No. 363. <https://wssaabstracts.com/user/home.php>. Accessed: October 2012.
- Yin, X., J. Goudriaan, E. A. Lanting, J. Vos, and H. J. Spiertz. 2003. A flexible sigmoid function of determinate growth. *Ann. Bot.* 91:361–371.
- Yu, Q., H. Han, M. Li, M. J. Walsh, and S. B. Powles. 2012. Resistance evaluation for herbicide-resistance-endowing acetolactate synthase (ALS) gene mutations using *Raphanus raphanistrum* populations homozygous for specific ALS mutations. *Weed Res.* 52:178–186.
- Yu, Q., H. Han, M. M. Vila-Aiub, and S. B. Powles. 2010. AHAS herbicide resistance endowing mutations: effect on AHAS functionality and plant growth. *Exp. Bot.* 61:3925–3934.

Received August 3, 2012, and approved October 26, 2012.