FINAL REPORT

Glyphosate-resistant Kochia (*Kochia scoparia*) in Saskatchewan

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Previous surveys have documented the occurrence of glyphosate-resistant (GR) kochia in Alberta in 2011 and 2012. To determine the incidence of GR kochia in the neighbouring province of Saskatchewan, a stratified-randomized survey of 342 sites (one population per site) in southern and central regions of the province was conducted in the fall of 2013. Mature plants were collected, seed threshed, and progeny screened by spraying with a discriminating glyphosate dose of 900 g ae ha^{-1} under greenhouse conditions. Screening confirmed 17 GR kochia populations in nine municipalities in west-central or central Saskatchewan. While the majority of GR kochia populations originated in chemical-fallow fields, some populations were found in cropped fields (wheat, lentil, GR canola) and non-cropped areas (oil well site, roadside ditch). Agronomic and economic impact of this GR weed biotype is compounded because of consistent multiple resistance to acetolactate synthase-inhibiting herbicides. However, GR kochia is susceptible to dicamba, an increasingly important auxinic herbicide used for control of this multiple-resistant biotype.

Nomenclature: dicamba; glyphosate; kochia, *Kochia scoparia* (L.) Schrad. KCHSC, synonym: *Bassia scoparia* (L.) A.J. Scott.; canola, *Brassica napus* L.; lentil, *Lens culinaris* Medik.; wheat, *Triticum aestivum* L.

Keywords: ALS-inhibitor resistance, glyphosate resistance, herbicide resistance, multiple resistance.

In western Canada, glyphosate is a key herbicide for weed control in chemical fallow, preseeding in zero-tillage systems, pre- and post-harvest control, and in glyphosate-resistant (GR) canola, corn (*Zea mays* L.), soybean (*Glycine max* L. Merr.), and sugar beet (*Beta vulgaris* L.). Glyphosate was first introduced in Canada in 1974, and is the most widely used herbicide in the world. In western Canada, glyphosate usage surpasses that of the next top 12 herbicides combined (Agdata, courtesy N. Harker, personal communication). Frequent glyphosate use has selected for GR weeds – currently 28 weed species in several countries, including eastern Canada (Heap 2014; Vink et al. 2012). Until 2011, GR weeds had not been identified in western Canada.

Kochia is the 10th most abundant weed across the Canadian prairies, but fourth most abundant weed in the southern semiarid Grassland region (Leeson et al. 2005). A competitive tumbleweed with early emergence (Schwinghamer and Van Acker 2008), abundant seed production, and stress tolerance, it occurs in agricultural areas, waste lands, and rangelands (Friesen et al. 2009). Kochia resistant to acetolactate synthase (ALS) inhibitors was first reported in the Canadian prairies in 1988 (Heap 2014). Beckie et al. (2011b, 2013b) found that 20 yr later, about 90% of prairie populations tested were ALS inhibitor-resistant. Resistant genes may be transmitted via pollen flow; however, long-distance transport of resistant alleles occurs via seed dispersal from mature plants tumbling across the landscape (Stallings et al. 1995).

GR kochia was first identified in Kansas in 2007 (Waite 2008; Waite et al. 2013), and is now present in seven states (Heap 2014). In 2011, kochia with multiple resistance to glyphosate and ALS inhibitors was discovered in southern Alberta (Beckie et al. 2013a). Initially, three populations were identified in chemical-fallow fields, with an additional seven populations confirmed later in the year from a survey conducted within a 20-km radius of the intial sites. Resistance level was considered low to moderate, with a resistance factor (ratio of the rates required for 50% control of the resistant and susceptible populations) of 4 to 7.

An expanded survey was conducted across southern Alberta in 2012 to determine the distribution and abundance of this GR weed biotype. GR kochia was identified at 13 of 309 sites surveyed (4% of fields) (Hall et al. 2014). Seven sites were located in Warner County, where GR kochia was previously confirmed in 2011 (Beckie et al. 2013a). Besides those 13 sites, GR kochia from 9 sites was also confirmed in Alberta that year from samples submitted by growers; moreover, 10 kochia samples submitted by growers in west-central and southwestern Saskatchewan in 2012 were confirmed as GR (Hall et al. 2014). Most populations originated in chemical fallow where glyphosate was typically applied alone, and at multiple times during the season to control vegetation. However, in the 2012 Alberta survey, two of the locations where GR kochia was found were non-agricultural areas (ditch and railway rights-of-way) adjacent to agricultural areas. The GR kochia populations identified in the 2011 and 2012 surveys were consistently resistant to ALS-inhibiting herbicides (Beckie et al. 2013a; Hall et al. 2014).

To determine the geographical extent of this GR weed, surveys similar to that conducted in Alberta in 2012 were needed in the neighbouring provinces of Saskatchewan and Manitoba in regions where the weed is abundant. Although GR kochia has been confirmed in Saskatchewan based on samples submitted by growers, its distribution and abundance in the province can only be determined in a random survey. Accordingly, a survey was conducted in the fall of 2013 across southern and central regions of Saskatchewan to determine the distribution and abundance of GR kochia. A concurrent GR kochia survey was conducted across southern Manitoba, and is reported elsewhere.

Materials and Methods

Survey Methodology. A survey of GR kochia was conducted post-harvest in September and October, 2013 in Saskatchewan. Similar to the 2012 Alberta survey, a stratified-randomized design was used to select sites (Hall et al. 2014). The number of survey sites was stratified, proportional to cultivated land area per ecodistrict (geographic area within an ecoregion similar in landform, soil, vegetation, and land use); sites were located mainly within the Mixed Grassland and Moist Mixed Grassland ecoregions (Agriculture and Agri-Food Canada 2003; Leeson et al. 2005). Therefore, the proportional allocation of sites in each municipality was the same as that of the previous general weed surveys (Leeson et al. 2005). Surveyors drove to 380 randomly predetermined sites in Saskatchewan during the ca. 4-wk survey period. Approximately 10 to 20 mature kochia plants were randomly collected at each site, and placed in a cotton bag to form a composite sample. Populations were sampled in field border areas and ruderal areas such as roadsides/ditches, railway rights-of-way, and oil well sites.

Sample Processing and Resistance Screening. Samples were threshed under contained conditions, and seed samples screened for herbicide resistance (detailed in Beckie et al. 2013a) at Saskatoon, SK from December, 2013 to June, 2014. For each population, seeds were planted in 52- by 26- by 5-cm flats containing potting soil. A minimum total of 100 seedlings per population (three flats or replicates per experiment run and repeated) were sprayed with glyphosate, tribenuron/thifensulfuron, or dicamba at discriminating doses of 900 g ae ha^{-1}, 15 (5+10) g ai ha^{-1}, and 280 g ai ha^{-1}, respectively, when seedlings were 3 to 5 cm tall. Herbicides were applied using 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. Three weeks after treatment, plant response to herbicide application was visually scored as susceptible (dead or nearly dead) or resistant (some injury but new growth, or no injury). Assessments were made relative to herbicide-treated and -untreated susceptible and resistant check populations.

Results and Discussion

Viable seeds were collected and seedlings tested for resistance from 342 sites in Saskatchewan. Of the 342 kochia populations screened in the greenhouse, 17 (5%) were confirmed as GR (Figure 1). As expected based on previous survey findings (e.g., Beckie et al. 2011b), all populations were also resistant to tribenuron/thifensulfuron, an ALS-inhibiting herbicide (Table 1). These populations were found in nine municipalities, mainly in west-central Saskatchewan where 10 previously reported populations originated (Hall et al. 2014). However, GR kochia was not previously found in six of the nine municipalities. Together with the previously confirmed populations, GR kochia is present in a total of 14 municipalities in Saskatchewan.

While the majority of GR kochia populations originated in chemical-fallow fields (10 of 17), some populations were found in cropped fields (one wheat, two lentil, one canola) and non-cropped areas (one oil well site, two roadside ditches) (Figures 2 and 3). One municipality with GR kochia near Moose Jaw is spatially isolated from the cluster of municipalities in west-central

Saskatchewan with the biotype (Figure 1). This field was cropped to lentil with glyphosate applied preseeding and preharvest, which is common practice. This is the first report of GR kochia in lentil and GR canola, although immigration from adjacent land cannot be ruled out. No in-crop herbicides are available to control GR plus ALS inhibitor-resistant kochia in lentil (Beckie et al. 2013a). Other than growing a glufosinate-resistant canola cultivar, there are no in-crop herbicide options to control this multiple-resistant biotype in this oilseed crop. Although glyphosate is not applied to ditch areas that are planted to forages, the herbicide may be applied along road edges adjacent to ditches to control weeds. Glyphosate is frequently used around oil well sites to control vegetation.

The frequency of glyphosate resistance in confirmed populations varied from 12 to 96% (Table 1). Differences may be due to the time since glyphosate resistance was selected or introduced (either via seed or pollen), the amount of glyphosate selection that occurred in that population over time, or recent treatments that removed susceptible individuals from the population. Only four populations tested had a resistance frequency < 50% to the ALS-inhibiting herbicide tribenuron/thifensulfuron (Table 1). High frequency of resistance to ALS inhibitors in kochia populations had been previously reported in the prairies (Beckie et al. 2011b).

All GR kochia populations in Saskatchewan were susceptible to dicamba (data not shown). Dicamba-resistant kochia has not been identified previously in western Canada, but has been reported in the midwestern USA (Cranston et al. 2001; Preston et al. 2009). Because resistance to glyphosate and ALS-inhibiting herbicides in kochia is target-site based (5-enolypyruvyl-shikimate-3-phosphate synthase (EPSPS) gene amplification (Wiersma 2012; E. Westra, personal communication) and ALS mutation (Beckie, unpublished data)), we did not expect populations of this biotype to be resistant to herbicides of other sites of action. Effectiveness of alternative herbicides on populations of this multiple-resistant biotype was confirmed in greenhouse studies (Burton et al. 2014).

The value (cost-benefit) of chemical fallow needs to be closely evaluated. GR kochia is strongly associated with chemical fallow, based on this survey and those in Alberta in 2011 and 2012 (Beckie et al. 2013a; Hall et al. 2014). The main reason for including fallow in rotation is water conservation in these semiarid regions. However, efficiency of water conservation is poor because of evaporation or leaching. Moreover, the Canadian prairies has generally experienced a prolonged wet cycle as well as strong crop prices, which weakens the justification for including fallow in the rotation. One reason for having chemical fallow is the inability to seed land in the relatively narrow planting window from late April (but usually early May) to the first week in June because of cold or wet soil conditions.

Bare-ground land where tillage is not practiced is at elevated risk of selecting for glyphosate resistance because of lack of crop competition and often multiple glyphosate (only) applications (Preston 2014). Even when glyphosate is tank-mixed with another herbicide, some key weed species may be controlled only by glyphosate. Furthermore, reliance on the same tank-mix partner from among the few site-of-action herbicide groups available to prairie farmers will inevitably select for multiple-resistant populations. For example, growers rely on auxinic or protoporphyrinogen oxidase (PPO)-inhibiting herbicides such as dicamba, saflufenacil, or carfentrazone to tank-mix with glyphosate to control GR plus ALS inhibitor-resistant kochia in chemical fallow (Beckie et al. 2014). How long will it take for kochia populations to evolve resistance to three site-of-action herbicides? In the future, better cover crops where fallow is practiced would lessen selection pressure for GR and multiple-resistant populations (Shaner and Beckie 2014).

Kochia was the first of several species predicted to be at risk for glyphosate resistance in western Canada (Beckie et al. 2011a, 2013a). Other abundant species selected during preseeding or in-crop/fallow applications are also at risk, including wild oat (*Avena fatua* L.), green foxtail (*Setaria viridis* L. Beauv.), cleavers (*Galium* spp.), and wild buckwheat (*Polygonum convolvulus* L.) (Beckie et al. 2013a). Like kochia, these weeds have already been selected for resistance to herbicides with different sites of action used in-crop. Worldwide, the incidence of multiple-resistant weed biotypes is increasing at an alarming rate (Heap 2014). Across the prairies, multiple-resistant weeds will continue to challenge growers, especially when one of those sites of action is glyphosate.

Surveillance of GR kochia across western Canada will continue in the future, through periodic surveys as described herein and testing suspected samples submitted by growers each year. A 7-d seed assay (Pratchler et al. 2014) may facilitate more timely confirmation of GR kochia, thereby aiding resistant weed management. We expect GR kochia to rapidly spread across the prairies, similar to ALS inhibitor-resistant populations. During these surveys, it was common to see putative GR kochia populations in fields adjacent to the survey-targeted field, suggesting seed spread via tumbleweed movement or by farm equipment. The ease of mobility of resistance alleles from field to field demands a collective regional response in proactively or reactively managing this multiple-resistant biotype.

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Table 1 Percentage of plants in a population resistant (R) to glyphosate or tribenuron/ thifensulfuron, and the habitat (CF = chemical fallow) and municipality where populations were located in Saskatchewan. All populations were susceptible to dicamba.

Municipality	Habitat	Glyphosate-R	Tribenuron/thifensulfuron-R
161	Lentil field	71	91
228	Canola field	92	95
228	CF	31	93
228	Ditch	73	90
230	CF	51	82
230	CF	85	85
230	Ditch	86	61
231	CF	85	32
257	CF	70	92
259	Wheat field	41	95
259	CF	32	46
260	CF	96	17
260	CF	95	60
261	CF	72	95
261	CF	80	81
261	Oil well site	91	80
285	Lentil field	12	7

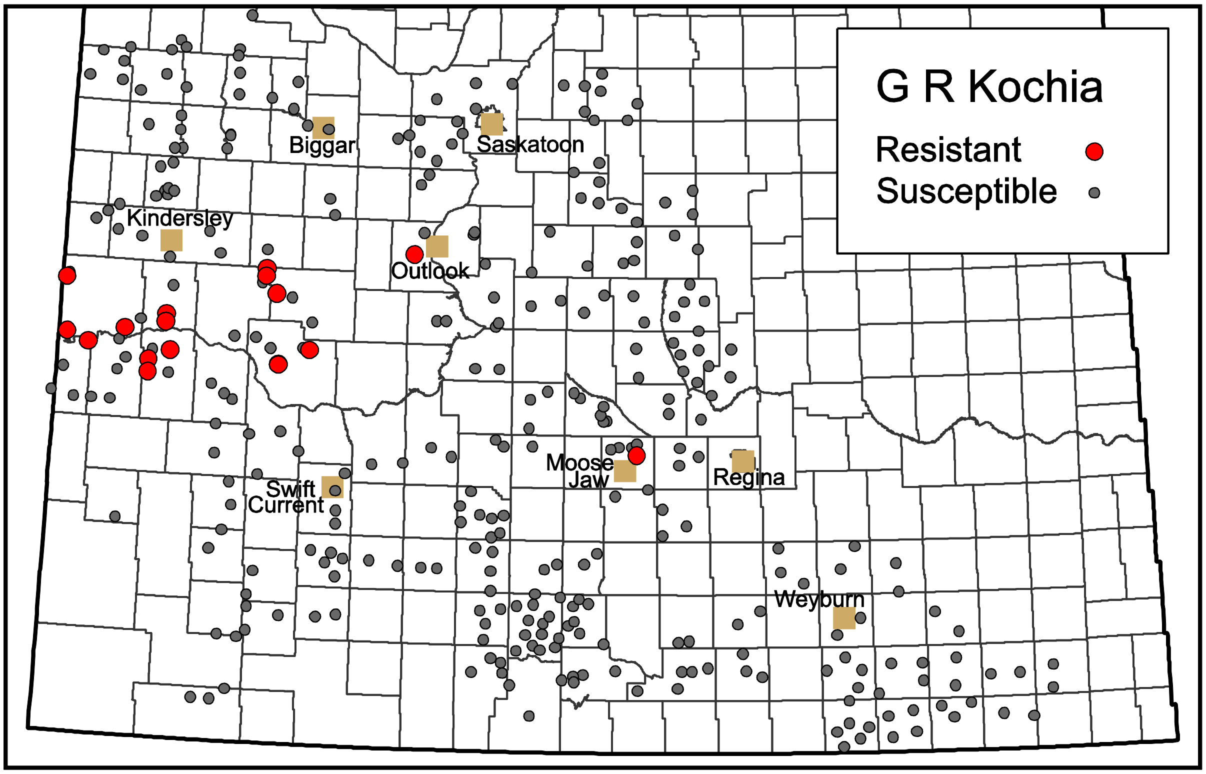

Figure 1. Glyphosate-resistant kochia in Saskatchewan in 2013.

Figure 2. Glyphosate-resistant kochia in lentil.

Figure 3. Glyphosate-resistant kochia post-harvest in canola in fall, 2013.