

Negative Cross-Resistance of Acetolactate Synthase Inhibitor–Resistant Kochia (*Kochia scoparia*) to Protoporphyrinogen Oxidase– and Hydroxyphenylpyruvate Dioxygenase–Inhibiting Herbicides

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This greenhouse experiment examined the response of homozygous susceptible and acetolactate synthase (ALS) inhibitor–resistant plants from six Canadian kochia accessions with the Pro197 or Trp574 mutation to six alternative herbicides of different sites of action. The null hypothesis was ALS-inhibitor–resistant and –susceptible plants from within and across accessions would respond similarly to herbicides of different sites of action. This hypothesis was accepted for all accessions except that of MBK2 with the Trp574 mutation. Resistant plants of that accession were 80, 60, and 50% more sensitive than susceptible plants to pyrasulfotole, mesotrione (hydroxyphenylpyruvate dioxygenase [HPPD] inhibitors), and carfentrazone (protoporphyrinogen oxidase [PPO] inhibitor), respectively. However, no differential dose response between resistant and susceptible plants of this kochia accession to bromoxynil, fluroxypyr, or glyphosate was observed. A previous study had found marked differences in growth and development between resistant and susceptible plants of this accession, but not of the other accessions examined in this experiment. Negative cross-resistance exhibited by resistant plants of accession MBK2 to PPO and HPPD inhibitors in this experiment may be a pleiotropic effect related to the Trp574 mutation.

Nomenclature: Bromoxynil; carfentrazone; fluroxypyr; glyphosate; mesotrione; pyrasulfotole; kochia, *Kochia scoparia* (L.) Schrad. KCHSC, synonym: *Bassia scoparia* (L.) A.J. Scott.

Key words: ALS-inhibitor resistance, herbicide resistance, pleiotropic effect, target-site mutation.

Este experimento de invernadero examinó la respuesta a seis herbicidas alternativos con diferentes sitios de acción de plantas de seis accesiones canadienses de *Kochia scoparia*, homocigotas susceptibles y resistentes a herbicidas inhibidores de acetolactate synthase (ALS) con las mutaciones Pro197 o Trp574. La hipótesis nula fue que plantas resistentes y susceptibles a inhibidores de ALS dentro y entre accesiones responderían en forma similar a herbicidas con diferentes sitios de acción. Esta hipótesis fue aceptada para todas las accesiones excepto MBK2 con la mutación Trp574. Comparadas con las plantas susceptibles, las plantas resistentes de esta accesión fueron 80, 60 y 50% más sensibles a pyrasulfotole, mesotrione (inhibidores de hydroxyphenylpyruvate dioxygenase [HPPD]) y carfentrazone (inhibidor de protoporphyrinogen oxidase [PPO]), respectivamente. Sin embargo, no se observó una respuesta a dosis diferenciada entre plantas resistentes y susceptibles de esta accesión de *K. scoparia* al ser tratadas con bromoxynil, fluroxypyr o glyphosate. Un estudio previo había encontrado diferencias marcadas en crecimiento y desarrollo entre plantas resistentes y susceptibles de esta accesión, pero no en las otras accesiones examinadas en este experimento. La resistencia cruzada-negativa que mostraron las plantas resistentes de la accesión MBK2 a inhibidores PPO y HPPD en este experimento podría ser un efecto pleiotrópico relacionado a la mutación Trp574.

Kochia is a common and economically important weed in crop and ruderal areas in the southern Canadian prairies and Great Plains of the United States. Across the prairies, kochia has increased in relative abundance rank (index based on field frequency, plant density, and field uniformity) by 14 places during the past 40 yr, and is currently the 10th most abundant weed (Leeson et al. 2005). The species is highly competitive, largely because of early season emergence, rapid growth, and tolerance to heat, drought, and salinity; kochia interference can reduce crop yields by up to 60% (reviewed in Friesen et al. 2009).

Acetolactate synthase (ALS) inhibitor–resistant populations were first reported in western Canada in 1988 (Morrison and Devine 1994). Currently, most (> 90%) populations across

the prairies are resistant to ALS-inhibiting herbicides (Beckie et al. 2011). Amino-acid substitutions identified for target-site (ALS) mutations in Canadian populations, in order of decreasing occurrence, are Trp574Leu, Pro197 (with substitution by one of nine amino acids), and Asp376Glu (Beckie and Tardif 2012; Beckie et al. 2011; Warwick et al. 2008). Although ALS-inhibiting herbicides are commonly used to control kochia in field crops (Saskatchewan Ministry of Agriculture 2012), the rapid increase in resistance occurrence in this weed since 1988 is a consequence of gene flow of resistance alleles via seed (tumbleweed) and, to a lesser extent, pollen (reviewed in Friesen et al. 2009).

Negative cross-resistance, i.e., herbicide-resistant plants being more sensitive to herbicides than susceptible plants, has been documented in several triazine-resistant weed biotypes (Dabaan and Garbutt 1997; Gadamski et al. 2000; Jordon et al. 1999; Parks et al. 1996). Some herbicides that inhibit photosystem II bind more efficiently to the mutant triazine binding domain than to the wild (susceptible) type. Triazine-resistant weeds frequently show negative

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Table 1. Herbicides used in the dose-response experiments, applied at 0, 0.125, 0.25, 0.5, 1, and 2 times the field-recommended dose.

Herbicide	Site of action ^a	Formulation ^b	Recommended dose g ai(e) ha ⁻¹
Fluroxypyr	Synthetic auxin	Attain A, 180 g L ⁻¹ EC	105
Glyphosate	EPSPS inhibitor	Roundup Transorb HC, 540 g L ⁻¹ K salt	450
Carfentrazone	PPO inhibitor	Aim, 240 g L ⁻¹ EC ^c	8.9
Pyrasulfotole + bromoxynil	HPPD inhibitor + Photosystem-II inhibitor	Infinity, 31 + 174 g L ⁻¹ EC	205
Bromoxynil	Photosystem-II inhibitor	Pardner, 280 g L ⁻¹ EC	280
Mesotrione	HPPD inhibitor	Callisto, 480 g L ⁻¹ SC	144

^a Abbreviations: EPSPS; 5-enolpyruvylshikimate-3-phosphate synthase; PPO, protoporphyrinogen oxidase; HPPD, hydroxyphenylpyruvate dioxygenase.

^b Manufacturers (in order of herbicides listed): Dow AgroSciences, Calgary, Alberta; Monsanto, Winnipeg, MB; Nufarm Agriculture, Calgary, Alberta; Bayer CropScience, Calgary, Alberta (pyrasulfotole + bromoxynil; bromoxynil); Syngenta Crop Protection, Regina, Saskatchewan.

^c Ag-Surf nonionic surfactant added to carfentrazone at 0.25% (v/v); Agral 90 nonionic surfactant added to mesotrione at 0.25% (v/v). EC, emulsifiable concentrate; SC, suspension concentrate.

cross-resistance to other photosystem-II inhibitors, such as bentazon and pyridate; triazine-resistant weeds can also exhibit negative cross-resistance to herbicides that do not affect photosystem II (Gadamski et al. 2000). Explanations for this phenomenon depend on the specific herbicide, but are largely speculative. Negative cross-resistance combined with the frequent fitness penalty of target site-based triazine-resistant populations can facilitate resistance management (Gadamski et al. 2000). For example, pyridate has been mixed with triazine herbicides and applied to millions of hectares annually, especially in Europe, to control triazine-resistant populations (Gressel 2002).

Negative cross-resistance has also been observed in non-triazine-resistant biotypes. For example, an imidazolinone-resistant smooth pigweed (*Amaranthus hybridus* L.) population was 10-fold more sensitive to cloransulam, another ALS inhibitor, compared with a susceptible population (Poston et al. 2001). A subsequent study found that the ALS enzyme of three imidazolinone-resistant populations exhibited increased sensitivity to inhibition by chlorimuron, thifensulfuron, or pyrithiobac compared with ALS from a susceptible population (Poston et al. 2002).

Homozygous susceptible and resistant plants from three accessions with the Pro197 mutation, and five accessions with the Trp574 mutation, were obtained by molecular characterization of field-collected parental plants (Beckie et al. 2011). This plant material has been used to identify growth differences between ALS inhibitor-resistant and -susceptible plants of an accession, and determine whether differences varied according to mutation or geographic origin (Légère et al. 2010a). Additionally, we examined the response of resistant and susceptible plants from six of these eight accessions to increasing doses of six alternative herbicides of different sites of action. The null hypothesis was ALS-inhibitor-resistant and -susceptible plants from within and across accessions would respond similarly to herbicides of different sites of action. That experiment is described herein.

Materials and Methods

Herbicide Dose-Response Experiments. The herbicide dose-response experiments included homozygous susceptible and resistant kochia plants from three accessions with the Pro197

mutation (ABK56, ABK75, MBK6) and three accessions with the Trp574 mutation (ABK82, SKK4, MBK2). Accessions originated from southern regions of the provinces of Alberta (AB), Saskatchewan (SK), and Manitoba (MB) in western Canada. Experiments were conducted in the greenhouse in the winter of 2011 at Saskatoon, Saskatchewan and repeated once. The experiments were arranged in a completely randomized design with four replications (one pot per replicate) per treatment. The dose response of susceptible and resistant plants of the six accessions to each of the six herbicides (fluroxypyr, glyphosate, carfentrazone, pyrasulfotole + bromoxynil, bromoxynil, mesotrione; Table 1) were separate experiments, i.e., one herbicide per experiment. Because pyrasulfotole is formulated with bromoxynil and not available as a stand-alone herbicide, bromoxynil was included in the study to infer the response of the kochia accessions to pyrasulfotole alone. The sites of action of the herbicides are the following: fluroxypyr, synthetic auxin; glyphosate, enolpyruvyl-shikimate-3-phosphate synthase inhibitor; carfentrazone, protoporphyrinogen oxidase (PPO) inhibitor; bromoxynil, photosystem-II inhibitor; and pyrasulfotole and mesotrione, hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors.

Five seeds were planted 1 cm deep in 10-cm square pots containing a mixture of soil, peat, vermiculite, and sand (3 : 2 : 2 : 2 by volume) plus a controlled-release fertilizer (15–9–12, 150 g 75 L⁻¹; Scotts Osmocote PLUS, Mississauga, Ontario). Experiments were conducted under a 20/16 C day/night temperature regime with a 16-h photoperiod supplemented with 230 μmol m⁻² s⁻¹ illumination. Pots were watered daily to field capacity.

Herbicides were applied to seedlings at the five- to six-leaf stage. Herbicides were applied with the use of a moving-nozzle cabinet sprayer equipped with a flat-fan nozzle tip (TeeJet 8002VS; Spraying Systems Co., Wheaton, IL) calibrated to deliver 200 L ha⁻¹ of spray solution at 275 kPa in a single pass over the foliage. Each herbicide was applied at 0.125, 0.25, 0.5, 1, and 2 times the field-recommended dose plus a nontreated control. The recommended dose in western Canada of the herbicides were as follows (g ai[e] ha⁻¹): fluroxypyr, 105; glyphosate, 450; carfentrazone, 8.9; pyrasulfotole plus bromoxynil, 205; bromoxynil, 280; and mesotrione, 144 (Table 1). Commercial formulations of the herbicides

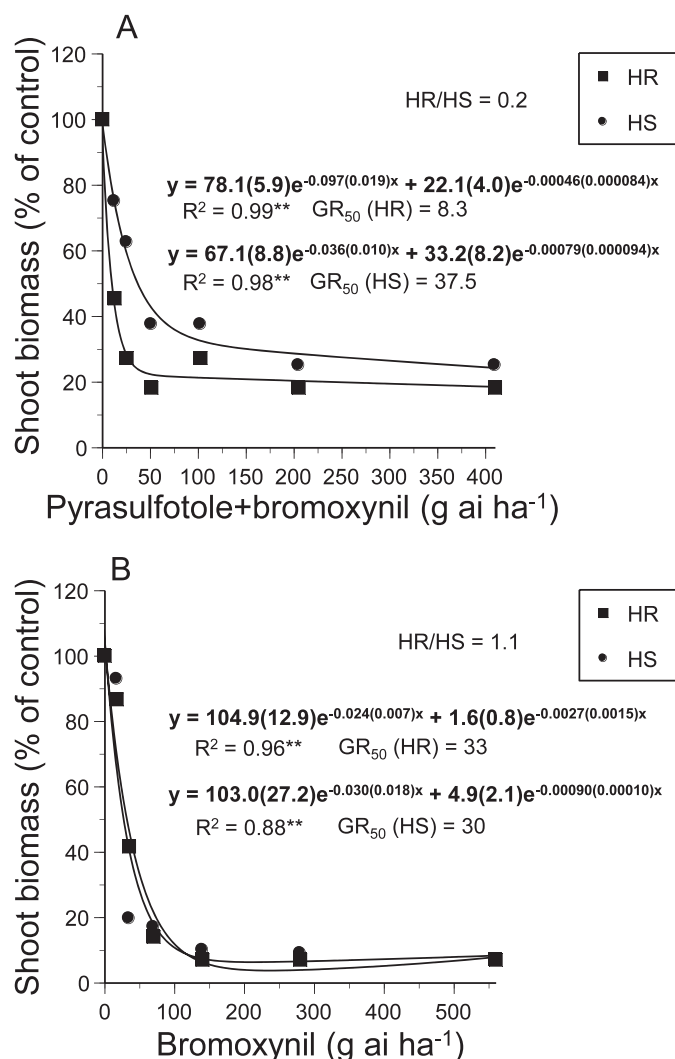


Figure 1. (A) Aboveground biomass response of acetolactate synthase (ALS) inhibitor-resistant (HR) and -susceptible (HS) plants of kochia accession MBK2 to increasing dose of the premixture pyrasulfotole (hydroxyphenylpyruvate dioxygenase inhibitor) plus bromoxynil (photosystem-II inhibitor). The double exponential decay model is described in the text (standard error in parentheses); double asterisks indicate significance at $P < 0.01$. (B) Aboveground biomass response of ALS-inhibitor-resistant (HR) and -susceptible (HS) plants of kochia accession MBK2 to increasing dose of bromoxynil. The double exponential decay model is described in the text (standard error in parentheses); double asterisks denote significance at $P < 0.01$.

were used. A nonionic surfactant at 0.25% (v/v) was added to the carfentrazone and mesotrione spray solutions (Table 1). Two weeks after treatment, shoot biomass was harvested. Harvested biomass was dried at 60 C for 3 d, and weighed.

Dose-Response Data Analysis. Results of each experiment were combined across runs upon confirmation of homogeneity of variances (Steel and Torrie 1980). The six experiments (each herbicide) were analyzed separately. Aboveground biomass (Y ; percentage of nontreated control) was regressed against herbicide dose (x , g ha⁻¹) using the double exponential decay model, which provided the best fit of the dose responses (Equation 1):

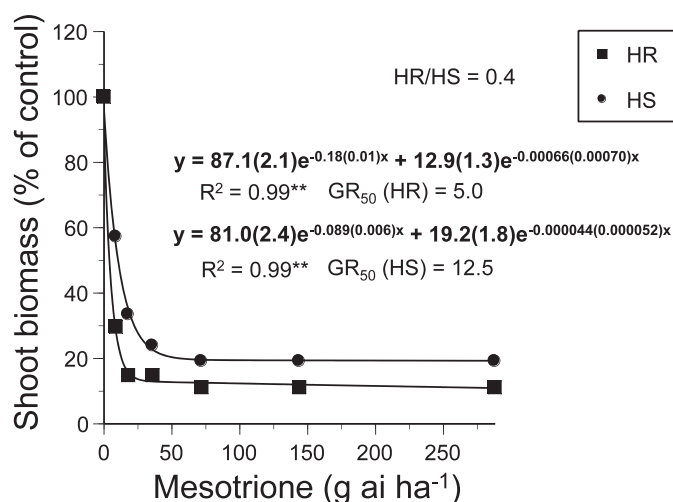


Figure 2. Aboveground biomass response of acetolactate synthase inhibitor-resistant (HR) and -susceptible (HS) plants of kochia accession MBK2 to increasing dose of mesotrione (hydroxyphenylpyruvate dioxygenase inhibitor). The double exponential decay model is described in the text (standard error in parentheses); double asterisks denote significance at $P < 0.01$.

$$Y = a \exp(-bx) + c \exp(-dx), \quad [1]$$

where $a + c$ is the intercept (% of nontreated control) and b, d quantify the slope (Yoshimura et al. 2006). Data were fitted to the model with the use of a derivative-free nonlinear regression procedure, provided with PROC NLIN (SAS 1999). Regression analyses were performed on treatment means averaged over replications as recommended by Gomez and Gomez (1984). Regression curves were statistically compared with the use of the lack-of-fit F test at the 0.05 level of significance, as outlined by Seefeldt et al. (1995). The resistance factor (index) was calculated as GR₅₀ of resistant plants divided by GR₅₀ of susceptible plants (within an accession), where GR₅₀ is the dose resulting in a 50% reduction in aboveground biomass relative to the nontreated control.

Results and Discussion

There was no difference in response between resistant and susceptible plants within or across accessions ABK56, ABK75, MBK6, ABK82, and SKK4 to any of the six herbicides (data not shown). The only accession where differential response to a herbicide was observed was MBK2 (resistant homozygous line MBK2-9R and susceptible homozygous line MBK2-5S). The lack-of-fit F test indicated a differential response of these lines to pyrasulfotole plus bromoxynil (Figure 1A), mesotrione (Figure 2), and carfentrazone (Figure 3). Because both lines responded similarly to bromoxynil (Figure 1B), we inferred that the differential response of the kochia lines to pyrasulfotole plus bromoxynil premixture was attributable to the pyrasulfotole component. Both lines also responded similarly to fluroxypyr and glyphosate (data not shown).

The double exponential decay model provided a good fit to the data, as indicated by the significance (**, $P < 0.01$) of the

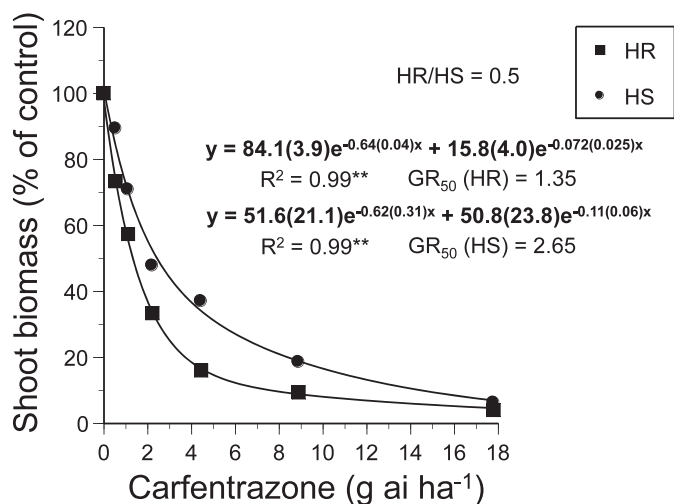


Figure 3. Aboveground biomass response of acetolactate synthase inhibitor-resistant (HR) and -susceptible (HS) plants of kochia accession MBK2 to increasing dose of carfentrazone (protoporphyrinogen oxidase inhibitor). The double exponential decay model is described in the text (standard error in parentheses); double asterisks denote significance at $P < 0.01$.

coefficients of determination (R^2). Parameter estimates are considered significant at the 0.05 level if the standard error is less than one-half the value of the estimate (Koutsoyiannis 1977). In most instances, the parameter estimates of the regression equations were significant.

Resistant plants of accession MBK2 were more sensitive than susceptible plants to pyrasulfotole (resistance factor = 0.2; Figure 1A), mesotrione (resistance factor = 0.4; Figure 2), and carfentrazone (resistance factor = 0.5; Figure 3). Pyrasulfotole and mesotrione are both HPPD inhibitors, whereas carfentrazone is a PPO inhibitor. Thus, resistant plants of that accession were 80, 60, and 50% more sensitive than susceptible plants to pyrasulfotole, mesotrione, and carfentrazone, respectively. Further studies are required to determine herbicide sensitivity differences between the kochia lines of this accession under field conditions.

In greenhouse replacement series experiments, Légère et al. (2010a) had examined the growth of resistant and susceptible plants from accessions ABK56, ABK75, ABK82, SKK4, MBK2, and MBK6. Resistant plants of accession MBK2, which possesses the Trp574Leu substitution, developed slower than susceptible plants of the accession; moreover, resistant plants produced significantly more shoot and root biomass, but much less seed biomass than susceptible plants (Légère et al. 2010b). Growth differences were also noted between resistant and susceptible plants of MBK6 (Pro197Gln substitution), but differences were much smaller than those observed for MBK2. Differential growth between resistant and susceptible plants was not apparent for the remaining accessions.

Previous research found no or negligible fitness penalty in kochia plants with Pro197Thr or Arg substitutions (Thompson et al. 1994a). In contrast, the Trp574Leu substitution had major pleiotropic effects on Powell amaranth (*Amaranthus powellii* S. Wats.) growth; similar to resistant plants of kochia accession MBK2, resistant biotypes of Powell amaranth produced much less seed than susceptible biotypes (Tardif

et al. 2006). Fecundity is a key criterion of plant fitness, as it directly affects population demographics or the evolutionary success of a weed biotype.

Some mutations result in decreased ALS sensitivity to feedback inhibition by the branched-chain amino acids Val, Leu, and/or Ile. In a study by Thompson et al. (1994b), kochia biotypes with the Pro197 mutation germinated faster than susceptible biotypes at low temperatures. As a consequence of reduced feedback sensitivity to inhibition, branched-chain amino acids accumulate in plant tissues; this higher concentration of free amino acids is correlated with higher seed germination rates at low temperatures (Dyer et al. 1993).

Powles and Yu (2010) concluded that some resistance mutations have a negligible effect on ALS activity or functionality, whereas others can alter such properties and/or have other pleiotropic effects on the plant. Moreover, fitness costs or pleiotropic effects may vary among ALS inhibitor-resistant weed populations that possess the same mutation (Vila-Aiub et al. 2009). Negative cross-resistance exhibited by resistant plants of accession MBK2 to PPO and HPPD inhibitors used in this study may be such a pleiotropic effect related to the Trp574 mutation. The exact physiological or biochemical processes involved remain to be elucidated.

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