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## Rapid increase in glyphosate resistance and confirmation of dicamba-resistant kochia (*Bassia scoparia*) in Manitoba

Charles M. Geddes, Mattea M. Pittman, Robert H. Gulden, Tammy Jones, Julia Y. Leeson, Shaun M. Sharpe, Scott W. Shirriff, and Hugh J. Beckie

**Abstract:** Increased adoption of crops with stacked traits conferring glyphosate and dicamba resistance, and recent confirmation of kochia [*Bassia scoparia* (L.) A.J. Scott] biotypes resistant to these herbicides in Alberta and Saskatchewan, warrant surveillance of herbicide-resistant kochia in Manitoba. A randomized-stratified survey of 315 sites in Manitoba was conducted in the fall of 2018. Overall, 58% of the kochia populations tested were glyphosate-resistant, while 1% were dicamba-resistant. This survey documents rapid increase in glyphosate-resistant kochia over a five-year time frame, and also confirms the first cases of kochia in Manitoba with dicamba resistance alone and in combination with glyphosate resistance.

**Key words:** gene flow, herbicide resistance, surveillance, survey, synthetic auxin.

**Résumé :** La culture de variétés cumulant la résistance au glyphosate et au dicamba ainsi que la confirmation récente de biotypes de kochie [*Bassia scoparia* (L.) A.J. Scott] résistants à ces herbicides, en Alberta et en Saskatchewan, justifient qu'on vérifie s'il n'y a pas de kochie résistante à ces herbicides au Manitoba. À l'automne 2018, les auteurs ont effectué une étude stratifiée aléatoire à 315 endroits de la province. Globalement, 58 % des peuplements de kochie testés résistaient au glyphosate, contre 1 % au dicamba. Cette enquête montre que la kochie résistante au glyphosate a vu sa population augmenter rapidement en cinq ans. Elle confirme de surcroît l'existence des premiers spécimens de kochie résistants au dicamba et aux deux herbicides, au Manitoba. [Traduit par la Rédaction]

**Mots-clés :** flux génétique, résistance aux herbicides, surveillance, enquête, auxine synthétique.

### Introduction

Kochia [*Bassia scoparia* (L.) A.J. Scott] is a summer-annual tumbleweed that is present in many cropped and non-cropped areas in the Great Plains of North America. In Canada, it is most abundant in southern Manitoba, Saskatchewan, and Alberta (Friesen et al. 2009; Leeson et al. 2017). In the most recent survey

conducted in Manitoba in 2016, kochia was the 30th most abundant weed among 127 species surveyed following post-emergence herbicide application in annual field crops, and was most abundant in the Aspen Parkland, followed by the Southwest Manitoba Uplands, and the Lake Manitoba Plain ecoregions (Leeson et al. 2017; ecoregions depicted in Fig. 1). Kochia infestation can

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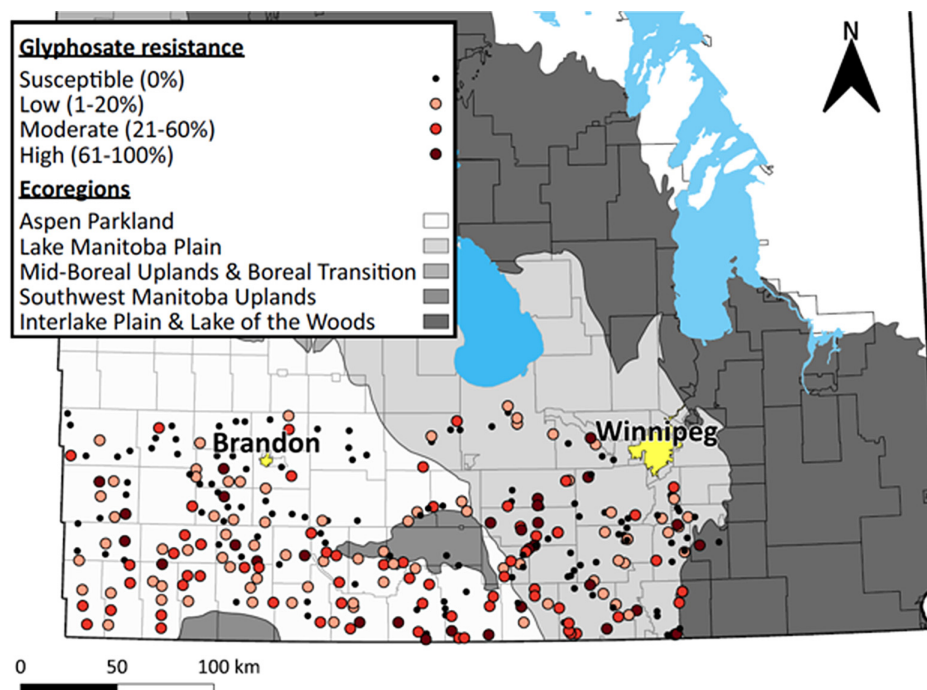
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**Fig. 1.** Glyphosate-resistant kochia in Manitoba in 2018. Low (1%–20%), moderate (21%–60%), and high (61%–100%) resistance indicate the percentage of resistant individuals within each population. Base layers: Ecoregions (Agriculture and Agri-Food Canada, A National Ecological Framework for Canada: GIS data, [https://sis.agr.gc.ca/cansis/nsdb/ecostrat/gis\\_data.html](https://sis.agr.gc.ca/cansis/nsdb/ecostrat/gis_data.html)); Manitoba Topographic Base Map, Scale 1 to 1 000 000 (Government of Manitoba, Lands and Geomatics Branch, <https://mli2.gov.mb.ca/adminbnd/index.html>). Map projection: NAD83/UTM Zone 14N. [Colour online.]



impede crop harvest and cause significant yield losses, in addition to adverse impacts on pastures, rangelands, roadside ditches, railway rights-of-way, oil well sites, and other ruderal areas (Friesen et al. 2009).

Several unique biological characteristics of kochia allow this  $C_4$  weed to evade herbicide control and thrive under conditions of abiotic stress like heat, drought, and salinity (Friesen et al. 2009). Kochia is among the first weed species to emerge in the spring, and emergence continues into late summer (Schwinghamer and Van Acker 2008). While the majority of the kochia population may be targeted for herbicidal control before planting, emergence often continues up to post-emergence herbicide application or even later in the season. Kochia plants emerging prior to the fourth week of August ( $\sim 2140$  growing degree-days,  $T_{base} 0^\circ C$ ) can produce viable seed before the end of the growing season and contribute to seedbank replenishment (Geddes and Davis 2021). Short (1–2 yr) kochia seed longevity in the soil seedbank causes rapid population turnover (Beckie et al. 2018), and prolific seed production (up to 120 000 seeds  $plant^{-1}$ ) results in ample seedbank inputs from plants that evade management efforts (Friesen et al. 2009; Beckie et al. 2016). Furthermore, tumbleweed seed dispersal enables long-distance transportation of kochia within and between fields, while protogynous flowering aids transfer of herbicide resistance alleles through an initial period of obligate cross-pollination followed by

self-pollination which provides reproductive assurance (Beckie et al. 2016).

Resistance to multiple herbicide modes-of-action in kochia is a growing problem resulting in lack of control in many field crops in the southern Canadian Prairies and western United States. In Canada, acetolactate synthase (ALS) inhibitor-resistant kochia was first discovered in Manitoba and Saskatchewan in 1988, and subsequently in Alberta in 1989 (Heap 2021). After about two-decades, 85% of kochia populations sampled across western Canada were ALS inhibitor-resistant, while this biotype has been present in all populations tested in recent years (Beckie et al. 2015, 2019). Glyphosate-resistant kochia was initially reported in Canada in 2011, where it was discovered in chemical fallow fields in southern Alberta (Hall et al. 2014). A subsequent 2012 survey of Alberta identified that 4% of the 309 sites sampled contained glyphosate-resistant kochia (Hall et al. 2014). Five years later, this frequency of resistance had increased to 50% of sampled sites in Alberta (Beckie et al. 2019). The 2017 survey of Alberta also reported that 18% of the kochia populations were dicamba-resistant (Beckie et al. 2019), while 13% were fluroxypyr-resistant (Geddes et al. 2021). Surveys of Saskatchewan and Manitoba in 2013 confirmed glyphosate resistance in 5% and 1% of the kochia populations, respectively; while no populations exhibited dicamba resistance (Beckie et al. 2015). Unlike the initial confirmations of glyphosate-

resistant kochia in chemical fallow in Alberta, the first reports of this biotype in Manitoba were in glyphosate-resistant cultivars of soybean [*Glycine max* (L.) Merr.] and corn (*Zea mays* L.), suggesting selection for glyphosate resistance in contrasting cropping systems.

Due to the speed with which glyphosate resistance evolved or spread in Alberta, initial reports of dicamba-resistant kochia in Alberta and Saskatchewan (Beckie et al. 2019; Heap 2021), and increasing adoption of soybean with stacked trait resistance to both of these herbicides in Manitoba, there is a need to understand the status of herbicide-resistant kochia within the province. This study was conducted to determine whether the frequency of glyphosate resistance had increased among kochia populations 5 yr after the initial confirmation in Manitoba, and also to determine whether dicamba-resistant kochia was present in the province.

## Materials and Methods

A randomized-stratified survey of 315 sites in Manitoba was conducted in the fall of 2018, five years after the preceding survey (Beckie et al. 2015). The sites sampled were stratified based on arable land area within each ecodistrict (area of similar soil, vegetation, landform, and land use within an ecoregion), and the majority of sample locations were within the ecoregions where kochia was most abundant (Leeson et al. 2017). Sites were randomly predetermined and visited post-harvest during a 3 wk period in early October. The surveyors collected reproductive biomass from 10–20 kochia plants at each site, and the biomass was composited to represent the kochia population present. Surveyed sites included cropped fields and also non-cropped areas, including roadside ditches, railway rights-of-way, and oil well sites. The location and characteristics (e.g., habitat and crop stubble type) of each site were documented.

The kochia populations were threshed under isolation to prevent cross-contamination at the University of Manitoba and Agriculture and Agri-Food Canada (AAFC) Saskatoon Research and Development Centre (RDC), then screened for resistance to glyphosate and dicamba in the greenhouse at the AAFC Lethbridge RDC following the procedures outlined by Beckie et al. (2015). Homogenized subsamples of seeds from each population were planted in 52 × 26 × 5 cm flats filled with modified Cornell soilless potting mixture with 756 mg N, 958 mg P, and 505 mg K·L<sup>-1</sup> mixture. The kochia seedlings within each flat were counted prior to herbicide treatment. A minimum of 72 individuals from each population were treated in separate experiments with either glyphosate (Roundup WeatherMax®, Bayer CropScience, Calgary, AB) or dicamba (XtendiMax™ with VaporGrip™ Technology, Bayer CropScience, Calgary, AB) at 900 or 280 g a.e.·ha<sup>-1</sup>, respectively. The herbicides were applied using a moving-nozzle cabinet sprayer when the seedlings were 3–5 cm tall. The sprayer was equipped with a flat-fan TeeJet® 8002VS nozzle (Spraying Systems Co.,

Wheaton, IL) which applied 200 L·ha<sup>-1</sup> spray solution at 275 kPa and a speed of 2.4 km·hr<sup>-1</sup>. The flats were placed in the greenhouse in a completely randomized design where they were watered daily under a 20/18 °C day/night temperature regime with a 16-h photoperiod and supplemented with 230 µmol·m<sup>-2</sup>·s<sup>-1</sup> light.

Plant responses within each kochia population were evaluated 3 or 4 wk after treatment with glyphosate or dicamba, respectively. The plants were scored individually as resistant (dead, or nearly dead) or susceptible (some injury but new growth, or no injury) relative to herbicide-treated and -untreated resistant and susceptible control populations (Beckie et al. 2015). The incidence of resistant individuals within each population was determined as a percentage of the number of individuals exposed to the herbicide. The populations were categorized as susceptible, or low, moderate, or high resistance based on a resistance incidence of 0%, 1%–20%, 21%–60%, or 61%–100%, respectively (Geddes et al. 2021). The frequency of resistant populations was determined within each rural municipality as a percentage of populations tested within the municipality. The incidence and frequency of resistance were mapped using QGIS 3.12 (QGIS Geographic Information System, Open Source Geospatial Foundation).

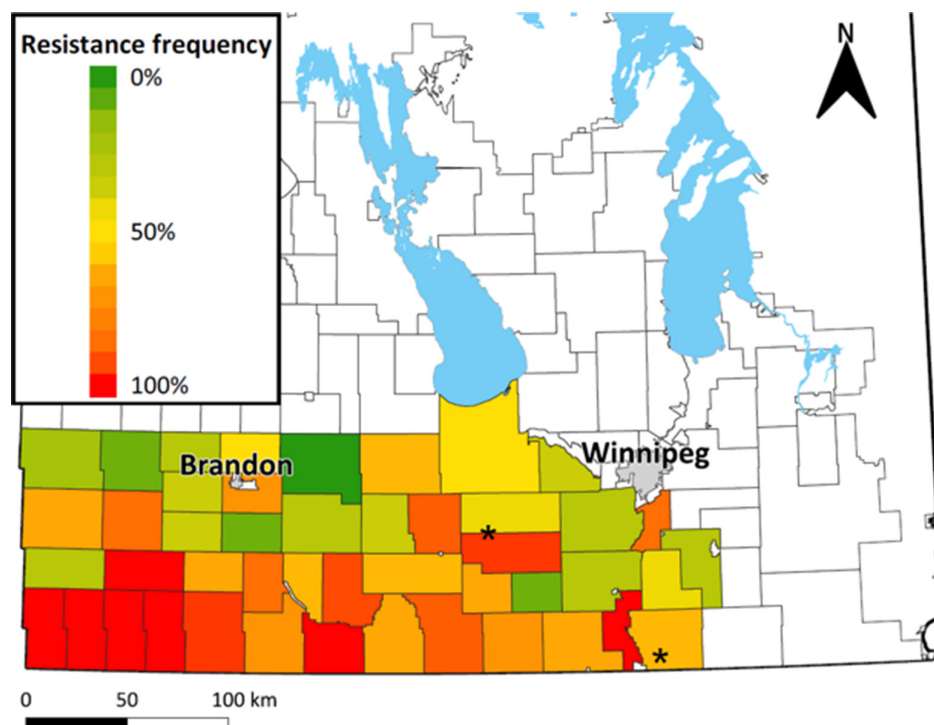
## Results and Discussion

Kochia populations from 300 of the 315 sites contained enough viable seed for resistance testing. The 2018 survey documented rapid increase of glyphosate resistance in Manitoba, with 58% of the kochia populations (175/300) containing a glyphosate-resistant biotype (Fig. 1). This number increased from 1% of the kochia populations (2/283) sampled in Manitoba just five years previous (Beckie et al. 2015). Of the 175 glyphosate-resistant populations in 2018, 44% (77/175) exhibited low resistance, while 38% (66/175) exhibited moderate resistance, and 18% (32/175) displayed high resistance (Fig. 1). The mean incidence of glyphosate resistance among these populations was 32%, while the median was 25% (SD = 27%) (data not shown). Low resistance (1%–20%) often goes undetected by farmers or agronomists as the threshold of herbicidal control by pesticide regulators is 80%. These low resistance populations are being selected for resistance and this indicates future problems with control under continued selection pressure (Geddes et al. 2021). However, the majority of resistant populations (98/175 or 56%) exhibited moderate or high resistance (Fig. 1) and therefore are not likely to be controlled when treated with glyphosate alone.

The greatest frequencies of glyphosate-resistant kochia populations were found in the rural municipalities located in the south and western areas of Manitoba where kochia is most abundant (Fig. 2). These municipalities were also those located in the southern extent of the Aspen Parkland ecoregion of Manitoba (Fig. 1). Glyphosate-resistant kochia was found in all rural



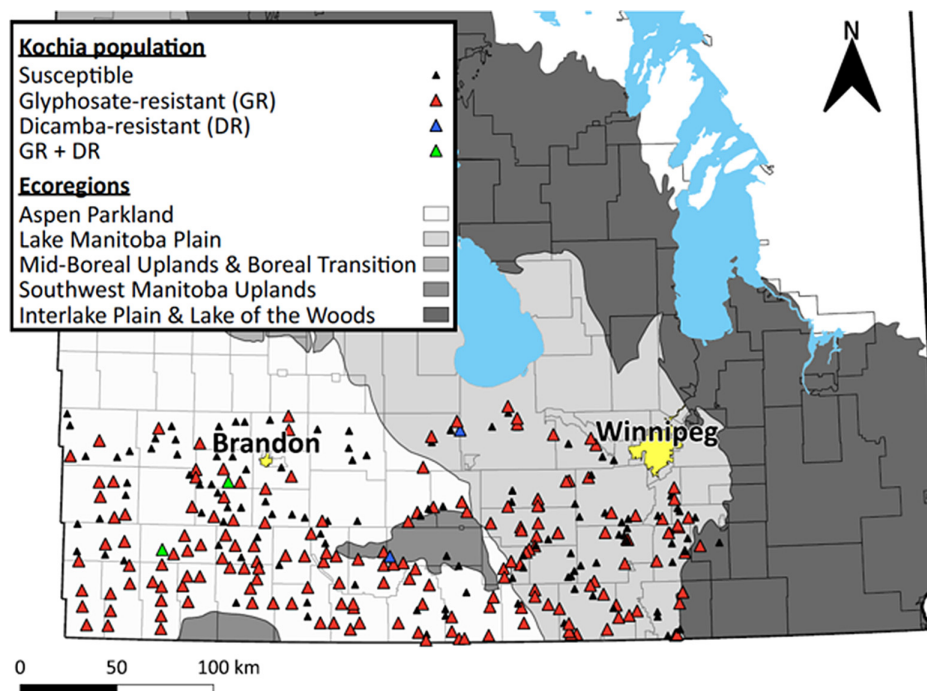
**Fig. 2.** Frequency of glyphosate-resistant kochia populations confirmed within each Rural Municipality sampled during a 2018 survey of Manitoba. Asterisks indicate the locations of the two glyphosate-resistant kochia populations confirmed in 2013 [Adapted from Beckie et al. (2015)]. Base layer: Manitoba Topographic Base Map, Scale 1 to 1 000 000 (Government of Manitoba, Lands and Geomatics Branch, <https://mli2.gov.mb.ca/adminbnd/index.html>). Map projection: NAD83/UTM Zone 14N. [Colour online.]



municipalities sampled, with the exception of North Cypress (i.e., present in 45/46 municipalities; Fig. 2). The two initially-confirmed populations in 2013 were located in two separate municipalities south or southwest of Winnipeg (Beckie et al. 2015; see asterisks marked in Fig. 2). Among sample sites in the current survey, the greatest frequency of glyphosate-resistant kochia was found in the glyphosate-resistant crops soybean (78% of kochia populations were glyphosate-resistant) and corn (70%), followed by other oilseeds [67%; including flax (*Linum usitatissimum* L.) and sunflower (*Helianthus annuus* L.)], canola (*Brassica napus* L.) (52%), cereals [49%; including wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.)], ruderal areas (29%), pulse crops [20%; including dry bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.)], and alfalfa (*Medicago sativa* L.) (0%). These results indicate that glyphosate-resistant soybean and corn contribute the greatest selection pressure for glyphosate-resistant kochia. In conjunction with *in situ* selection and evolution in any habitat under glyphosate selection pressure (glyphosate-resistant and -susceptible crops, and ruderal areas), resistance alleles from such populations can subsequently be dispersed across the landscape via seed or pollen (Martin et al. 2020). The rapid increase in frequency of glyphosate resistance in kochia between 2013 and 2018 strongly suggests that dispersal of herbicide-resistant biotypes was a dominant factor in addition to *in situ* selection.

This survey confirmed the first cases of dicamba-resistant kochia in Manitoba, and also the first cases of kochia with multiple herbicide resistance to glyphosate and dicamba. Four (about 1%) of the 300 populations tested exhibited dicamba resistance (Fig. 3). Two of these dicamba-resistant kochia populations were also glyphosate-resistant. Even though ALS inhibitor resistance was not tested in the current survey, past surveys in western Canada – including Manitoba – showed that all kochia populations tested in this region contain ALS inhibitor-resistant biotypes (Beckie et al. 2015, 2019). Together, these results suggest that triple herbicide-resistant kochia has been present in Manitoba since 2018, only one year following the first confirmation of this biotype in Alberta (Beckie et al. 2019). The two glyphosate- and dicamba-resistant kochia populations were found in soybean and canola in the municipalities of Whitehead and Cameron, respectively (Fig. 3); while the two dicamba-resistant but glyphosate-susceptible populations were found in canola and sunflower in the municipalities of Portage la Prairie and Lorne, respectively (Fig. 3). These respective paired municipalities are not adjacent to one another, suggesting independent selection and evolution of resistance. All dicamba-resistant populations exhibited low resistance (3%–11% incidence), and thus were being selected for or developing resistance, but likely not manifesting noticeable loss of herbicidal control at the field scale.

**Fig. 3.** Glyphosate-resistant (GR), dicamba-resistant (DR), and multiple herbicide-resistant (GR + DR) kochia in Manitoba in 2018. Base layers: Ecoregions (Agriculture and Agri-Food Canada, A National Ecological Framework for Canada: GIS data, [https://sis.agr.gc.ca/cansis/nsdb/ecostrat/gis\\_data.html](https://sis.agr.gc.ca/cansis/nsdb/ecostrat/gis_data.html)); Manitoba Topographic Base Map, Scale 1 to 1 000 000 (Government of Manitoba, Lands and Geomatics Branch, <https://mli2.gov.mb.ca/adminbnd/index.html>). Map projection: NAD83/UTM Zone 14N. [Colour online.]



Documentation of glyphosate- and dicamba-resistant kochia in Manitoba corresponds with previous reports in the Canadian Prairie Provinces and western United States (Beckie et al. 2019; Westra et al. 2019; Heap 2021). For example, a 2012–2014 survey of herbicide-resistant kochia in Colorado found resistance to glyphosate and dicamba in 49% and 33% of the 171 populations tested, respectively (Westra et al. 2019), while a 2017 survey of Alberta identified resistance to glyphosate and dicamba in 50% and 18% of the populations, respectively (Beckie et al. 2019). While synthetic auxin resistance in kochia is a relatively recent phenomenon in Canada (first confirmed in a wheat field in Saskatchewan in 2015), this type of resistance has been present in the United States since 1993/1994 (Heap 2021).

With increasing adoption of stacked trait glyphosate- and dicamba-resistant soybean in Manitoba, and the dominant use of synthetic auxin herbicides for kochia control in cereal crops (Torbiak et al. 2021), farmers should be cognizant of the potential risk for selection of glyphosate- and dicamba-resistant kochia biotypes in these crop production systems. A key strategy to reducing selection for multiple herbicide-resistant kochia in these production systems will be greater integration of non-chemical weed control tools for proactive mitigation of these biotypes (Geddes and Gulden 2018). Such strategies should focus on mitigating kochia seed production and return to the soil seedbank (Geddes and Davis 2021), therefore exploiting the short-lived

seedbank persistence of this species (Beckie et al. 2018). While these strategies may be effective at reducing or mitigating localized kochia populations, a community-based strategy will be required to mitigate reinfestation and transfer of herbicide-resistant biotypes of this tumbleweed among farms and fields.

### Competing Interests

The authors declare there are no competing interests.

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### Contributors' Statement

CMG: methodology, investigation, supervision, validation, formal analyses, visualization, writing – original draft, reviewing and editing. JYL, MMP, SMS, SWS, TJ, and RHG: investigation, writing – reviewing and editing. HJB: conceptualization, methodology, funding acquisition, investigation, writing – reviewing and editing.

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