Control of Glyphosate-Resistant Canada Fleabane (*Conyza canadensis*) and Waterhemp (*Amaranthus tuberculatus* var. *Rudis*) in Glyphosate/Dicamba-Resistant Soybean (*Glycine max*)

By

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A Thesis

presented to

The University of Guelph

In partial fulfillment of requirements

For the degree of

Master of Science

In

Plant Agriculture

Ridgetown, Ontario, Canada

ABSTRACT

CONTROL OF GLYPHOSATE-RESISTANT CANADA FLEABANE (CONYZA CANADENSIS) AND WATERHEMP (AMARANTHUS TUBERCULATUS VAR. RUDIS) IN GLYPHOSATE/DICAMBA-RESISTANT SOYBEAN (GLYCINE MAX)

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This thesis is an investigation of the control of glyphosate-resistant (GR) Canada fleabane and waterhemp. Glyphosate/dicamba was applied to Canada fleabane at three postemergent (POST) application timings (5, 15 and 25 cm) and 4 herbicide rates (0, 900, 1350 and 1800 g ae ha^{-1}). An increase in plant height at the application timing, led to a decrease in control of Canada fleabane; an increase in herbicide rate led to an increase in efficacy. There was an interaction between application timing and rate for density and biomass only. This same study was conducted with GR waterhemp, the same trend followed except there was no interaction between application timing and herbicide rate. Control of GR Canada fleabane with several preplant herbicide tankmixes was evaluated; there was no increase in control with the addition of a second-effective site-of-action. Similarly, GR waterhemp control did not increase with the addition of a second-effective mode-of-action. Acceptable control was not obtained with glyphosate/dicamba applied preemergent (PRE), therefore, another mode-of-action is needed for effective control of GR waterhemp. A two-pass program of an effective PRE herbicide followed

by glyphosate/dicamba provided season-long control of GR waterhemp and was more efficacious than a PRE or POST program alone.

ACKNOWLEDGEMENTS

As one chapter ends, and another begins, I would like to acknowledge several people who supported me throughout my MSc. degree. This journey began with Dr. Peter Sikkema and Dr. Darren Robinson taking me on as a graduate student. From the beginning, they challenged my thinking, helped improve my writing, knowledge of herbicides, weed species, and even statistics. They both run outstanding research programs and take time out of their busy schedule to answer questions related to research or agriculture in general. Words cannot express how grateful I am for all the help, and kind words throughout the past 2 years. Now enough of the mushy stuff. I would also like to thank my committee member Dr. David Hooker for all his help and suggestions. His insight was invaluable throughout the process, challenging my thoughts and reasoning. Thank you to Dr. Nader Soltani for all the assistance in publishing manuscripts and support when presenting at conferences.

There are many different people who assisted throughout the research process. I would like to start by thanking my technician Chris Kramer for all his help in establishing trials, harvesting trials and everything in between. Your knowledge, cool headedness and comradery was very appreciated as I'm sure you appreciated my calendars. Thank you to all my fellow graduate students, Mike Schryver, Taiga Cholette, Andrea Smith, Brendan Metzger, Lauren Benoit, Elizabeth Buck, Tessa De Boer, Sydney Wardell and Meghan Grguric. The office was a better place with all of you in it, even when we did get off track talking about bridge safety. I really appreciate the help you gave during the field season. I would like to thank the summer students who tirelessly helped with the field work, especially weed harvests, Meghan Dilliot, Shelby Johnston and Nicole Mellford. A huge thank you to Michelle Edwards for all her help with statistics. The meetings, workshops and prompt replies to e-mails were a huge help.

Thank you to the funding partners Monsanto, Growing Forward 2 and Grain Farmers of Ontario.

Last but not least, I would like to thank my friends and family for all the support and kind words, not just in the last two years but throughout my life.

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Chapter 1: Literature Review: Control of Glyphosate-Resistant Canada Fleabane (*Conyza canadensis*) and Waterhemp (*Amaranthus tuberculatus var. Rudis*) in Glyphosate/Dicamba-Resistant Soybean (*Glycine max*)

1.1 Biology of *Conyza canadensis*

1.1.1. Introduction

Conyza canadensis (L.) *Cronq.*, commonly known as Canada fleabane, horseweed, or marestail, is a member of the Asteraceae (Compositae) family, and native to North America (Loux et al. 2006). Canada fleabane was reclassified from *Erigeron canadensis* to *Conyza canadensis* because it has less hermaphroditic flowers, relatively more pistillate flowers compared to other *Erigeron* species and due to the absence of ligules (Cronquist 1943). Canada fleabane is an annual weed that has a winter or summer annual life cycle (Loux et al. 2006; Weaver 2001). It is considered a ruderal species found south of N55 in Canada (excluding Newfoundland), although there is limited invasiveness and seed production observed at N52 and above (Archibold 1981). Because of its ruderal nature, Canada fleabane is found growing in orchards, recently abandoned fields, pastures, vineyards, roadsides, and in fields with reduced or no tillage (Weaver 2001). Canada fleabane is a native weed found throughout ruderal habits in North America.

Canada fleabane is considered a troublesome weed globally due to high seed production and widespread dispersal (Loux et al. 2006). The main explanations for increased Canada fleabane prevalence are "lack of crop rotation, reduced tillage and herbicide resistance" (Loux et al. 2006). Canada fleabane's physiology and the lack of diversity in crop production systems, and in weed management programs has contributed to its rapid increase in agricultural fields.

1.1.2. Identification and Characteristics

Canada fleabane has its own defining characteristics to distinguish it from similar species. Cotyledons of Canada fleabane are smooth, ovate, hairless, lack noticeable veins, and are 2.0 to 3.5 mm long and 1.0 to 2.0 mm wide (Royer and Dickinson 1999; Bryson and DeFelice 2009). The first true leaves are hairy on the upper surface and spatula-shaped (Royer and Dickinson 1999). The leaves are alternate and narrow with entirely or slightly toothed margins (Loux et al.

2006). Stems of Canada fleabane are erect, hairy, can grow up to 180 cm tall and tend to be unbranched at the base of the plant with flowering branches near the top (Weaver 2001; Loux et al. 2006). Mature plants have no petioles, and smaller leaves at the top of the plant (Loux et al. 2006). Flowers are white ray and yellow disk florets that range from 1.6 to 3.2 mm in length and 3.0 to 5.0 mm in diameter and are arranged in a loose cluster near the top of the plant (Weaver 2001; Loux et al. 2006). The seeds of Canada fleabane are small achenes (fruit), with a pappus of bristles that are twice as long as the achenes, allowing for efficient dispersal by the wind (Weaver 2001). Canada fleabane has a short taproot with secondary fibrous laterals (Weaver 2001). The distinct characteristics of Canada fleabane aid in its identification.

1.1.3. Germination and Emergence

Canada fleabane is an annual weed that can germinate as soon as it is released from the mother plant. Due to the absence of seed dormancy, germination can occur throughout the year, but the majority of germination occurring during two periods; September to October and April to June (Buhler and Owen 1997; Loux et al. 2006; Nandula et al. 2006; Main et al. 2006). In southern Ontario, Tozzi and Van Acker (2014) found that most plants emerged between August 27 and September 9 in the fall and from May 14 to May 27 in the spring. Previous research observed 5 to 32% of Canada fleabane emerged in the spring (Tozzi and Van Acker 2014; Buhler and Owen 1997). Variable emergence can occur and is partially dependent on tillage practices (Bhowmik and Bekech 1993). Weaver (2001) reported that only a small percentage of Canada fleabane seedlings emerged from March to May while Davis et al. (2008) found 90% of seedlings emerged in the spring in Indiana. Tozzi and Van Acker (2014) found there was a difference in Canada fleabane height and fecundity when comparing early-emerging fall and spring plants to late-emerging fall and spring plants. Nevertheless, when combining the early and late emerging cohorts and comparing spring to fall emerging plants, there was no difference between the two (Tozzi and Van Acker 2014). Emergence of Canada fleabane occurs after seed shed predominantly from September to October and March to May.

Seed bank recruitment of Canada fleabane is low. Annual recruitment from the seed bank of Canada fleabane is estimated at 6.5% (Regehr and Bazzaz 1979). This is attributed, in part, to no primary dormancy required for germination, the small seed size of Canada fleabane and corresponding limited energy reserves (Tozzi et al. 2014). Germination of 84 to 93% has been

documented in the first year after seed shed (Tozzi et al. 2014). The seeds of Canada fleabane are estimated to remain viable for 2-3 years under laboratory conditions and 3 years under field conditions with only 1% of seed remaining viable afterwards (Hayashi 1979). Viable Canada fleabane seed was found in the seed bank 20 years after a pasture was abandoned, and in a 10-year-old abandoned agricultural field (Tsuyuzaki and Kanda 1996; Leck and Leck 1998). In general, Canada fleabane seed viability is 2 to 3 years.

Microsite conditions such as temperature, light, soil texture and moisture play a major role in Canada fleabane germination (Nandula et al. 2006). Buhler and Owen (1997) and Buhler and Hoffman (1999) found optimal germination was at a day/night temperature of 22°C/16°C. This is consistent with Nandula et al. (2006), who observed maximum germination at 24°C/20°C, and Yamashita and Guimaraces (2011), who reported 97% germination at 25°C. In contrast, only 2% germination was observed at 15°C and no germination was observed at day/nighttime temperature of 12°C/6°C (Yamashita and Guimaraces 2011; Nandula et al. 2006). Tozzi et al. (2014) observed differential emergence among biotypes from Iran, Canada, Spain, and the United Kingdom. Ontario biotypes germinated at 8-9.5°C, which was the lowest germination temperature range across the four biotypes. Temperature requirements for Canada fleabane germination are biotype dependent.

Canada fleabane germination is affected by soil moisture, burial depth, soil pH and soil texture. Nandula et al. (2006) observed a drastic decrease in emergence with an increase in seed depth. Seeds at the soil surface germinated more readily while no seedlings emerged at a depth of 0.5 cm or greater. Other studies reported that Canada fleabane germination was reduced by 60 to 90% when buried at 1 cm compared to surface seeds (Bhowmik and Bekech 1993). No germination was found when Canada fleabane seed was buried at a depth of 6 cm (Bhowmik and Bekech 1993). Greater emergence has been observed on coarse soils compared to fine-textured soils (Nandula et al. 2006). Canada fleabane has greater emergence at a depth of 1 cm in coarse sandy soils than fine clay soils (Bhowmik and Bekech 1993). Canada fleabane emergence was similar among soil types at seed burial depths greater than 1 cm and less than 0.5 cm. This is further substantiated since Canada fleabane has been shown to grow optimally in well-drained coarse, stony, sandy, or fertile loam soils (Weaver 2001). Soil moisture is an important factor for germination; although, Canada fleabane is able to germinate under moderate water-stress conditions, giving this species an advantage against plants that do not germinate readily under

water-stress conditions (Shrestha et al. 2008; Nandula et al. 2006). Soil pH did not impact Canada fleabane seed germination when soil pH ranged from 4 to 10; however, an increase in germination occurred in neutral to alkaline soil conditions compared to acidic soils (Nandula et al. 2006). Soil microsite conditions affect the ability of Canada fleabane in the seed bank to germinate and emerge.

Crop residue and light affect Canada fleabane germination and emergence. Canada fleabane has shown variable responses to light for germination. Nandula et al. (2006) reported 14 and 60% germination under dark and light conditions, respectively. This is in contrast to Riemens (2003) who observed 18.6 and 0.8% germination in the light and dark conditions, respectively. Crop residue can influence Canada fleabane emergence. Bowmik and Bekech (1993) found that crop residue on the soil surface delayed emergence by four weeks and reduced emergence by 80%. Canada fleabane can germinate with a single flash of light, therefore crop residue movement and deterioration may allow for light to activate germination later in the growing season (Riemens 2003). Crop residue can reduce Canada fleabane germination and emergence due to light requirements for germination.

When germination has occurred in the fall, Canada fleabane will form a basal rosette for winter survival; spring-emerged seedlings do not form a rosette (Loux et al. 2006). "The rosette deteriorates when the stem begins to elongate in May and bloom around the middle of July" (Weaver 2001). Weaver (2001) found that Canada fleabane seedlings emerge in the fall from late August through October, depending on weather conditions. The larger the rosette is prior to the onset of winter, the greater the chance of survival due to a more expansive root system to tolerate frost heaving (Buhler and Owen 1997). Rosettes that overwinter are able to fix carbon at a significant rate even at low temperatures (Regehr and Bazzaz 1976). Up to 91% of fall-emerging Canada fleabane rosettes survive until spring in Tennessee, allowing for a competitive advantage for space, water, light, and nutrients compared to spring emerging plants (Main et al. 2006). Canada fleabane plants that emerge in the fall have a competitive advantage against spring seeded annual crops but mortality is increased.

1.1.4. Reproduction and Dispersal

Pollen dispersal of Canada fleabane is variable between fall and spring emerging plants, with production peaking at different times within the day and growing season. There is a

difference in flowering timing between spring and fall-emerging plants with spring-emerging plants flowering significantly earlier than fall-emerging plants (Tozzi and Acker 2014). Previous research observed 79% of Canada fleabane pollination occurs between 9:00 am and 7:00 pm, with a peak in pollination occurring at 1:30 pm (Ye et al. 2016). They also observed an average daily release of Canada fleabane pollen was 95,000 grains per plant per day, this is higher than ragweed which releases 60,000 grains per day. Pollen release has been shown to last approximately two months peaking in the first week of September (Ye et al. 2016). Short dispersal studies show that Canada fleabane pollen concentration decreases significantly between 8 and 10 m from the plant source (Ye et al. 2016). Overall, there is a 50% reduction in pollen concentration at 22 m from the pollen source. Canada fleabane releases pollen before the captula are fully opened, supporting studies that have shown Canada fleabane mainly self-pollinate (Weaver 2001; Loux et al. 2006). Outcrossing within a population in Ontario ranged from 1.2 to 14.5%, with an estimated outcrossing average of approximately 4% (Weaver 2001; Smisek 1995). Canada fleabane is a primarily self-pollinating plant with a short pollen dispersal distance.

Seeds of Canada fleabane mature about three weeks after fertilization (Weaver 2001). A single Canada fleabane plant has been reported to produce approximately 200,000 seeds, with 60 to 70 seeds per flower and an average seed weight of 0.072 mg (Fenner 1983; Smisek 1995). Seed production has been suggested to remain relatively constant per unit area, changing per plant instead of through density dependent mortality (Weaver 2001). Canada fleabane produces a high amount of small seeds.

Canada fleabane seeds have unique characteristics that aid in seed dispersal. Seed dispersal is facilitated by a small 2-3 mm pappus, resulting in a slow settlement velocity of 0.278 m sec^{-1} (Anderson 1993). Height of seed release and settling velocity play an important role in seed dispersal of Canada fleabane (Dauer et al. 2006; Regehr and Bazzaz 1979). Regehr and Bazzaz (1979) found seed more than 122 m from the seed source in a corn field. Dauer et al. (2006) suggested that seed may move hundreds of metres from the source and Shield et al. (2006) found seed 500 km from the source. Canada fleabane seed is also dispersed by water (Weaver 2001). Widespread seed dispersal of Canada fleabane is facilitated by a pappus.

1.1.5. Herbicide-Resistance

Canada fleabane has evolved resistance to multiple classes of herbicides. Currently,

Canada fleabane is resistant to five different modes of action, with resistance to at least one mode of action in 16 countries around the world (Heap 2016). Herbicide resistant Canada fleabane was first reported to paraquat in 1980 in Japan and was found in Essex County, Canada in 1998 (Loux et al. 2006; Smisek et al. 1998). Resistance to atrazine, glyphosate, cloransulam-methyl, linuron, chlorsulfuron, imazapyr, metribuzin, pyrithiobac-sodium, sulfometuron-methyl, simazine, chlorimuron-ethyl, diuron, iodosulfuron-methyl-sodium, metsulfuron-methyl, rimsulfuron, thiencarbazone-methyl, thifensulfuron-methyl, and tribenuron-methyl have been found around the world (Heap 2016). In Michigan, resistance to more than one herbicide has been reported to atrazine, simazine and diuron (Loux et al. 2006). Biotypes in Ohio, Indiana and Ontario have resistance to glyphosate and acetolactate synthase (ALS) inhibiting herbicides (Trainer et al. 2005; Kruger et al. 2009; Byker et al. 2013a). Canada fleabane has developed resistance to many different herbicide modes-of-action.

Glyphosate previously provided excellent control of Canada fleabane. The extensive use of glyphosate and rapid seed dispersal has increased the number of glyphosate-resistant (GR) populations around the world (Bruce and Kells 1990; VanGessel 2001; Shrestha et al. 2008). Glyphosate-resistant biotypes were first reported in Delaware in 2000 (VanGessel 2001). Glyphosate-resistant Canada fleabane was selected in a field that had 14 consecutive years of soybean with only glyphosate used for weed management and in as few as 3 years of consecutive soybean and intensive glyphosate use (VanGessel 2001; Loux et al. 2006). The rate of glyphosate required for 90% control of glyphosate-susceptible and resistant biotypes was 0.28 to 0.84 kg/ha, and 4.4 to 8.8 kg/ha, respectively (VanGessel 2001). Different resistance levels to glyphosate have been reported among different biotypes of Canada fleabane. VanGessel (2001) reported 8- to 13-fold resistance in Delaware, Main et al. (2004) reported 4-fold resistance, and Dinelli et al. (2006) a 4- to 5-fold resistance to glyphosate. No differences in growth and seed production have been observed between GR and susceptible biotypes (Davis et al. 2009a). There is widespread GR Canada fleabane with varying levels of resistance but no known fitness penalty.

Canada fleabane has evolved resistance to glyphosate through a number of mechanisms of resistance. Target-site resistance to glyphosate is due to an incompletely dominant single locus gene that has been suggested to be dependent on the growth stage and plant biotype (Dinelli et al. 2006). The non-target mechanisms of GR in Canada fleabane are reduced translocation to

meristematic tissues, over-expression of 5-enolpyruvylshikimate-3-phosphate synthase in plant cells, target-site mutation, vacuole sequestration of glyphosate, metabolic deactivation, and emergence of new branches after herbicide application (Flessner 2015; Dinelli et al. 2006; Ge et al. 2010; Gonzalez-Torralva et al. 2012). Studies have concluded that resistance is not due to reduced absorption of glyphosate (Feng et al. 2004). Different mechanisms confer resistance to glyphosate in Canada fleabane populations.

1.1.6. Impact

Canada fleabane interference can result in substantial crop yield losses. Bruce and Kells (1990) reported that Canada fleabane competition can result in soybean yield losses of up to 83%. When soybean was grown in competition with Canada fleabane, escapes of Canada fleabane protruding through the canopy produced 31 000 to 72 000 seeds per plant (Davis and Johnson 2008). Davis and Johnson (2008) observed an 83 to 93% decrease in soybean yield due to Canada fleabane interference. Poor control of GR Canada fleabane with glyphosate 900 g ha^{-1} resulted in a 35 to 40% soybean yield loss (Byker et al. 2013b). Canada fleabane escapes can have a huge negative impact on soybean yield.

1.1.7. Control

Crop rotation, cover crops, and tillage are integral components of a diversified Canada fleabane control program. Davis et al. (2009a) found no reduction in Canada fleabane densities during the first 2 years of a study comparing continuous soybean to a rotation of corn and soybean when an effective herbicide program was used; but after 4 years there was greater Canada fleabane density in the continuous soybean field (Davis et al. 2009b). Main et al. (2006) observed significant differences between Canada fleabane emergence when comparing crop residues. Corn residue reduced Canada fleabane density by 75%, while soybean residue did not reduce Canada fleabane density to the same degree. Soybean fields provided significantly more residue cover and reducing Canada fleabane emergence compared to land left fallow (Main et al. 2006). After harvest, crop residue cover may reduce winter survival by delaying fall emergence (Buhler and Owen 1997). Cover crops containing rye (*Secale cereale L.*), crimson clover (*Trifolium incarnatum* L.), or hairy vetch (*Vicia villosa* Roth.) have been shown to decrease emergence of Canada fleabane (Przepiorkowski and Gorski 1994; Brown and Whitwell 1988).

Spring tillage can effectively control fall-emerged Canada fleabane (Brown and Whitwell 1988). Johnson et al. (2004) reported that 92, 79 and 39% of conventional-till, reduced-till and no-till fields were absent of Canada fleabane, respectively. Fall tillage followed by cover crop seeding eliminated Canada fleabane establishment in research by Brown and Whitwell (1988). Non-chemical weed management tactics are an integral part of an integrated weed management program for the control of GR Canada fleabane.

Herbicides can be used in an integrated management program to control Canada fleabane. In no-till soybean, emerged Canada fleabane should be controlled prior to planting with tank-mixes that contain a residual herbicide in order to control late-emerging plants (Loux et al. 2006). Pre-plant (PP) residual herbicides applied in the spring reduced Canada fleabane densities more than fall applications due to emergence occurring after application (Davis et al. 2009b). Davis et al. (2008) found that 90% of Canada fleabane seedlings emerged in the spring in Indiana, which influences the optimal herbicide application timing. Canada fleabane has been controlled in summer fallow with 2,4-D, 2,4-D + atrazine, dicamba, chlorosulfuron, metsulfuron and thifensulfuron, even at heights of 30 cm (Wiese et al. 1995). Kruger et al. (2010) reported that 2,4-D provided 90 to 97% control of Canada fleabane that was up to 30 cm in height. Multiple studies have shown that Canada fleabane height influences herbicide efficacy. Dicamba (600 g ha^{-1}) applied preplant followed by 300 g ha^{-1} applied postemergence (POST) reduced Canada fleabane density in glyphosate/dicamba-resistant soybean (Byker et al. 2013b). Flessner (2015) reported that dicamba provided 97% control of GR Canada fleabane 4 weeks after application. Bruce and Kells (1990) found variable Canada fleabane control with paraquat, linuron, imazaquin, imazethapyr and metribuzin when applied PP or PRE. Similarly, Byker et al. (2013c) observed unacceptable control of GR Canada fleabane with linuron and imazethapyr. Paraquat provided 15 to 60% control of Canada fleabane, but when tank-mixed with glyphosate control increased to 59 to 95% (Bruce and Kells 1990; Byker et al. 2013c). Weaver (2004) observed soybean chlorosis with linuron applied POST and bleaching with diquat, therefore these herbicides do not have an acceptable margin of crop safety in soybean. Glyphosate (900 g ae ha^{-1}) applied POST in soybean provided 99 to 100% of glyphosate-susceptible Canada fleabane control; control has been reduced to 20% in a field with GR biotypes (Flessner et al. 2015; Byker et al.2013d). Glyphosate plus dicamba applied POST controlled GR Canada fleabane 65 to 96% while a sequential application reduced Canada fleabane densities 95 to 100%

(Byker et al. 2013b). Davis et al. (2009b) observed effective control of GR Canada fleabane with non-glyphosate herbicides caused a shift in the ratio of GR to susceptible biotypes after 4 years of using residual PP herbicide followed by a non-glyphosate POST herbicide program. Alternate herbicides, with different modes-of-action, provide varying levels of control of GR Canada fleabane.

1.2 Biology of Waterhemp (*Amaranthus tuberculatus* var. *rudis*)

1.2.1 Introduction

Waterhemp, also known as *Amaranthus tuberculatus* var. *rudis*, *Amaranthus rudis*, *Amaranthus tuberculatus*, *Amaranthus tuberculatus* var. *tuberculatus*, tall waterhemp, or common waterhemp, is a member of the Amaranthaceae family (Costea et al. 2005). Waterhemp is an annual, dioecious, C4 species native to the Great Plains of North America; it is absent from the western region of Canada and some of the western states (Nordby et al. 2007; Costea et al. 2005; Sauer 1972). Waterhemp has become more prevalent within agricultural ecosystems in Ontario, and the southern and midwest United States due to reduced tillage systems that favour small-seeded species, herbicide resistance, species with a rapid growth rate, high seed production, prolonged emergence, and seed dormancy (Nordby et al. 2007; Costea et al. 2005). Waterhemp is increasing in prevalence and is a troublesome weed primarily in the US corn belt and the province of Ontario.

The classification of common waterhemp and tall waterhemp as two separate species is controversial within the botany community. Riddell (1835) first separated the two species into *Amaranthus altissimus* and *Amaranthus miamiensis* after inspecting plants from western Ohio. He stated that the classification was only temporary until further investigation could be performed (Pratt and Clark 2001). Due to the invalidity of temporary names, classification into two separate species was also suggested by Nuttall (1837) and Moquin-Tandon (1849); however, descriptive features were not precisely identified and hybridization between Amaranthus species caused sterile plants to be mistakenly included in the nomenclature (Pratt and Clark 2001). Recent comparisons of *Amaranthus rudis* and *Amaranthus tuberculatus* indicated that morphologically pistillate and staminate show slight extremes between western and eastern populations with a continuum between the two populations (Pratt and Clark 2001). Fruit

dehiscence was previously suggested as a tool to differentiate between the two species, but after further research Pratt and Clark (2001) found segregation of dehiscence occurs within populations and therefore cannot be used to differentiate common and tall waterhemp into two species. Furthermore, molecular data found no fixed allele differences between any of the tested waterhemp populations (Pratt and Clark 2001). Seedlings of both species in Canada were observed to have a difference in hypocotyl length, cotyledon shape and size, presence or absence of tepals and fruit that were dehiscent versus indehiscent (Costea et al. 2005). Nonetheless, Sauer (1955) and Costea et al. (2005) acknowledge two separate species, with Costea et al. (2005) recognizing species separation at the varietal level. Regardless of the controversy among botanists, a single species of common waterhemp, *Amaranthus tuberculatus* var. *rudis* (Costea et al. 2005) will be referred to as waterhemp for the remainder of this literature review.

1.2.2 Identification and Characteristics

Waterhemp has specific characteristics that differentiate it from similar Amaranthus species. Cotyledons have a width of 2-4 mm, length of 12 to 14 mm and are ovate (egg-shaped) to linear-lanceolate in shape with first true leaves being ovate-lanceolate (Nordby et al. 2007). The hypocotyl of waterhemp seedlings is glabrous and 2.5 to 5 cm in length (Nordby et al. 2007). Mature plant stems are erect, up to 3 m in height, smooth, green or reddish in colour, with the ability to branch and become prostrate depending on environmental conditions and injury (Costea et al. 2005; Horak and Loughin 2000). Waterhemp can grow 0.11 to 0.16 cm per growing degree day, which can equate to up to 2.5 cm of growth per day during the growing season (Nordby et al. 2007; Buhler and Hartzler 2001). Waterhemp has a taproot root structure and indeterminate growth (Costea et al. 2005). Leaves of mature waterhemp are alternate, ovate to lanceolate in shape, petiolated, glossy, smooth, and are 2 to 10 cm in length with a width of 1 to 3 cm (Nordby et al. 2007; Horack and Loughin 2000; Pratt and Clark 2001). Waterhemp is dioecious with female and male flowers on separate plants (Costea et al. 2005). Male flowers have 1.5 to 2 mm long bracts, an extended midrib and five unequal tepals while female bracts are 1.5 to 2.5 mm in length, have a more prominent midrib, and 1 to 2 tepals (Costea et al. 2005). Seeds of waterhemp are red to black, elliptic to obovate, about 1 mm and 2.01 to 2.35 g weight range per 10,000 seeds (Costea et al. 2005; Wu and Owen 2014). Waterhemp has distinct features.

Waterhemp is adapted to a wide range of environmental conditions. Waterhemp is a C4 plant that is able to tolerate a wide range of habitats due to higher water use efficiency and seed production; although conducive climatic conditions for optimal plant growth do exist (Long 1999; Lovelli et al. 2010). Waterhemp grows best in hygrophyte to mesophyte habitats including but not limited to the margins of fresh waters, rivers, lakes, ponds, marshes, bogs and wet areas of fields with the ability to tolerate temporary anaerobiosis (Costea et al. 2005; Nordby et al. 2007). While being able to tolerate a wide range of soil textures, waterhemp grows best in nitrogen rich (nitrophilous) soils with a pH range of 4.5 to 8.0 (Costea et al. 2005). Costea et al. (2005) states waterhemp is a thermophyte and heliophyte; meaning plants prefer warm to hot climates and sunlight. Further evidence to support a preference for warm to hot climates is in a study performed by Guo and Al-Khatib (2003) that showed higher biomass, height and root volume occurred at a daytime temperature of 25 and 30°C 4 weeks after emergence compared to a daytime temperature of 15°C. Waterhemp has a preference for warmer climates, nitrophilous soils and is adapted to soils with varying moisture levels.

1.2.3 Germination and Emergence

Waterhemp requires a period of dormancy before germination occurs. Leon et al. (2004) observed germination after 12 weeks of wet stratification at 4°C. Waterhemp seeds that were chilled had 4 times greater germination than non-chilled seeds (Leon and Owen 2003). Wu and Owen (2014) observed that plants grown in stressed environments (moisture, density, and temperature) were more likely to produce heavier seeds with reduced dormancy than those grown under less stressed environments. Light quality was also shown to be a significant factor in breaking dormancy. Leon and Owen (2003) observed an increase in germination by three times when seeds were exposed to red light (R) than far-red light (FR); however, high temperatures and temperature alternation reduced the light requirement observed. Studies have shown that waterhemp plants and populations differ in their dormancy, and consequently have different environmental cues required to break dormancy and initiate germination (Leon et al. 2006).

Waterhemp germinates and emerges throughout the growing season. Greater waterhemp emergence has been observed in no-tillage systems due to seeds being closer to the soil surface; emergence is hindered at deeper seed burial depths due to a relatively short hypocotyl compared

to other weed species (Costea et al. 2005; Nordby et al. 2007). Germination of waterhemp seeds was observed between 10 to 50°C and plateaued when temperature was a constant 32°C (Leon et al. 2004; Guo and Al-Khatib 2003). Because of natural day to day, and, day to night, temperature variation, Leon et al. (2004) conducted experiments to determine waterhemp germination under varying temperature regimes. Varying temperatures, when compared with constant temperatures, increased germination from 30% to 90% in a study conducted on a two-way thermogradient plate. Amplitude of temperature alternation was also seen to significantly increase germination, plateauing at an amplitude of 18°C. Increasing mean environmental temperature was shown to reduce the time required to obtain 50% germination (Leon and Owen 2003). Buhler and Hartzler (2001) observed up to 7% emergence in one year from the seed bank, with total emergence not exceeding 15% of the seed bank after four years. Emergence was 5% in year one, 7% in year two, 1% in year three and 2% in year four. In the same study, the percentage of recovered seed was 65% in year one, 45% in year two, 28% in year three and 12% in year four, which is due to seed predation and decomposition (Buhler and Hartzler 2001). Viability of recovered waterhemp seed after four years was 95% which was an increase from year 3, which had 71 to 80% seed viability (Buhler and Hartzler 2001). In a long-term study, 3% of the original waterhemp seedbank still germinated after the seed was buried for 17 years in the soil (Burnside et al. 1996). It is important to note that while only a small percentage of seeds emerge in any given year, one million seeds per plant can be produced, enter the soil seed bank and may germinate and emerge in subsequent years (Steckel et al. 2003). In soybean, waterhemp have been reported at densities between 100-220 plants m^{-2} in Ontario, 346-480 plants m^{-2} in Iowa, and 480 plants m^{-2} where no herbicides or other weed controls were applied (Felix and Owen 1999; Hartzler et al. 1999). Steckel et al. (2013) observed waterhemp emergence under 40, 68 and 99% artificial shade and seed production of May emerged plants was reduced by 51, 75 and 99%, respectively. Plants that emerged in June had a 41, 51 and 77% reduction in seed production for the 0, 40 and 68% shade environments compared to May-emerged plants. These results correlate with Horak and Loughin's (2000) study that late emergence reduces biomass and leaf area compared to plants that emerge early in the growing season. In Ontario, emergence has been observed from the mid-May to beginning of September (Vyn et al. 2007). Emergence of waterhemp increased up to 102% in rows marked by tractor wheels than those without (Jurik and Zhang ShuYu 1999; personal observation 2016). Waterhemp appears to have varying seed dormancy requirements

that are based on environmental signals (Leon et al. 2003). The varying germination and emergence patterns of waterhemp allow this plant to germinate, emerge and grow in a wide range of environmental conditions throughout the growing season.

1.2.4 Reproduction and Dispersal

Waterhemp is a dioecious species that results in wide genetic diversity. Waterhemp pollen is spherical and small, having a diameter of 20 um (Franssen et al. 2001a). Waterhemp pollen mostly fertilizes female plants within 25 m of a male plant (Liu et al. 2003). Pollen from long-distance sources has reduced viability compared to closer plants (Fromhage and Kokko 2010). Liu et al. (2012) reported that waterhemp pollen was viable for up to 120 hours (5 days) post-anthesis and found pollen up to 800 m away from the source. Seed production decreases if the male plant is more after 25 m away, with a 90% decrease in production seen at 50m, but seed production has been documented at distances of 400 and 800m. Assuming a height of 0.5 and 1 m and wind velocity of 10 and 40 km h^{-1}, it is estimated pollen grains of waterhemp could travel 30 to 50 and 300 to 325 m, respectively (Costea et al. 2005). Resistance can be cross-bred between Amaranthus species and has been observed from *Amaranthus palmeri* (Palmer Amaranth) to waterhemp and from *Amaranthus hybridus* (smooth pigweed) to waterhemp (Franssen et al. 2001b). Waterhemp pollen can travel relatively long distances, transferring resistance traits from the same species and genetically similar species. Waterhemp pollen dispersal aids in the rapid spread of herbicide resistance.

Seed production of waterhemp is affected by environmental conditions. Due to an indeterminate growth habit, flowering and seed set can continue until the first frost (Costea et al. 2005). Waterhemp that emerged later in the growing season requires fewer days after emergence to flower than plants that emerged earlier (Wu and Owen 2014). In the absence of competition, it has been documented that waterhemp can produce up to 4.8 million seeds per plant (Hartzler et al. 2004). In contrast, waterhemp seed production decreased to 600,000 seeds per plant when grown in soybean (Schwartz et al. 2016). Seed production in the absence and presence of soybean was much lower than in the previous study, with more conservative values of up to 305,831 seeds per plant in the absence of competition, and 180,700 seeds per plant when plants emerged with soybean (Schwartz et al. 2016; Uscanga-Mortera et al. 2007). Delayed emergence and increased shade reduces waterhemp seed production. For example, from a study by Steckel

et al. (2013), seed production under 40, 68 and 99% shade was 646 900, 401 200 and 8 seeds per May-emerging plant, respectively, and 315 500, 90 400 and 14 seeds, respectively, for June-emerging plants. Hartzler et al. (2004) observed 309 000, 64 000, 17 000 and 3 000 waterhemp seeds per plant for plants that emerged 0, 27, 40 and 50 days after planting soybean, respectively. Seed production was reduced from 34 450 seeds seen under no water stress to 27 775, 10 194 and 4 469 seeds per plant under light, moderate and high water stress, respectively (Sarangi et al. 2016). Schwartz et al. (2016) reported a correlation between aboveground biomass and seed production. Waterhemp plants that emerged in the first week of July had a 50 to 75% reduction in seed production compared to plants that emerged in mid- to late-May (Wu and Owen 2014). Seed production per plant decreased with increasing plant density up to 100 plants m^{-2}; seed production per plant plateaued afterwards (Schwartz et al. 2016; Wu and Owen 2014). As density decreased, the ratio of males to females changed from an expected 1:1 to 1:5 or 1:10; this was close to the ratio of 1:3 in Illinois (Lemen 1980; Costea et al. 2005; Schwartz et al. 2016). A higher ratio of female to male plants will result in higher seed production and greater density in future years since waterhemp is a dioecious species. Waterhemp has reduced seed production under stressed environments such as drought, increased shade and high densities.

Waterhemp seed can disperse through several different methods. Dispersal of waterhemp seeds can occur through wind, waterways, animals, machinery, and manure or compost (Costea et al. 2004). The fruit and seeds of waterhemp can float, allowing for seed dispersal in rivers and streams (Costea et al. 2005). Dispersal of waterhemp occurs through a number means which is facilitated by its seed characteristics.

1.2.5 Herbicide-Resistance

Waterhemp has been documented to be resistant to multiple herbicide modes of action. Triazine-resistant waterhemp was first reported in Nebraska in 1990; since then, populations resistant to protoporphyrinogen oxidase (PPO) inhibitors, Photosystem ll (PSII) inhibitors, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors, synthetic auxins and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors have been reported throughout the United States and in Ontario, Canada (Anderson et al. 1996; Heap 2016). Populations resistant to glyphosate (EPSPS inhibitor) have been found after 7 to 8 years of continuous soybean that had at least two applications of glyphosate per year (Nordby et al. 2007). Multiple resistant

populations of waterhemp were first identified in 1998 to ALS and PSII inhibiting herbicides, with three-way resistance between ALS, PSII and PPO inhibiting herbicides, and glyphosate, ALS and PPO inhibiting herbicides being discovered in 2002 and 2005, respectively (Heap 2016; Nordby et al. 2007). Four and five-way resistance has been observed in Missouri, with 52% of the populations studied resistant to at least two modes-of-action (Schultz et al. 20015). Only one population was resistant to five different modes of action; PSII, ALS, EPSPS, PPO and HPPD inhibitors (Schultz et al. 2015). Some waterhemp populations are resistant to the ALS inhibiting herbicides imazethapyr, flumetsulam, chlorimuron-ethyl, nicosulfuron and thifensulfuron; PPO inhibiting herbicides lactofen, acifluorfen, fomesafen, flumiclorac, flumioxazin and sulfentrazone; HPPD inhibitor topramezone; and PSII inhibiting herbicide atrazine (Heap 2016). In 2002, multiple resistance to imazethapyr and atrazine was reported within Ontario, and multiple resistance to glyphosate and imazethapyr was discovered in 2014 (Heap 2016). Waterhemp is resistant to multiple herbicide modes of action within North America.

Research was conducted on the frequency of PPO resistance alleles (Wuerffel et al. 2015). They observed that fomesafen, especially at high application rates, increased the frequency of PPO resistant alleles within a population when applied alone. However, when applied with *S*-metolachlor, the frequency of PPO resistant alleles did not increase. Therefore, resistance management should always be taken into consideration when applying herbicides. Successful control of GR waterhemp must incorporate many of the tenets of an integrated weed management program.

There are several mechanisms that confer resistance to glyphosate in waterhemp. Reduced translocation, EPSPS gene amplification and an altered target site are the known mechanisms of resistance to glyphosate in some waterhemp populations (Bell et al. 2013; Nandula et al. 2013; Chatham et al. 2015). High glyphosate rates in previous years has been correlated with a higher average EPSPS copy number conferring higher fold resistance to glyphosate (Chatham et al. 2015). Multiple mechanisms-of-resistance have been found within waterhemp populations. Schultz et al. (2015) observed both elevated EPSPS copy number and a Pro106Ser substitution being the cause of resistance within a waterhemp plant. Single and multiple mechanisms of resistance confer resistance to glyphosate in waterhemp.

1.2.6 Impact

Waterhemp can reduce soybean yield. When waterhemp competed with soybean throughout the growing season, yields were reduced by 44% in 76 cm rows and 37% in 18 cm rows (Steckel and Sprague 2004). This is further corroborated by Hager et al. (2002) who reported a 43% reduction in soybean yield in Illinois when waterhemp emerged at the unifoliate stage at a plant density of 89 to 360 plants m^{-2}. In southern Ontario, soybean yield was reduced up to 73% with season-long competition waterhemp, but only 13% reduction was observed when waterhemp was removed 4 weeks after soybean emergence (Vyn et al. 2007). Steckel and Sprague (2004) observed a 10% yield reduction when waterhemp emerged at the V4 growth stage of soybean. When allowed to compete with soybean, waterhemp interference can result in substantial yield losses.

1.2.7 Control

Control of waterhemp can be achieved through a combination of cultural, mechanical, biological and chemical weed management strategies. Reducing row spacing is a cultural strategy that increases the ability of the crop to compete with waterhemp while also accelerating canopy closure (Nordby et al. 2007; Steckel and Sprague 2004). Reduction of sunlight to the soil surface due to canopy closure greatly reduces biomass and seed production of waterhemp (Steckel and Sprague 2004). Buhler et al. (2001) found that mechanical weed control such as moldboard plowing and inter-row cultivation deceased waterhemp seed bank during a 5-year soybean and corn rotation. Biological control of waterhemp is through seed predation by insects and decomposition due to bacteria and fungi in the soil (Costea et al. 2005). Chemical weed control includes the use of efficacious herbicides. Waterhemp can be controlled with a diversified crop production system that takes advantage of a range of weed control methods.

The use of efficacious herbicides is one component in managing waterhemp. Pre-emergence (PRE) herbicides are critical in soybean since waterhemp is extremely competitive when allowed to grow with soybean (Uscanga-Mortera et al. 2007). Bensch et al. (2003) observed a 25% reduction in competitiveness when waterhemp emerged at the V2 to V3 stage of soybean compared to plants that emerged at the same time as soybean emergence. A 30 cm reduction in plant height was observed per week of delayed waterhemp emergence, resulting in the majority of emerging plants not extending above the soybean canopy; therefore, it can be

inferred that the use of an effective PRE herbicide is important in the control of waterhemp (Hartzler et al. 2004). While late-emerging plants may not extend above the canopy or affect crop yields, plants may still produce seed which will add to the seed bank (Costea et al. 2005). Therefore, PRE herbicides should be applied as close to soybean seeding as possible to increase efficacy (Hager et al. 2003). Preemergence herbicides that have been reported to control waterhemp are sulfentrazone, flumioxain, dimethenamid, *S*-metolachlor, pendimethalin, acetochlor, linuron, imazethapyr, chlorimuron, imazamox, and metribuzin (Sweat et al. 1998; Niekamp and Johnson 2001). Control with the aforementioned herbicides is only achieved if populations are not resistant. Sulfentrazone (280 g ha^{-1}) and *S*-metolachlor (1540 g ha^{-1}) + metribuzin (360 g ha^{-1}) provided 80 and 94% control waterhemp, respectively (Legleiter et al. 2009; Vyn et al. 2007). Sulfentrazone, metribuzin and metolachlor provided >97% control of waterhemp (Sweat et al. 1998). Glyphosate, although ineffective for the control of GR populations, has been shown to control glyphosate-susceptible waterhemp plants up 30 cm in height (Hoss et al. 2003). Postemergence applications of lactofen, fomesafen, and acifluorfen have been reported to provide 75-90% control of up to 10 cm waterhemp (Hager et al. 2003). In southern Ontario, Vyn et al. (2007) observed 95% control of waterhemp 28 days after application (DAA) with acifluorfen and 88% control at 70 DAA. In the same study, fomesafen was observed to control 98% of waterhemp 28 and 70 DAA. Soltani et al. (2009) reported 92% control of waterhemp with dimethenamid applied PRE followed by acifluorfen POST and 94% control with dimethenamid PRE followed by fomesafen POST. To prevent a decrease in soybean yield, waterhemp needs to be controlled from soybean emergence through the critical period of weed control using diversified weed management tactics.

1.3 Glyphosate

1.3.1 Introduction

Glyphosate or N-phosphonomethylglycine, is a herbicide in the organophosporus family (Shaner et al. 2014). Glyphosate is the active ingredient in herbicides such as Roundup ™, Touchdown™, Durango™, and Aquamaster™; each of these herbicides have different salts of glyphosate (potassium**,** isopropylamine, diammonium and dimethylamine) and different

surfactant blends. (Franz et al. 1997). Glyphosate is an organophosphorus salt that is formulated in a variety of ways.

Glyphosate is a non-selective, systemic herbicide that is applied after weeds have emerged since it exhibits no soil activity (Powles 2008). Glyphosate controls a wide range of annual, biennial and perennial broadleaf and grass weeds along with small trees and shrubs (Ibrahim 2016). Glyphosates molecular formula is $C_3H_8NO_5P$ (Franz et al. 1997). It is a white, odourless acid, with a molecular weight of 169.07 g/mole and density of 1.74 g/ml (Shaner et al. 2014). Glyphosate is a non-selective, systemic herbicide that controls a wide range of weeds, trees and shrubs.

Chemists synthesize new compounds that are vigorously tested for herbicidal activity and efficacy (Franz et al. 1997). In the 1950s, Monsanto started testing different chemicals for use as a herbicide; after years of testing tens of thousands of chemicals, three were commercialized (Franz et al. 1997). One of the chemicals tested was glyphosate, which was originally synthesized by Cilag, a pharmaceutical company (Duke and Powles 2008). In 1974, glyphosate was commercialized globally, as a "highly effective, non-selective, systemic, postemergence herbicide with low mammalian toxicity and environmental safety" (Franz et al. 1997). Glyphosate has herbicidal activity and has been commercialized globally.

Glyphosate is the most widely used herbicide in cropland, plantations, orchards, forestry, residential and industrial areas (Franz et al. 1997). Previously, preemergence herbicides with residual activity were used, the introduction of glyphosate simplified and changed weed control strategies (Gulden et al. 2010). Following the introduction of GR crops, postemergence applications of glyphosate were made two to four times within a growing season to control weeds since glyphosate has no residual activity (Gulden et al. 2010). Holm et al. (1977) published a list of the most troublesome weeds globally, and glyphosate controlled the top 18. Glyphosate has a unique chemical structure, is rapidly absorbed by susceptible species, is translocated via the symplast and apoplast, and has a unique mode of action; these characteristics make it an ideal herbicide (Dill 2005; Duke and Powles 2008). Glyphosate is an efficacious herbicide with many unique attributes that have contributed to its widespread use.

1.3.2 Mode-of-Action

Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is found within bacteria, some fungi and in the chloroplasts and root plastids of plants (Dill 2005; Duke and Powles 2008). EPSPS is an important enzyme in the shikimate biosynthetic pathway. The shikimate pathway synthesizes aromatic plant metabolites which are postulated to make up 35% of a plant's dry weight (Dill 2005; Franz et al. 1997). Aromatic amino acids are involved in plant metabolism, and are precursors for hormones, proteins and secondary metabolites (Tzin and Galili 2010). One of the intermediaries in the shikimate pathway is chorismate. Chorismate is used in the synthesis of the three aromatic amino acids – phenylalanine, tyrosine and tryptophan. The shikimate pathway begins by converting phosphoenolpyruvate (PEP) and erythrose into 3-deoxy-o-arabino-heptulosonate 7-phosphate through 3-Deoxy-o-arabino-heptulosonate 7 phosphate synthase (DAPH) (Herrmann 1995). 3-dehydroquinate synthase (DHQS) then catalyzes 3-deoxy-o-arabino-heptulosonate 7-phosphate into 3-dehydroquinate. The next step of the pathway uses the enzyme 3-dehydroquinanate dehydratase (DHQD) to catalyze 3-dehydroquinate into 3-Dehydroshikimate. 3-dehydroshikimate is converted into shikimate through the enzyme shikimate dehydrogenase (SDH). Shikimate kinase (SDK) converts shikimate into shikimate 3-phosphate (S3P). S3P is converted to 5-enolpyruvylshikimate 3-phosphate by binding with PEP using EPSP synthase. In the final step of the shikimate pathway, 5-enolpyruvylshikimate 3-phosphate is catalyzed into chorismate through the chorismate synthase enzyme. Glyphosate inhibits growth by binding more readily to EPSPS and S3P, in place of the substrate PEP creating a EPSPS-S3P-glyphosate compound (Franz et al. 1997). It is important to note that glyphosate is the only known molecule that inhibits EPSPS (Duke and Powles 2008). After inhibition of the shikimate pathway, there is a build-up of S3P, and aromatic amino acid production is halted (Gomes et al 2014). Photosynthesis has been shown to be inhibited by glyphosate most likely due to the lack of amino acids, which leads to oxidative stress and mineral deprivation within the cell (Gomes et al. 2014). Glyphosate inhibits the shikimate pathway by binding to the EPSPS and S3P.

1.3.3 Behaviour in Plants and Soil

Glyphosate is readily absorbed and translocated throughout the plant. After application, glyphosate enters the plant through rapid diffusion into the cuticle and absorption across the cell

membrane (Franz et al. 1997). Glyphosate is translocated in the apoplast and symplast, accumulating in the roots, leaves, and actively growing tissues or organs (Duke and Powles 2008; Franz et al. 1997). Weed growth is inhibited through binding of glyphosate to EPSPS which causes chlorosis and necrosis (Franz et al. 1997). Chlorosis occurs within 4 to 7 days after application for more susceptible species such as annual grasses, and within two weeks for less susceptible species such as perennial broadleaf weeds (Franz et al. 1997). Glyphosate is an efficacious herbicide which is attributed to its relatively rapid absorption, symplastic and apoplastic translocation, and limited degradation in plants (Duke and Powles 2008).

Glyphosate is strongly adsorbed to soil colloids. Glyphosate has moderate soil persistence with a half-life of 47 days from studies conducted in the field and and a half-life of 25 days from studies conducted in the lab (Shaner et al. 2014). Glyphosate rapidly and tightly binds to the soil; therefore, glyphosate has low mobility and potential for runoff (Shaner et al. 2014). If there is dust in the air or on the leaf surface, a substantial amount of glyphosate will be deactivated within the first hour after application (Sprankle et al. 1975). Glyphosate is decomposed through microbial interactions, photochemical reactions (UV light), and hydrolytic and oxidation processes (Shaner et al. 2014). Glyphosate is metabolized to aminomethlyphosphonic acid (AMPA), which is degraded more slowly than glyphosate but is more mobile within the soil (Franz et al. 1997; Duke and Powles 2008). Aminomethlyphosphonic acid is degraded further into phosphate, carbon dioxide, amino acids, carbohydrates, ammonia and formaldehyde (Franz et al. 1997). The degradation of glyphosate is influenced by its chemical structure, environmental conditions, soil type, phosphate level of the soil and soil micro-organisms (Franz et al. 1997; Sprankle et al. 1975). Increased phosphate levels increase glyphosate degradation in the soil (Sprankle et al. 1975). Organic matter, pH, silt and sand content of soil have a limited effect on glyphosate soil adsorption. Glyphosate is adsorbed more rapidly to clay than sand and is more mobile in coarse textured, high pH soils (Franz et al. 1997; Sprankle et al. 1975). Due to the non-volatile nature of glyphosate, there is no contamination of the atmosphere (Duke and Powles 2008). Within waterways, glyphosate is rapidly adsorbed by soil colloids, silt, suspended soil particles, and degraded by microorganisms and UV light (Franz et al. 1997). Most glyphosate residues are found in sediment on the bottom of waterways. Glyphosate binds tightly to soil particles and rate of degradation is influenced by soil properties and micro-organisms.

1.3.4 Environmental Interactions and Toxicology

The efficacy of glyphosate is affected by environmental and application interactions. Environmental conditions like drought, heat, cold, and time of day influence glyphosate efficacy due to an increase in the thickness of the cuticle and changes in leaf orientation (Hartzler et al. 2006). Glyphosate efficacy is influenced by the amount absorbed into living plant tissue and loaded into the phloem (Sammons and Gaines 2014). The rate of glyphosate required for acceptable weed control is influenced by weed species, weed size and weather conditions (Hartzler et al. 2006). Glyphosates efficacy is affected by weed species, weed size, and environmental conditions.

Toxicological data of glyphosate, while recently controversial, have been shown to pose no harm to human health when label directions are followed. Studies on glyphosate and its first breakdown metabolite, AMPA, have been extensively studied for their effects on human and environmental health. No genotoxic, carcinogenic, reproduction affects or neurotoxic effects have been documented in laboratory studies on mammals (Ibrahim 2016). Glyphosate has a lower LD_{50} (5400 mg/kg) than other common chemicals that humans ingest such as sodium chloride (3700 mg/kg), and caffeine (331 mg/kg) (Ibrahim 2016). Glyphosate does not bio-accumulate or get metabolized in humans and is instead excreted from the body (Ibrahim 2016). Chronic effects of glyphosate have not been observed at higher concentrations than those used when following the products label and multiple government organizations do not view glyphosate as carcinogenic (Ibrahim 2016). The International Agency for Research on Cancer (IARC) has classified glyphosate as probably carcinogenic to humans in 2015. This is despite a vast amount of scientific research on glyphosate that has found that it is not carcinogenic (Williams et al. 2000). Glyphosate is considered to have no detrimental mammalian health effects when applied according to label directions.

Commercial glyphosate formulations include surfactants and other additives. The main surfactant is polyethyloxylated tallowamine (POEA), and while more toxic than glyphosate with an LD_{50} 0f 1200 mg/kg, at low concentrations (20%) it not harmful to humans at current use patterns (Williams et al. 2000). Additives in commercial glyphosate formulations influence its toxicological effects.

1.3.5 Glyphosate-Resistant Crops

Glyphosate-resistant crops were first introduced in 1996 in soybean and canola (Dill 2005). Further research has led to additional GR crops including corn, sugarbeet, sweet corn, cotton, and alfalfa (Dill 2005; Duke and Powles 2008). Glyphosate resistance in crops is conferred through an insensitive target enzyme in the shikimate pathway (CP4 EPSPS) and enhanced glyphosate metabolism (glyphosate oxidoreductase) (Dill 2005; Duke and Powles 2008). While several ways to create GR crops has been studied, achieving acceptable levels of resistance through single point mutations were difficult due to the close binding sites of PEP and glyphosate on the EPSPS enzyme (Dill 2005). Resistance to glyphosate was eventually achieved through a single missense mutation caused by mutagenesis of a cell line. Monsanto introduced GR crops under the Roundup Ready® brand, which use an insensitive EPSPS mechanism to confer resistance instead of a mutation (Dill 2005). This involved the insertion of the bacterial EPSPS gene called CP4 EPSPS from *Agrobacterium tumefaciens* into the genome of various crops (Dill 2005). This is due to the CP4-EPSPS protein being structurally different at the PEP binding region so that glyphosate cannot bind to the protein (Dill 2005). While this mechanism of resistance is common in most GR crops, a GOX gene from *Ochrobactrum* was inserted into the genome of canola to confer a higher level of tolerance to glyphosate (Duke and Powles 2008). Multiple GR crops have been created through two different mechanisms.

Glyphosate-resistant crops have significantly changed farming. Glyphosate-resistant crops and glyphosate was rapidly adopted by growers due to potentially excellent weed control, a wider margin of crop safety, potentially low cost of weed control, the ability to spray glyphosate postemergence, potentially fewer herbicide applications, flexibility to switch to no-tillage, and earlier seeding (glyphosate has no soil activity) (Powles 2008; Shaner 2000). Less reliance on tillage for weed control has reduced wind and water erosion, fuel and equipment costs, and improved soil organic matter content and and soil water retention (Dill 2005). While there are many benefits to glyphosate, over-reliance on one herbicide can lead to herbicide resistant weeds, specifically weed species present in crop production fields; this has occurred extensively with the over-use of acetolactate synthase (ALS) inhibitors, photosystem II inhibitors (PSII) and acetyl CoA carboxylase (ACCase) inhibitors (Powles 2008; Gulden et al. 2010; Heap 2016). The ability to spray glyphosate on GR crops has drastically changed crop production, adding benefits while also creating new challenges.

1.4 Herbicide-Resistance

1.4.1 Introduction

Weed management involves the inclusion of at least one of three main goals: prevention, control and eradication. Prevention involves utilizing various practices to reduce the chance of introducing weed species to non-native areas, controlling weeds is, "the suppression or reduction of weed species to an economically acceptable level", while eradication is when weed species are made extinct in a specific area (Holt 2013). The use of herbicides is a common method of weed control that provides short-term control of weeds, but a shift to herbicide-tolerant, resistant or perennial weed species has been shown to occur (Holt 2013). The development of herbicide resistance in a weed species that was previously controlled, enables survival and reproduction following exposure to a herbicide; herbicide tolerance is different in that it doesn't occur through selection pressure but is already present in the plant species (Cobb and Reade 2010). When essential cell processes are controlled by more than one gene, it is thought that herbicide resistance is less likely to occur (Cobb and Reade 2010). Acetolactate synthase inhibitor (ALS) herbicides are the highest risk as they affect processes within the plant that are regulated by one gene (Cobb and Reade 2010). The first herbicide resistant weed discovered was 2,4-D-resistant wild carrot (*Daucus carota L.*) in Ontario, Canada (Whitehead 1963). The second major weed to develop resistance was common groundsel (*Senecio vulgaris L.*) in the USA in 1968 to the triazine herbicides atrazine and simazine (Ryan 1970). In general, the primary method of weed management is through the use of herbicides, and their overreliance has led to the evolution of herbicide-resistant biotypes.

There are many factors that can influence the progression of a weed population to develop resistance. Some of these factors include: initial frequency of the resistant alleles in the population, herbicide mode-of-action, herbicide persistence in the soil, the number of mutations required to confer resistance, genetic variability, gene flow among individual plants (i.e., dioecious species), selection pressure (application frequency of same herbicide or mode-of-action), fitness of resistant and susceptible plants, fecundity, seed bank longevity, and mode of inheritance (i.e., dominance of the resistance trait) (Cobb and Reade 2010; Diggle et al. 2003). Diggle et al. (2003) modelled the probability of a weed developing resistance; they concluded that population size/pollen flow was a major factor. A larger plant population or one with greater

pollen/seed travel will be more likely to become resistant due to a higher chance of resistant alleles being present to one or more herbicides. Currently, the families: Poaceae, Asteraceae, Brassicaceae, Amaranthaceae, and Chenopodiaceae account for about 61% of all cases of herbicide-resistance species, and approximately 48% of agriculturally important weed families from around the world (Heap 2017). Canada fleabane is in the Asteraceae family which is in the top three relevant weed species in commercial fields, accounting for approximately 16% of herbicide-resistant species in the world. Waterhemp, a member of the Amaranthaceae family, accounts for 4% of herbicide-resistant species (Heap 2017). According to Heap (2018), the herbicide group with the most resistant species are ALS inhibitors (160), followed by triazines (74), ACCase inhibitors (48) and glycines (41). The probability of herbicide resistance is dependent on weed characteristics and herbicide properties.

1.4.2 Mechanisms of Resistance

There are many known mechanisms of herbicide-resistance. The two main classifications are target site resistance and non-target site resistance. Target-site resistance develops when a herbicide can no longer bind to a site within a biological pathway or there is a reduction in lethality due to an overwhelming increase in the number of target-sites (Cobb and Reade 2010; Sammons and Gaines 2014). In susceptible plants, the herbicide may bind to the target site and disrupt growth, leading to plant death (Cobb and Reade 2010). Non-target site resistance occurs when there is a reduction in the amount of herbicide that reaches the target site. Non-target site resistance can be further divided into enhanced metabolism, decreased absorption and/or translocation, herbicide sequestration and gene amplification/duplication (Cobb and Reade 2010); however, gene amplification is considered to involve both target and non-target resistance mechanisms (Sammons and Gaines 2014). Research by Yuan et al. (2007) suggests that a high herbicide dose is more likely to cause target site resistance, while low doses are more likely to cause non-target site resistance. Additionally, cross-resistance and multiple-resistance can occur. Cross-resistance is when a single mutation confers resistance to more than one herbicide family within a group, while multiple-resistance occurs when two or more herbicide modes-of-action are selected within a weed population; it is more probable for herbicide-resistance to occur sequentially (Cobb and Reade 2010). Herbicide resistance is due to target and non-target mechanisms.

1.4.3 Target and Non-Target Resistance

Target and non-target herbicide resistance are due to many different changes in biological processes. Target site resistance is often due to a single-point mutation on the gene coding for the protein. Gene amplification, also known as gene duplication, occurs when there is more than one copy of a gene related to important biological processes, increasing production of enzymes coded by the gene; which can reduce herbicide efficacy below the lethal threshold (Powles 2010). Enhanced metabolism resistance occurs when a plant can break down herbicides faster than susceptible plants within the population, leading to survival and seed production (Cobb and Reade 2010). This can be achieved by a more active enzyme, increased amounts of enzyme or genes coding for certain enzymes that break down herbicides (Yuan et al. 2007). Examples of these enzymes are glutathione S-transferases, glycosyl transferases and cytochrome P450s (Yuan et al. 2007). These increased enzyme concentrations within the plant are constant, unlike in susceptible plants that increase production too late for survival. Enhanced sequestration (compartmentalization) is the movement of herbicides from the cytoplasm to the cell vacuole directly or indirectly when herbicides are attached to sugars which are moved into the vacuole (Cobb and Reade 2010). Decreased absorption can occur when the epicuticular wax on the leaf surface is thick enough to prevent movement of herbicide into the cell (Ferreira and Reddy 2000). There are several different mechanisms of resistance in weed species, involving multiple biological processes and genes within the plant.

1.4.4 Glyphosate-Resistance

The recent evolution of GR weed species is a major issue in crop production. The presence of GR weeds increases the cost of weed management in Roundup Ready® crops and reduces crop yield if weeds are not effectively controlled (Holt 2013). The first case of GR was rigid ryegrass (*Lolium rigidum Gaud.*) in an Australian apple orchard in 1996 (Powles et al. 1998). According to Heap (2018), there are currently 41 weed species with biotypes that are resistant to glyphosate globally. Known mechanisms of GR in these weed species include: target site mutation of EPSPS, reduced glyphosate translocation, gene amplification of the EPSPS gene, enhanced metabolism, and vacuolar sequestration (Feng et al. 2003; Nandula 2010; Shaner 2009; Gonzalez-Torralva et al. 2012). Reduced translocation of glyphosate has been observed in many different GR weed species, with glyphosate seemingly confined in the distal region of the

leaf (Shaner 2009). Reduced glyphosate translocation in the phloem has been attributed to movement of glyphosate into the vacuole or apoplast, binding of glyphosate to the cell wall, and movement of glyphosate out of the chloroplast into the cytoplasm away from the target site; however, the most likely reason is sequestration in the cell vacuole, either due to more transporters or new, more efficient transporters (Shaner 2009). Interestingly, research has suggested that GR plants with reduced translocation are more resistant to glyphosate than plants with a mutation at Proline 106 of the EPSP synthase (Nandula 2010). Many different plant species have evolved resistance to glyphosate by several different mechanisms of resistance.

1.4.5 Glyphosate-Resistant Canada fleabane

Populations of Canada fleabane have evolved resistance to glyphosate throughout the world. Glyphosate-resistant Canada fleabane was confirmed in 2000 in the United States and the estimated cost of controlling GR Canada fleabane in soybean is \$30.46 USD ha^{-1} (Mueller et al. 2005). Glyphosate-resistant Canada fleabane negatively affects the profitability of farms.

Glyphosate-resistance in some Canada fleabane populations throughout the world is due to a target site mutation. Powles and Preston (2006) observed that an amino acid substitution of the EPSPS enzyme at site 106 from proline (Pro) to serine (Ser), alanine (Ala) or threonine (THR) caused a reduction in the ability of glyphosate to bind to EPSPS, conferring resistance to glyphosate in Canada fleabane. Resistance to glyphosate through both altered target site and reduced translocation has been observed in rigid ryegrass. In Africa, where this was observed, glyphosate was used continuously for 25 years (Yu et al. 2007). There are target site mutations that confer resistance to glyphosate in some Canada fleabane biotypes.

Reduced translocation of glyphosate in Canada fleabane has also been confirmed as a mechanism of weed resistance to glyphosate (Feng et al. 2004). Koger and Reddy (2005) also observed different rates of translocation between susceptible and resistant Canada fleabane populations in Mississippi, Tennessee, Arkansas and Delaware, but no differences in absorption of glyphosate. The authors observed reduced translocation ranging from 28 to 45% of absorbed glyphosate among different resistant populations. Due to the nature of these studies, reduced translocation may be due to enhanced sequestration of glyphosate, since the movement of glyphosate was studied at on the whole plant level. Further research is needed to determine if vacuolar sequestration and reduction in phloem loading is the reason for reduced translocation.

Reduced translocation of glyphosate from treated leaves is one mechanism of glyphosate resistance in Canada fleabane.

Vacuolar sequestration is a mechanism of GR in some Canada fleabane biotypes. Ge et al. (2010) observed a reduction in the amount of glyphosate that was able to reach the target site to a non-lethal concentration (vacuolar sequestration). Research on the mechanism behind vacuolar sequestration in Canada fleabane has led to the theory that an ATP-dependent ABC transporter moves glyphosate from the cytoplasm into the cell vacuole (Ge et al. 2014). These tonoplast-membrane proteins are assumed to be the method of transport for glyphosate into the cell vacuole in both resistant and susceptible plants (Ge et al. 2014). In a study by Ge et al. (2014), the amount of glyphosate transported was 85% in resistant biotypes compared to 15% in susceptible biotypes under normal conditions. This difference may be due to genes coding for more proteins or more efficient proteins. Interestingly, Ge et al. (2011) observed that vacuolar sequestration of glyphosate by Canada fleabane is inhibited by cool temperatures. The authors also observed differences in glyphosate sequestration was initially observed between plants sprayed in 30°C and 20°C environments, with a significant increase in glyphosate efficacy occurring at 8°C. The cool environments seem to suppress the transport of glyphosate across the tonoplast; therefore, there is very little advantage to genetic control of transport unless the environment warms within several days after application. Additionally, plants grown in warm conditions and then exposed to cold conditions were still resistant when sprayed with glyphosate (Ge et al. 2014). Some Canada fleabane populations have evolved resistance to glyphosate through enhanced vacuolar sequestration.

Enhanced metabolism conferred resistance to glyphosate in Canada fleabane populations in Spain (González-Torralva 2012). This is in contrast to Feng et al. (2004) whom observed no metabolism of glyphosate in Canada fleabane. Nonetheless, de Carvahlho et al. (2008) observed metabolism of glyphosate in sourgrass (*Digitaria insularis L.)*. These are rare cases of glyphosate metabolism; further studies with different analytical methods are suggested within the literature to confirm the occurrence of glyphosate metabolism as a mechanism of resistance (Sammons and Gaines 2014). Other studies looking at the mechanism of resistance in specific weed species have found metabolism is not the mechanism of GR (Heap 2016).

1.4.6 Glyphosate-Resistant Waterhemp

Control of waterhemp with glyphosate is variable among waterhemp biotypes; a 3.5-fold increase in resistance has been reported after only two generations of selection (Zelaya and Owen 2002). Research by Baylis (2000) observed variable responses of waterhemp and other Amaranthus species to glyphosate. Additionally, control of waterhemp with glyphosate has been less than satisfactory in some environments (Nordby et al. 2007). For example, Shaner (2000) observed that three applications of glyphosate were needed before an acceptable level of control was achieved. Glyphosate-resistant waterhemp was first recorded in 2005 in Missouri, where soybean was grown continuously for 7 or 8 years and glyphosate was applied two or more times per year (VanGessel 2001; Nordby et al. 2007). The estimated cost of controlling GR waterhemp with herbicides is \$48 USD ha^{-1} in Missouri (Legleiter et al. 2009). Glyphosate-resistant waterhemp is an economically important weed, that was previously controlled by multiple applications of glyphosate.

Resistance of waterhemp to glyphosate is due to a number of different mechanisms. Chatham et al. (2015) reported that resistance to waterhemp populations in Illinois, Kansas, Missouri and Nebraska was due to EPSPS gene amplification, while a population in Kentucky was due to a target-site mutation at P106S. Lorentz et al. (2014) also reported that EPSPS gene amplification was the mechanism of glyphosate resistance in six populations of waterhemp from Missouri and Illinois. The resistance level of a population with EPSPS gene amplification has been found to increase with the number of EPSPS gene copies (Chatham et al. 2015). A waterhemp population in Mississippi was found to be GR due to an altered target-site P106S and reduced translocation (Nandula et al. 2013). Gaines et al. (2011) observed movement of the genes related to gene amplification from Palmer amaranth (*Amaranthus palmeri*) to other Amaranthus species. Movement of herbicide-resistant genes between Palmer amaranth and waterhemp has been observed with an ALS resistant gene (Wetzel et al. 1999). In summary, resistance to glyphosate in waterhemp may be due to EPSPS gene amplification, reduced translocation and/or a target-site mutation.

1.4.7 Strategies to Combat Herbicide Resistance

In order to delay herbicide resistance an integrated weed management (IWM) program should be implemented. Integrated weed management involves the use of cultural, biological,

mechanical, and chemical methods of weed control, although all may not be feasible in each individual field. Some IWM methods that can be used are crop rotation, tillage, narrow rows, rotating herbicide modes of action, herbicide mixtures, and flaming (OMAFRA 2009). Norsworthy et al. (2012) stated that the following practices should be implemented to reduce the selection of herbicide-resistant biotypes: scout fields to observe what weeds are present and understand how to control them, prevent weed seed bank return or introduction from crop seed, equipment or field borders, use cultural, biological and mechanical methods to suppress weeds, and multiple herbicides with different modes-of-action at the labelled rate. Multiple methods of weed control reduces the selection pressure for the development of herbicide-resistant weeds.

Herbicide tank mixes with multiple modes-of-action are commonly suggested to prevent herbicide resistance. Powles et al. (1996) modelled the evolution of herbicide resistance in a four-year study and observed resistance was less likely to occur in a tank mixture than herbicide rotation when there was no fitness penalty. A fitness penalty occurs when plants of resistant biotypes are not as vigorous in the absence of the herbicide selection compared to susceptible biotypes (Vila-Aiub et al. 2009). This penalty has been observed in GR rigid ryegrass (Preston et al. 2009). A model by Diggle et al. (2003) found that herbicides with different modes-of-action were more likely to delay the onset of herbicide resistance when applied together than herbicides rotated annually. The model assumed there was no fitness penalty, adequate control of weeds to prevent seed return, and the resistant-population had infested less than 100 hectares. Research by Beckie and Reboud (2009), also observed that herbicide combinations delayed the onset of herbicide resistance to ALS inhibitors compared to herbicide rotations. Herbicide mixtures may delay the onset of herbicide resistance.

1.5 Roundup Ready 2 Xtend® Soybean and Roundup Xtend™

1.5.1 Roundup Ready 2 Xtend® Soybean

The profitability of new herbicide-resistance traits is greater than the discovery and registration of new herbicides. Agricultural companies, with the use of biotechnology, are advancing weed management through the development of genetically modified crops that are resistant to registered herbicides. For example, a gene encoding a Rieske nonheme monoxygenase called dicamba O-methylase is capable of conferring resistance to dicamba

(Behrens et al. 2007). The dicamba monoxygenase (DMO) gene, found in *Pseudomonas maltophilia*, inactivates dicamba by removal of a methyl group, thereby converting dicamba into 3,6-dichlorosalicylic acid (Behrens et al. 2007). Dicamba monoxygenase has been inserted into soybean and cotton to confer resistance to dicamba. From field soybean experiments, Behrens et al. (2007) reported that the soybean cultivars with the DMO gene had acceptable tolerance to dicamba up to 2.8 kg ha^{-1}, which was the highest rate tested. Roundup Ready 2 Xtend® soybean has stacked traits, CP4 EPSPS and DMO, which confers resistance to glyphosate and dicamba, respectively. Herbicide-resistant traits can be used as a weed management strategy.

1.5.2 Dicamba

Dicamba is a synthetic auxin or growth regulator herbicide. Dicamba, chemically known as 3,6-dichloro-2-methoxybenzoic acid or 2-methoxy-3,6-dichlorobenzoic acid, is a benzoic acid and classified as a synthetic auxin herbicide. The synthetic auxin herbicides include phenoxyalkanoic acids, benzoic acids, pyridine derivatives, aromatic carboxylmethyl derivatives and quinoline carboxylic acids (Christoffoleti et al. 2015). There are many different herbicide formulations of dicamba such as dimethylamine salt, diglycolamine salt and dicamba sodium salt (Nishimura et al. 2015). Synthetic auxins are group 4 herbicides that mimic natural auxins such as indole-3-acetic acid (IAA) (Nishimura et al. 2015). Auxins play an important role in cell division, differentiation and elongation which can affect seedling morphology, leaf senescence, and flowering (Cobb and Reade 2010). Auxins can inhibit or stimulate plant growth due to degradation of two transcription factors and a protein complex involved in the cell cycle (Cobb and Reade 2010). Plant death occurs due to unregulated RNA and protein synthesis, increasing cell division and differentiation; leading to breakdown of intercellular membranes, organelles and vascular tissue (Cobb and Reade 2010). With a vapour pressure of 4.5 mPa at 25°C, dicamba is considered a moderately volatile compound (Nishimura et al. 2015). Previous research on dicamba volatility has observed an increase in foliar damage of soybean from 15 to 30°C, indicating temperature is a factor in dicamba volatility (Behrens and Lueschen 1979). Low persistence in the environment, low cost, and little to no toxicity in wildlife and humans has led to widespread use of dicamba (Shaner et al. 2014). Dicamba is a synthetic auxin herbicide that mimics auxins within the plant.

The chemical properties of dicamba affect its absorption, translocation and degradation. Dicamba is absorbed through the roots, stems and leaves and is transported across the plasmalemma by either active transport or passive diffusion similar to IAA (Shaner et al. 2014). Translocation of dicamba is symplastic and apoplastic, meaning it can move through the phloem and xylem, accumulating in the meristematic regions (Shaner et al. 2014). Since dicamba is, in part, xylem mobile, it can be absorbed through the roots and translocated acropetally to above ground plant tissues (Shaner et al. 2014). Dicamba is metabolized more rapidly in tolerant species like grasses (Shaner et al. 2014). Symptomology of dicamba injury on susceptible plants includes epinasty (ie., twisting and curling of the stems and petioles), stem swelling and elongation, and leaf cupping (Shaner et al. 2014). New plant growth has a higher concentration of IAA; therefore, injury is most pronounced in the meristematic regions (Shaner et al. 2014).

The half-life of dicamba in the soil is estimated to be 14 days (Shaner et al. 2014). Dicamba is primarily degraded by microbial degradation with very little photo decomposition (Shaner et al. 2014). Dicamba is degraded to 3,6-dichlorosalicylic acid then 2,5-dihydroxy-3,6-dichlorobenzoic acid, two compounds with no herbicidal activity (Menasseri et al. 2004). The half-life of dicamba is dependent on soil conditions since it is soil mobile. In aerobic conditions the half-life decreased to 6 days, while under anaerobic conditions it increase to 141 days (Nishimura 2015). Soil temperature is also a factor in dicamba degradation. Comfort et al. (1992) observed a half-life of 151, 38 and 23.5 days when the temperature was 12, 20 and 28°C, respectively. This is further corroborated by Ochsner (2006) who observed that an increase in temperature and water content of soil decreased the half-life of dicamba in the soil. Voos and Groffman (1997) observed a positive correlation between organic carbon and dicamba degradation. Dicamba is a synthetic auxin, systemic herbicide with a relatively short half-life in the soil.

1.5.3 Roundup Xtend™ and Xtendimax™

Roundup Xtend™ and Xtendimax™ are two dicamba herbicide formulations that are sold by Monsanto for use on Roundup Ready 2 Xtend® soybean and Bollgard II® XtendFlex® cotton. Xtendimax™ is a diglycolamine salt formulation of dicamba at a concentration of 350 g ae L^{-1}. Roundup Xtend™ is a two-to-one formulation of glyphosate and dicamba. The formulation contains 240 g ae L^{-1} of glyphosate and 120 g ae L^{-1} of dicamba (Anonymous 2013).

On Roundup Ready 2 Xtend® soybean cultivars, these two herbicide formulations can be sprayed PP, PRE and POST up to early flower (R1). Xtendimax™ can also be applied on spring and winter wheat, spring barley, oats, spring rye, field corn, pasture and non-crop areas (Anonymous 2013). Xtendimax™ is registered for use on a wide range of broadleaf weed species. Roundup Xtend™ can be applied to corn hybrids containing the Roundup Ready 2® technology since corn is tolerant to dicamba. Roundup Xtend™ is registered for use on a wide range of grass and broadleaf weed species. Roundup Xtend™ and Xtendimax™ can be applied to Roundup Ready 2 Xtend® soybean in Canada.

A concern with the use of dicamba for weed management is off-site movement due to physical or vapour drift. Physical drift is the movement of droplets by wind onto non-target species and vapour drift is the movement of chemical after it changes from a liquid to a gas (Cobb and Reade 2010). In order to minimize off-site injury from dicamba there are a plethora of application recommendations and regulations, along with improvements in herbicide formulation. The chemical formulation of dicamba in Xtendimax™ and Roundup Xtend™ reduces volatility through VaporGrip® Technology by reducing the association of hydrogen and dicamba molecules, preventing the formation of dicamba acid, which is a highly volatile compound (Anonymous 2016). The herbicide label requires the applicator to adhere to specific requirements for nozzle selection, spray volume, maximum boom height, travel speed and specific weather conditions (Anonymous 2015). These requirements include using nozzles that create very coarse to ultra-coarse spray droplets, a minimum carrier volume of 100 L ha^{-1}, boom height of up to 51 cm above the ground, crop or weed canopy, equipment ground speed of <25 km h^{-1}, and spraying when winds speeds are between 3 and 16 km h^{-1} (Annonymous 2015). Through changes in formulation and stringent application requirements, off-site injury from dicamba can be minimized.

1.5.4 Control of Glyphosate-Resistant Canada Fleabane in Soybean

Consistent control of GR Canada fleabane in soybean is difficult. Prior to the evolution of GR Canada fleabane, glyphosate (900 g ae ha^{-1}) provided acceptable control, but GR Canada fleabane has become a widespread issue (VanGessel 2001, Byker et al. 2013a). In a study conducted in Missouri, glyphosate at 840 and 1680 g ae ha^{-1} controlled GR Canada fleabane 33 and 64%, respectively (Bolte et al. 2015). Control options of GR Canada fleabane POST in

soybean are limited, especially in Canada, and control tends to be inconsistent (Bolte et al. 2015; OMAFRA 2016; Byker et al. 2013d). For instance, in a study reported by Byker et al. (2013d), POST applications of glyphosate plus fomesafen (900 + 240 g ae ha^{-1}), glyphosate plus acifluorfen (900 + 600 g ae ha^{-1}) and glyphosate plus chlorimuron-ethyl (900 + 9 g ae ha^{-1}) controlled GR Canada fleabane 19 to 24, 19 to 22 and 44 to 58%, respectively, 4 weeks after application (WAA). Glyphosate-resistant Canada fleabane is a troublesome weed to control.

Control of GR Canada fleabane is affected by plant height, biotype, density and herbicide. McCauley and Young (2016) found that dicamba (280 g ai ha^{-1}) applied POST provided acceptable control of GR Canada fleabane up to 24 cm in height. Halauxifen-methyl (5 g ae ha^{-1}) applied POST controlled GR Canada fleabane up to 19 cm (McCauley and Young 2016). Glyphosate (900 g ae ha^{-1}) plus saflufenacil (25 g ae ha^{-1}) applied preplant to soybean controlled GR Canada fleabane 88 to 100% 4 WAA (Byker et al. 2013c). Eubank et al. (2008) observed 90 and 99% control of Canada fleabane with glyphosate plus dicamba (860 + 280 g ha^{-1}) and glyphosate plus 2,4-D (860 + 840 g ae ha^{-1}), respectively, 4 WAA. Paraquat plus dicamba (840 + 280 g ae ha^{-1}) and paraquat plus metribuzin controlled GR Canada fleabane 78 and 94%, respectively at 4 WAA with no difference in soybean yield (Byker et al. 2013c). Glyphosate plus 2,4-D ester (900 + 500 g ae ha^{-1}), glyphosate plus paraquat (900 + 1100 g ae ha^{-1}), glyphosate plus saflufenacil (900 + 25 g ae ha^{-1}), and glyphosate plus saflufenacil/dimenthenamid-*P* (900 + 245 g ae ha^{-1}) applied PP, controlled GR Canada fleabane 78 to 92, 59 to 95, 88 to 100, and 87 to 100%, respectively (Byker et al. 2013c). Paraquat and saflufenacil controlled GR Canada fleabane 80 and 95%, respectively, 3 WAA (Bolte et al. 2015). An acceptable level of Canada fleabane control has been achieved with different herbicides.

Control of GR Canada fleabane has been extensively researched. Glufosinate (590 g ai ha^{-1}) applied preplant (PP) controlled GR Canada fleabane 94% at 4 WAA; the addition of a tankmix partner did not improve control or soybean yield (Byker et al. 2013c). This is in contrast to research by Bolte et al. (2015) who reported that glufosinate (594 g ai ha^{-1}) controlled GR Canada fleabane 65%. Bolte et al. (2015) observed a decrease in GR Canada fleabane control with saflufenacil, paraquat, dicamba, and 2,4-D when plant density increased from 57 plants m^{-2} to 124 plants m^{-2}. The variable control with glufosinate may be due to weed size at application, weed density, air temperature or time of day. Dicamba at 280 g ae ha^{-1} (diglycolamine or dimethylamine salt) controlled 98% of GR Canada fleabane when plants were less than 30 cm

tall (Kruger et al. 2010). Spaunhorst et al. (2013), and Johnson et al. (2010) both observed an increase in GR Canada fleabane control with the addition of glyphosate to dicamba. In contrast, Flessner et al. (2015) did not observe an increase in control with the addition of glyphosate to dicamba. The reason for the contrasting results with the addition of glyphosate to dicamba, may be a reflection of the proportion of GR biotypes in the fields where the studies were conducted. Dicamba plus glyphosate (420 + 840 g ae ha^{-1}) applied POST at heights of 10 to 20 cm, 20 to 30 cm and 30 to 40 cm controlled GR Canada fleabane 46 to 93, 68 to 76, and 54 to 60%, respectively, in 2013 and 80 to 84, 75 to 87 and 67 to 77%, respectively in 2014 at 4 WAA (Bolte et al. 2015). Control was reduced when Canada fleabane was ≥20 cm in height. Research indicates that chemical control of GR Canada fleabane with dicamba and glufosinate in soybean is variable, depending on plant size or density, and temperature at time of application.

In Roundup Ready 2 Xtend® soybean, when the rate of dicamba applied PP was increased from 300 to 600 g ae ha^{-1}, there was an increase in GR Canada fleabane control (Byker et al. 2013b). Dicamba (280 g ae ha^{-1}) applied PRE in dicamba-resistant soybean controlled GR Canada fleabane 90% (Johnson et al. 2010). Flessner et al. (2015) observed that GR Canada fleabane that was not controlled 6 WAA produced seed and I_{50} values of dicamba were 32 to 237 g ai ha^{-1}. In general, dicamba has shown to be effective in controlling GR Canada fleabane in Roundup Ready 2 Xtend® soybean.

1.5.5 Control of Glyphosate-Resistant Waterhemp in Soybean

The control of GR waterhemp in soybean is challenging mainly because it emerges throughout the growing season; therefore, herbicides with longer residual activity are predicted to be more efficacious. Flumioxazin reduced waterhemp emergence by 87 and 99% after 0.13 and 1 cm of rainfall, respectively (Luke and Smeda 2016). Dicamba applied at 140 and 1120 g ae ha^{-1} reduced waterhemp emergence by 17 and 69%, respectively, 18 days after application (Scott et al. 2016). In addition, after 0 and 1 cm of rainfall, emergence of waterhemp was reduced by 58 and 84%, respectively, at the high rate of dicamba (1120 g ae ha^{-1}). Rainfall is important to dissolve dicamba in the soil water solution so that it can be absorbed by developing weed seedlings. Pyroxasulfone (178 g ai ha^{-1}) applied PRE provided greater control of GR waterhemp than *S*-metolachlor (1792 g ai ha^{-1}), acetochlor (1817 g ai ha^{-1}), atrazine (1680 g ai ha^{-1}) or metribuzin (426 g ai ha^{-1}) in a study completed by Fleitz et al. (2016). In the above study, they

observed that herbicide programs with 4 different herbicide sites-of-action provided 98% control while herbicide programs with 3 sites-of-action provided 64%.

Two-pass herbicide programs of a PRE followed by (fb) POST provided 77% control of GR and PPO-resistant waterhemp and Palmer amaranth while PRE or POST alone provided 29 and 62% control, respectively (Fleitz et al. 2016). Behnken et al. (2016) observed that *S*-metolachlor, dimethenamid-*P* and acetochlor applied PRE controlled GR waterhemp 81, 71 and 62%, respectively; while a two-pass program (PRE fb POST) controlled GR waterhemp 95, 94 and 90%, respectively, in 2015. Into the last week of September in 2016, the same PRE herbicides controlled GR waterhemp 76, 79 and 79%, respectively, and the two-pass programs controlled GR waterhemp 94, 95 and 91%, respectively. Shryver et al. (2017a) found that pyroxasulfone/flumioxazin (240 g ai ha^{-1}), pyroxasulfone/sulfentrazone (300 g ai ha^{-1}) and *S*-metolachlor/metribuzin (1943 g ai ha^{-1}) controlled GR waterhemp 97, 95 and 93%, respectively at 8 WAA. Both Shryver et al. (2017a) and Sarangi et al. (2017) found that two-pass herbicide programs (PRE fb POST), provided greater GR waterhemp control than an application of a PRE or POST herbicide alone.

Control of GR waterhemp with dicamba and other herbicides has been studied. In a study reported by Schlichenmayer (2013), dicamba (560 g ae ha^{-1}) at one location controlled GR waterhemp >80% when the waterhemp was ≤25 cm in height, while at another location, waterhemp up to 36 cm was controlled. A higher rate of dicamba (840 g ae ha^{-1}) controlled GR waterhemp >90% at both locations up to 25 cm. In the same study, the rate of dicamba required for >90% control of GR waterhemp 5 to 10 cm tall, and 28 to 36 tall, was 560 and 1120 g ae ha^{-1}, respectively. Research by Johnson et al. (2010) observed variable control (15-82%) of GR waterhemp control with dicamba (280 g ae ha^{-1}) applied PP. Dicamba plus glyphosate (560 + 860 g ae ha^{-1}) applied to GR waterhemp that was 7.5, 15 and 30 cm reduced waterhemp biomass 78, 69 and 46%, respectively. When glyphosate and dicamba (840 + 280 g ae ha^{-1}) was applied PRE fb POST, the highest level of GR waterhemp control was observed. A sequential application of glyphosate plus dicamba (860 g ha^{-1} + 560 g ha^{-1}) decreased GR waterhemp biomass if applied <14 days after first application when plants were 7.5 cm tall (Schlichenmayer 2013). In RR2 Xtend® soybean, flumioxazin plus chlorimuron (60 + 20 g ai ha^{-1}) applied PP fb glyphosate + dicamba (860 + 560 g ae ha^{-1}) applied POST to 10 cm escapes controlled GR waterhemp 89% (Spaunhorst et al. 2014). A sequential application of dicamba plus glyphosate

plus acetochlor (560 + 860 + 1300 g ai ha^{-1}) applied PP fb POST controlled GR waterhemp >90% (Spaunhorst et al. 2014). Previous research has observed variable control of GR waterhemp with dicamba.

1.6 Hypotheses and Objectives

Dicamba is a benzoic acid, group 4, synthetic auxin herbicide that has activity on broadleaf weeds. The commercial release of glyphosate/dicamba-resistant soybean cultivars allows for dicamba to be sprayed PP, PRE and POST. Research is needed to evaluate the efficacy of dicamba for the control of GR Canada fleabane and waterhemp in Ontario. In addition to delaying the onset of dicamba-resistant Canada fleabane and waterhemp, weed management programs need to be developed that utilize multiple sites-of-action.

Hypothesis:

1. As height of GR Canada fleabane and waterhemp increases at the time of herbicide application, glyphosate/dicamba efficacy will decrease.
2. Control of GR Canada fleabane and waterhemp will increase as the rate of glyphosate/dicamba is increased.
3. The addition of glyphosate/dicamba, in a tank mixture with another effective site-of-action, will increase the control of GR Canada fleabane and waterhemp.
4. A two-pass program will be more efficacious for the control of GR Canada fleabane and waterhemp than a one-pass PRE or a one-pass POST program.

Objectives:

1. To ascertain the effect of GR Canada fleabane and waterhemp size at the time of application on the efficacy of glyphosate/dicamba.
2. To determine the effect of glyphosate/dicamba rate on the control of GR Canada fleabane and waterhemp.
3. To determine the most efficacious glyphosate/dicamba tank-mixtures for the control of GR Canada fleabane and waterhemp.
4. To determine the relative efficacy of one- and two-pass weed control programs for the control of GR waterhemp in glyphosate/dicamba-resistant soybean.

Chapter 2: Control of glyphosate-resistant waterhemp with two-pass weed control strategies in glyphosate/dicamba-resistant soybean

2.1 Abstract

Waterhemp is a small-seeded, dioecious, broadleaf weed that emerges throughout the growing season. If left uncontrolled, waterhemp interference can reduce soybean yield up to 73%. Glyphosate-resistant (GR) waterhemp was first discovered in one county in Ontario in 2014; as of 2017, it has been found in two other counties. Glyphosate/dicamba-resistant soybean can be sprayed with glyphosate and/or dicamba preplant (PP), preemergence (PRE) and/or postemergence (POST). The objective of this study was to determine the control of GR waterhemp in glyphosate/dicamba-resistant soybean with PRE residual herbicides, glyphosate/dicamba applied POST or a two-pass program of a PRE residual herbicide followed by glyphosate/dicamba applied POST. At 8 weeks after application (WAA), pyroxasulfone (150 g ai ha^{-1}), *S*-metolachlor/metribuzin (1943 g ai ha^{-1}), pyroxasulfone/sulfentrazone (300 g ai ha^{-1}) and flumioxazin/pyroxasulfone (240 g ai ha^{-1}), applied PRE, resulted in 71, 85, 82 and 90% GR waterhemp control, respectively. The same PRE herbicides, followed by glyphosate/dicamba (1800 g ae ha^{-1}) POST, improved control to >96%. This study concludes that a two-pass program of an effective soil applied residual herbicide followed by glyphosate/dicamba POST controlled GR waterhemp in glyphosate/dicamba-resistant soybean.

2.2 Introduction

Glyphosate is a 5-enolypyruvalshikimate 3-phosphate synthase (EPSPS) inhibitor that provides broad-spectrum weed control (Franz et al. 1997). Glyphosate is the most widely used herbicide in the world, and controls susceptible waterhemp biotypes up to 30 cm in height (Hoss et al. 2003). Currently, there are 41 weed species resistant to glyphosate globally, with four glyphosate-resistant (GR) weed species in Ontario (Heap 2018). The weed species resistant to glyphosate in Ontario are waterhemp [(*Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea and Tardif], Canada fleabane (*Conyza canadensis* L. Conq.), common ragweed (*Ambrosia artemisiifolia* L.) and giant ragweed (*Ambrosia trifida* L.) (Costea and Tardif 2003; Heap 2017). Glyphosate-resistant weeds are prevalent in southern Ontario, and a long-term,

diversified, integrated weed management strategies need to be developed for the control of GR weeds.

Glyphosate-resistant waterhemp was first discovered in Lambton County, Ontario in 2014 (Schryver et al. 2017a). By 2017, GR waterhemp had also been found in Essex County and Chatham-Kent County (Schryver et al. 2017a). All waterhemp populations surveyed in Ontario are resistant to Group 2 herbicides [acetolactate synthase (ALS) inhibitors], and frequently Group 5 herbicides [photosystem II (PSII) inhibitors]; populations with multiple-sites of resistance decreases the number of herbicides available for controlling this weed species (Schryver et al. 2017a). Globally, waterhemp has been found resistant to six different sites-of-action: groups 2, 4, 5, 9, 14 and 27 (Heap 2017). Herbicide resistance to five sites-of-action in one waterhemp biotype has been confirmed (Heap 2017). This limits the efficacious herbicides for waterhemp control in some populations.

Waterhemp is a dioecious weed species in the Amaranthus genus that can produce up to 4.8 million seeds per plant when grown in the absence of competition (Hartzler et al. 2004). In the presence of competition, one study reported waterhemp produced an average of 309,000 seeds per plant when it emerged at the time of soybean planting. Waterhemp has been observed to emerge from mid-May until the end of October in Ontario (Vyn et al. 2007; Schryver et al. 2017b). There is a decrease in waterhemp germination and emergence with an increase in row shading; therefore, herbicides that provide residual control until canopy closure are important for waterhemp control (Steckel et al. 2003). This highly competitive weed is a major issue in crop production, and when left uncontrolled, one study found it to decrease soybean yield by up to 73% (Vyn et al. 2007).

Schryver et al. (2017c) studied the efficacy of numerous preemergence (PRE) herbicides for the control of waterhemp in soybean. The most efficacious herbicides were pyroxasulfone (150 g ai ha^{-1}), *S*-metolachlor/metribuzin (1943 g ai ha^{-1}), pyroxasulfone/sulfentrazone (300 g ai ha^{-1}) and pyroxasulfone/flumioxazin (240 g ai ha^{-1}) which provided 87, 93, 95, and 97% control, respectively, 8 weeks after application (WAA). Additionally, the authors observed a two-pass herbicide programs of pyroxasulfone (150 g ai ha^{-1}) or *S*-metolachlor (1600 g ai ha^{-1}) applied PRE followed by (fb) fomesafen (240 g ai ha^{-1}) or acifluorfen (600 g ai ha^{-1}) applied postemergence (POST) provided 97 to 98% control (Schryver et al. 2017b). Meyer et al. (2016) found that dicamba + *S*-metolachlor + metribuzin (1120 + 1068 + 420 g ae/ai ha^{-1}),

pyroxasulfone (179 g ai ha^{-1}), and pyroxasulfone + flumioxazin (70 + 89 g ai ha^{-1}) controlled GR waterhemp 96, 96 and 97%, respectively, 4 WAA, in the absence of crop competition. At 8 WAA, pyroxasulfone (89 - 179 g ai ha^{-1}) controlled GR waterhemp 78% (Hay et al. 2017). Sarangi et al. (2017) reported an increase in control and decrease in density and biomass of waterhemp with a PRE fb POST program compared to a two-pass POST program. Sequential applications of glyphosate (560 - 860 g ae ha^{-1}) + dicamba (280 - 860 g ae ha^{-1}) applied 4 to 7 days apart controlled 7.5 cm tall GR waterhemp 72% (Spaunhorst and Bradley 2013). In general, two-pass herbicide programs in past research have provided more consistent control of GR waterhemp.

Glyphosate/dicamba-resistant soybean (Roundup Ready 2 Xtend® soybean) is a new biotechnology trait available in Canada that contains transgenes conferring resistance to glyphosate and dicamba. This technology allows for glyphosate and dicamba to be applied preplant (PP), PRE and POST to the soybean crop. In 2017, dicamba is sold alone as Engenia™, Fexapan™ or Xtendimax™, or as a premix of glyphosate/dicamba under the trade name Roundup Xtend™. New technologies may be important for the control of GR weeds, as they allow for the use of additional modes-of-action for the control of GR biotypes.

The objective of this study was to evaluate PRE herbicides, POST applications of glyphosate/dicamba, sequential POST applications of glyphosate/dicamba, and a PRE fb POST program with glyphosate/dicamba applied POST for the control of GR waterhemp in glyphosate/dicamba-resistant soybean. It is hypothesized that the two-pass programs of a PRE residual herbicide fb glyphosate/dicamba POST will provide the best control of GR waterhemp. This research will add to the existing knowledge on the control of GR waterhemp in Ontario, providing additional options for growers within the province to achieve acceptable control of GR waterhemp.

2.3 Materials and Methods

This study was completed over a two-year period (2016, 2017) at three locations in southwestern Ontario for a total of six site-years. Two sites were on Walpole Island, Ontario (42.592650, -82.476869) and the third site was near Cottam, Ontario (42.128549, -82.744135). Each trial consisted of 13 treatments that were arranged in a randomized complete block design with four replications; treatments included a weedy and weed-free control. The six one-pass

programs consisted of four different herbicides with residual activity applied PRE and two POST application timings of glyphosate/dicamba. The PRE herbicides were pyroxasulfone (150 g ai ha^{-1}), *S*-metolachlor/metribuzin (1943 g ai ha^{-1}), pyroxasulfone/sulfentrazone (300 g ai ha^{-1}), and pyroxasulfone/flumioxazin (240 g ai ha^{-1}). The POST treatments were glyphosate/dicamba (1800 g ae ha^{-1}) applied early POST (EPOST), or glyphosate/dicamba (1800 g ae ha^{-1}) applied late POST (LPOST). The five two-pass programs consisted of glyphosate/dicamba applied EPOST fb glyphosate/dicamba LPOST, and the above residual herbicides applied PRE fb glyphosate/dicamba LPOST. The PRE herbicides were applied after seeding soybean and before crop emergence; the EPOST herbicide applications were when waterhemp plants were up to 10 cm in height and the LPOST herbicide applications were when there were up to 10 cm waterhemp escapes in the pyroxasulfone treatment. All treatments were applied with a CO_2 pressurized backpack sprayer calibrated to deliver 200 L ha^{-1} at 275 kPa through a 1.5 m boom fitted with four TurboTeeJet Induction (TTI) nozzles spaced 50 cm apart (TeeJet Technologies, Wheaton, IL) resulting in a 2.0 m spray width. In-crop cover sprays of glyphosate (900 g ae ha^{-1}) were applied as needed to remove the confounding effect of other weed species in the experimental area.

Glyphosate was applied PP at the Walpole sites to control emerged weeds prior to seedbed preparation. Seedbed preparation at all sites included secondary tillage with a tandem disc followed by a cultivator. Soybean cultivars DKB14-41 and DKB10-01 (Monsanto, St. Louis, MO) were seeded in 2016 and 2017, respectively, at 400 000 seeds ha^{-1} to a depth of 4 cm. Plots were 2.25 m wide (3 soybean rows spaced 0.76 m apart) by 8 m in length. Trial location, year, seeding date, herbicide application dates and soybean growth stage are presented in Table 2.1.

Soybean injury and weed control assessments were completed on the same day as the LPOST application. Crop injury ratings were taken at 2 and 4 weeks after the LPOST application of glyphosate/dicamba. Injury was rated on a scale of 0 to 100, where 0% was no injury and 100% was soybean death. Visible weed control assessments were completed 2, 4, 8 and 12 weeks after application (WAA) of glyphosate/dicamba applied LPOST. Control at 2 WAA was from five site-years instead of six due to human error, and control at 12 WAA was from four site-years since soybean harvest occurred before the 12 WAA rating. Data from glyphosate/dicamba applied LPOST was from 5 site-years, due to waterhemp plants being too

tall at time of application for reliable weed control data to be determined. Visible weed control was rated on a scale of 0 to 100%, where 0 was no control and 100 was complete control. Waterhemp density and biomass measurements were determined 8 WAA and soybean yield was obtained at maturity. Waterhemp density was measured by counting the number of plants and removing the aboveground biomass in two, 0.25 m^{-2} subsamples within each plot. Plants samples were dried in a kiln at 60ºC for two weeks then weighed. In 2017, soybean yield was measured by harvesting two rows of each plot with an Almaco combine (Almaco, Nevada, IA). In 2016, soybean yield was measured from a two 1 m subsamples from two rows in the plot and threshed using a stationary Almaco thresher. Moisture of soybean seed was adjusted to 14.5% before analysis.

Statistical analysis was performed using the GLIMMIX procedure in SAS (Ver. 9.4, SAS Institute Inc., Cary, NC). The fixed effect was herbicide treatment, and the random effects were environment (combination of year and location) and block. Herbicide treatment means were separated using the Fisher's protected LSD test and adjusted using Tukey-Kramer. Alpha value was set at p=0.05. The weedy and weed-free control were removed for analysis of visible weed control data; the weed-free control was removed from the waterhemp biomass and density data. PROC UNIVARIATE was used to test residuals for a normal distribution, errors independent of one another and homogeneity. As a result, an arcsine transformation was fit to all visible weed control data and a lognormal distribution with the identity link was fit for waterhemp density and biomass data. Yield data were not transformed. For presentation purposes, all transformed means were back-transformed.

2.4 Results

2.4.1 Soybean Injury

There was no significant soybean injury (<5%) in this study (data not presented).

2.4.2 Waterhemp Control

The PRE herbicides pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, and pyroxasulfone/flumioxazin controlled GR waterhemp 95 to 99% prior to the LPOST application of glyphosate/dicamba. All PRE herbicides provided similar

GR waterhemp control. Glyphosate/dicamba (1800 g ae ha^{-1}) applied EPOST, controlled GR waterhemp by 70%, which was less than the PRE herbicides used alone (Table 2.2).

At 2 WAA, pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, and pyroxasulfone/flumioxazin applied PRE controlled GR waterhemp by 86, 90, 90 and 91%, respectively. Glyphosate/dicamba applied EPOST, LPOST and EPOST fb LPOST provided 85, 23 and 89% control of GR waterhemp, respectively. The sequential application of a PRE herbicide fb glyphosate/dicamba applied LPOST resulted in in the same level of GR waterhemp control as the PRE herbicide applied alone (Table 2.3).

At 4 WAA, the PRE herbicides used alone provided 85 to 95% visible control of GR waterhemp, depending on the PRE herbicide. Glyphosate/dicamba applied EPOST, LPOST and EPOST fb LPOST provided 86, 65 and 97% visible control of GR waterhemp, respectively. All two-pass programs of a PRE herbicide fb glyphosate/dicamba LPOST provided ≥97% visible control of GR waterhemp, and were more efficacious than the corresponding PRE treatment alone (Table 2.3).

At 8 WAA, visible control of GR waterhemp with the PRE herbicides alone declined to 71 to 90% depending on the PRE herbicide. Glyphosate/dicamba applied EPOST, LPOST and EPOST fb LPOST provided 77, 79 and 95% control of GR waterhemp, respectively (Table 2.3). The two-pass programs of a PRE herbicide fb glyphosate/dicamba LPOST controlled GR waterhemp ≥96% and provided greater control than the PRE herbicides alone and glyphosate/dicamba applied LPOST. There was no difference in GR waterhemp control among the two-pass programs evaluated.

At 12 WAA, visible control of GR waterhemp among the PRE herbicides alone declined to 51 to 85%; the decline of control with time may be attributed to the extended waterhemp emergence pattern (Vyn et al. 2007; Schryver et al. 2017b). Glyphosate/dicamba applied EPOST, LPOST and EPOST fb LPOST provided 71, 69 and 91% control of GR waterhemp, respectively. The two-pass programs consisting of a PRE herbicide followed by an application of glyphosate/dicamba LPOST controlled GR waterhemp ≥93% and provided greater control than the PRE herbicides alone and glyphosate/dicamba applied LPOST. The less than acceptable full season control using PRE herbicides alone highlights the finding that a two-pass program is necessary for controlling GR waterhemp through the growing season (Sarangi et al. 2017). There was no difference in GR waterhemp control among the two-pass programs evaluated.

2.4.3 Density and Biomass

Waterhemp density and biomass of all herbicide treatments were less than the weedy control. Pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone, and pyroxasulfone/flumioxazin reduced GR waterhemp density and biomass by ≥96 and ≥91%, respectively, compared to the weedy control. Glyphosate/dicamba applied EPOST, LPOST, and EPOST fb LPOST reduced GR waterhemp density by 89, 68, and 98%, respectively, and reduced waterhemp biomass by 92, 79 and 99%, respectively. The two-pass treatments consisting of a PRE residual herbicide fb glyphosate/dicamba applied LPOST reduced GR waterhemp density and biomass, ≥99 and ≥98%, respectively. Pyroxasulfone applied PRE fb glyphosate/dicamba LPOST was not as efficacious as the other two-pass programs evaluated.

2.4.4 Soybean Yield

Waterhemp interference in the weedy control reduced soybean yield 48% in this study. All treatments with herbicide(s) resulted in soybean yields that were similar to the weed-free control.

2.5 Discussion and Conclusion

Visible control of GR waterhemp with the four PRE herbicides evaluated declined from ≥95 at the time of the LPOST application to 51 to 85% at 12 WAA. Nonetheless, the level of visible control of GR waterhemp provided by some herbicide treatments depended on the field site. At two of the sites, the PRE herbicides used alone provided 100% control throughout the season; therefore, the LPOST application of glyphosate/dicamba was not necessary.

The LPOST application was applied prior to soybean canopy closure; however, the waterhemp escapes in the pyroxasulfone treatment had not reached 10 cm in height at the time of application. This decline in control supports the need for effective two-pass weed control programs for the control of GR waterhemp. The two-pass programs of a residual herbicide applied PRE fb glyphosate/dicamba applied LPOST controlled GR waterhemp ≥93% at 12 WAA, reduced density and biomass ≥98%, and resulted in soybean yields that were equivalent to the weed-free control. Waterhemp control with a single application of glyphosate/dicamba was unacceptable; glyphosate/dicamba applied EPOST or LPOST controlled GR waterhemp 69 and 71%, respectively at 12 WAA, reduced density 68 and 89% and biomass 79 and 92%,

respectively. Although the sequential application of glyphosate/dicamba provided >90% control, this weed management program is not recommended due to increased selection intensity for dicamba-resistant waterhemp.

Waterhemp is a highly prolific seed producer and even some escapes can add seed to the seedbank; therefore, season-long control is required to limit waterhemp seed return to the seedbank. The EPOST application of glyphosate/dicamba was more efficacious at 2 and 4 WAA than LPOST. At 8 and 12 WAA, there was no difference in control; however, there was a difference in density. It is important to note that the waterhemp populations at each field site were not 100% resistant to glyphosate; therefore, glyphosate was an effective mode-of-action to some degree. Crop competition is important to reduce the germination and establishment of waterhemp, therefore, narrow soybean row widths, competitive cultivars and crop rotation should be considered in a long-term diversified weed management program.

Two-pass herbicides programs provided greater than 91% control of GR waterhemp in this study. Although all two-pass systems were efficacious and there was no difference in yield, it is important to choose the most efficacious herbicide program to reduce weed seed return to the soil while also ensuring that multiple herbicide modes-of-action are used over time.

2.6 Tables

Table 2.1 Trial location, year, seeding date, herbicide application dates, and soybean growth stage at application in Ontario in 2016 and 2017.

Location	Year	Seeding date	PRE application date	EPOST application date	Soybean growth stage	LPOST application	Soybean growth stage
Walpole 1	2016	30-May	03-June	30-June	V3	12-July	V5
	2017	08-June	09-June	02-July	V2	28-July	R3
Walpole 2	2016	23-May	24-May	09-July	V5	17-June	R2
	2017	03-June	07-June	15-July	R2	03-August	R5
Cottam	2016	30-May	01-June	21-June	V2	29-June	V4
	2017	19-May	23-May	24-June	V3	02-July	R2

Abbreviations: PRE, preemergent; EPOST, early POST and LPOST, late POST

Table 2.2 Means for visible waterhemp control before LPOST application in Ontario in 2016 and 2017.

Treatment	Rate (g ai/ae ha^{-1})	Application timing	[ab]Control (%) at LPOST app.
Glyphosate/dicamba	1800	EPOST	70b
Pyroxasulfone	150	PRE	95a
S-metolachlor/metribuzin	1943	PRE	99a
Pyroxasulfone/sulfentrazone	300	PRE	98a
Pyroxasulfone/flumioxazin	240	PRE	99a

Abbreviations: PRE, preemergent; EPOST, early POST; LPOST, late POST; app, application

[a]Means followed by the same letter with a column are not statistically different according to Fisher's Protected LSD (P=0.05).

[b]Visible control estimates based on comparisons made to weedy and weed-free control treatments

Table 2.3 [a]Means for waterhemp visible control, density, biomass and soybean yield in Ontario averaged across six field sites in 2016 and 2017.

Treatment	Rate (g ai/ae ha^{-1})	App. Timing	[b]Visible control (%)				8 WAA		Soybean grain yield (t ha^{-1})
			2 WAA	4 WAA	8 WAA	12 WAA	Waterhemp density (plants m^{-2})	Waterhemp biomass (g m^{-2})	
Weedy control			0	0	0	0	270.0a	288.5a	1.0c
Weed-free control			100	100	100	100	0	0	1.9ab
Pyroxasulfone	150	PRE	86d	85c	71e	51e	10.5d	26.2bc	1.5abc
S-metolachlor/metribuzin	1943	PRE	90bcd	94bc	85cde	75cd	4.0de	18.6bc	1.6ab
Pyroxasulfone/sulfentrazone	300	PRE	90bcd	92bc	82de	69de	4.2de	19.8bc	1.6ab
Pyroxasulfone/flumioxazin	240	PRE	91bcd	95b	90bcd	85bcd	1.8ef	10.5cd	1.3bc
Glyphosate/dicamba	1800	EPOST	85d	86c	77de	71de	30.3c	21.3bc	1.4abc
Glyphosate/dicamba	1800	LPOST	23e	65d	79de	69de	84.9b	61.1b	1.4bc
Glyphosate/dicamba fb	1800	EPOST fb	89cd	97ab	95abc	91abc	4.9de	3.1edf	1.6ab
Glyphosate/dicamba	1800	LPOST							
Pyroxasulfone fb	150	PRE fb	91bcd	97ab	96ab	93ab	3.5e	4.2de	1.6ab
Glyphosate/dicamba	1800	LPOST							
S-metolachlor/metribuzin fb	1943	PRE fb	99a	99a	99a	97ab	0.3f	0.2f	2.0a
Glyphosate/dicamba	1800	LPOST							
Pyroxasulfone/sulfentrazone fb	300	PRE fb	97abc	99a	99a	97ab	0.6f	0.6ef	1.8ab
Glyphosate/dicamba	1800	LPOST							
Pyroxasulfone/flumioxazin fb	240	PRE fb	98ab	99a	99a	98a	0.3f	0.8ef	1.8ab
Glyphosate/dicamba	1800	LPOST							

Abbreviations: PRE, pre-emergent; EPOST, early POST; LPOST, late POST; app, application; WAA, weeks after application
[a]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (P=0.05).
[b]Visible control estimates based on comparisons made to weedy and weed-free control

Chapter 3: Control of Glyphosate-Resistant Waterhemp with Preemergence Herbicides in Glyphosate/Dicamba-Resistant Soybean

3.1 Abstract

Glyphosate-resistant (GR) waterhemp was first discovered in Ontario, Canada in 2014. In previous studies in Ontario, GR waterhemp interference reduced soybean yield up to 73%. Tankmixes of herbicides with multiple modes-of-action are important for delaying the evolution of herbicide resistance. The objective of this study was to evaluate the efficacy of pyroxasulfone (150 g ai ha^{-1}), *S*-metolachlor/metribuzin (1943 g ai ha^{-1}), pyroxasulfone/sulfentrazone (300 g ai ha^{-1}) and pyroxasulfone/flumioxazin (240 g ai ha^{-1}) applied preemergence (PRE) with and without the addition of glyphosate/dicamba (1800 g ae ha^{-1}) for the control of GR waterhemp in soybean. At 8 WAA, glyphosate/dicamba applied PRE controlled GR waterhemp 45%. Pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone and pyroxasulfone/flumioxazin, applied PRE, controlled GR waterhemp 79, 87, 91 and 95%, respectively. At 2, 4, 8 and 12 WAA, the addition of glyphosate/dicamba to the aforementioned PRE herbicides did not improve GR waterhemp control. There was no increase in GR waterhemp control with the addition of glyphosate/dicamba; however, multiple herbicide modes-of-action should be utilized to reduce the selection intensity for herbicide-resistant weeds.

3.2 Introduction

Waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea and Tardif)] is a small-seeded, dioecious weed species in the Amaranthaceae family, and is native to the Great Plains of North America (Costea and Tardif 2003; Costea et al. 2005). When grown in the absence of competition from neighbouring plants, waterhemp can produce up to 4.8 million seeds $plant^{-1}$; however, 309 000 seeds $plant^{-1}$ is a more realistic estimate when grown with soybean (Hartzler et al. 2004). Waterhemp seeds can remain viable in the soil seedbank for years; in one study, 3% of the original seedbank was still viable after 17 years (Burnside et al. 1996). Based on these assumptions, 9 000 seeds from one plant would still be viable in the soil after 17 years.

Waterhemp interference has been shown to reduce soybean yield up to 73% in Ontario (Vyn et al. 2007). Season-long control of waterhemp is challenging, even under competitive crop

canopies. New seedlings can emerge throughout the growing season, which in Ontario is from mid-May until the last week of October (Vyn et al. 2007; Schryver et al. 2017a). In the absence of competition, waterhemp emerging under 40, 60 and 99% artificial shade, had a reduction in seed production of 51, 75 and 99%, respectively (Steckel et al. 2003). The authors also observed that waterhemp seed production per plant was reduced as emergence was delayed through the growing season. Flowering and seed set can occur until the first frost; however, for the late season emerging cohorts, fewer days are required for plants to flower and set seed (Costea et al. 2005; Wu and Owen 2014). Early season weed control and rapid canopy closure are two ways to minimize soybean yield loss due to waterhemp interference.

The first known case of herbicide-resistance in waterhemp was to Group 5 herbicides [photosystem II (PSII) inhibitors] in 1990 (Anderson et al. 1996). Currently, that number has increased to six different sites-of-action, including Group 2 [acetolactate synthase (ALS) inhibitors], Group 4 (synthetic auxins), Group 5 [photosystem II (PSII) inhibitors], Group 9 [5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) inhibitors], Group 14 [protoporphyrinogen oxidase (PPO) inhibitors], and Group 27 [p-hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitors] (Heap 2017a). Waterhemp resistant to multiple sites-of-action was first documented in 1998 to Group 2 and 5 herbicides and up to five-way resistance within one biotype has been found in Missouri (Heap 2017). In Canada, multiple herbicide-resistant waterhemp (Groups 2 and 5) was first reported in Ontario in 2002, and three-way herbicide-resistance (Groups 2, 5 and 9) was confirmed in 2014 (Schryver et al. 2017b; Heap 2018). In addition to an integrated weed management program, herbicide programs containing multiple modes-of-action are essential to reduce the selection pressure for resistance to additional modes-of-action.

Glyphosate can control glyphosate-susceptible waterhemp up to 30 cm in height (Hoss et al. 2003). However, the evolution of herbicide-resistant waterhemp, and specifically glyphosate-resistance (GR), has led to the need for alternative control strategies. One strategy is to apply an efficacious preemergence (PRE) herbicide. Vyn et al. (2007) reported at 4 weeks after application (WAA), the most efficacious herbicides (≥96% waterhemp control) applied PRE included linuron (2250 g ai ha^{-1}), dimethenamid (1250 g ai ha^{-1}), and *S*-metolachlor + metribuzin (1600 + 658 g ai ha^{-1}). Other research found that alachlor (2800 g ai ha^{-1}), flumioxazin (90 g ai ha^{-1}), sulfentrazone (280 g ha^{-1}), and *S*-metolachlor + metribuzin (1540 + 360 g ai ha^{-1}) controlled waterhemp 45, 58, 80 and 80%, respectively (Legleiter et al. 2009). Sweat et al.

(1998) reported that sulfentrazone (350 g ai ha^{-1}), metribuzin (420 g ai ha^{-1}) and metolachlor (1680 g ai ha^{-1}) controlled waterhemp ≥97%. These studies demonstrated that alternative PRE herbicides are effective or partially effective in controlling GR waterhemp in soybean, reducing the reliance of glyphosate.

A strategy to control GR waterhemp, and to expand the number of herbicide options, is to plant a soybean cultivar resistant to multiple herbicides. One of these technologies is glyphosate/dicamba-resistant soybean (Roundup Ready 2 Xtend® soybean). Resistance is conferred by two transgenes, which code for CP4 EPSPS and dicamba monooxygenase (DMO) that confer resistance to glyphosate and dicamba, respectively. The resistance conferred by the CP4 EPSPS and DMO transgenes allow for glyphosate and dicamba to be applied preplant (PP), PRE and postemergence (POST) on Roundup Ready 2 Xtend® soybean. Low volatile dicamba formulations that have been developed for use on glyphosate/dicamba-resistant soybean include Xtendimax™, Fexapan™ and Engenia™. In addition, a premix formulation of glyphosate/dicamba is sold as Roundup Xtend™. The registration of this new technology provides new options for the control of herbicide-resistant weeds and will allow for multiple modes-of-action to be used on weed biotypes that are susceptible to glyphosate and dicamba.

It is hypothesized that the addition of glyphosate/dicamba to several PRE herbicides will provide improved control of GR waterhemp. The objective of this study was to evaluate the efficacy of pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone and pyroxasulfone/flumioxazin applied PRE with and without the addition of glyphosate/dicamba for the control of GR waterhemp. This research is important to assess the efficacy of additional modes-of-action for the control of GR waterhemp in order to delay the evolution of resistance to current effective modes-of-action in Ontario.

3.3 Materials and Methods

This study was conducted at three locations over a two-year period (2016, 2017) for six location-years in southwestern Ontario. In both years, one location was near Cottam, Ontario (42.128549, -82.744135) and the other two were on Walpole Island, Ontario (42.592650, -82.476869). The experiments were arranged in a randomized complete block design (RCBD) consisting of 11 treatments. The treatment list is presented Table 3.1, which included a weedy and weed-free control and 9 herbicide treatments. The herbicides were applied after planting and

before crop emergence (PRE). The weed-free control was maintained with pyroxasulfone/flumioxazin (240 g ai ha^{-1}) + glyphosate/dicamba (1800 g ae ha^{-1}) applied PRE; all weed escapes were removed manually. Glyphosate (1800 g ae ha^{-1}) was applied PP to the entire experimental area on Walpole Island to control emerged weeds. Seedbed preparation consisted of one pass with a tandem disc followed by a cultivator. Soybean was seeded at a rate of 400 000 seed ha^{-1} to a depth of approximately 4 cm. The soybean cultivars were DKB14-41 and DKB10-01 (Monsanto, St. Louis, MO) in 2016 and 2017, respectively. Plots were 2.25 m wide (three soybean rows spaced 0.76 m apart) by 8 m in length.

Soybean injury was assessed 2 and 4 weeks after soybean emergence (WAE) on a scale of 0 to 100%, where 0 was no injury and 100 was soybean death, relative to the weedy control treatment. Visible waterhemp control was assessed at 2, 4, 8 and 12 WAA on a scale of 0 to 100%, where 0 was no control and 100 was complete control when compared to the weedy control treatment. Waterhemp biomass and density were determined at 4 WAA. Waterhemp density was determined by counting the number of plants within two 0.25 m^{-2} subsamples in each plot. Waterhemp biomass was estimated by cutting the plants at the soil surface within the 0.25 m^{-2} subsample, placing them in a paper bag, drying them in a kiln at 60°C for two weeks, and recording the weight. Soybean yield was obtained at maturity. In 2016, a 1 m subsample from two rows within each plot was harvested by hand, and the soybean plants were threshed with a stationary Almaco thresher (Almaco, Nevada, IA). In 2017, two full rows were harvested with an Almaco combine. Seed yield data were adjusted to 14.5% moisture content before analysis.

Statistical analysis was completed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). The fixed effect was the herbicide treatment and the random effects were environment and block. Residuals were assessed using the UNIVARIATE procedure for normality, homogeneity and errors independent of each other. Weedy and weed-free treatments were not included in the analysis for waterhemp control or soybean injury, and the weed-free control was not included in the analysis for waterhemp density and biomass since they were artificially set to 0 or 100% control/injury and 0 plants m^{-2}, respectively. Control data at 2 WAA was fit to a gamma distribution using the logit link. Control data at 4 WAA was fit to a beta distribution and logit link. Control data at 8 WAA was fit to a normal distribution with the identity link and control 12 WAA was fit to a beta distribution utilizing the cumulative complementary log-log link. Data from 2, 4 and 12 WAA were backtransformed in SAS using

the ilink option. Different distributions and links were used to find a model that best fit the data (Bowley 2015). Density, and biomass data were analyzed using a lognormal distribution with the identity link and backtransformed within SAS. Soybean injury and yield data did not need transformation. Treatment means were separated by Fisher's Protected LSD and Tukey-Kramer adjustment with alpha set at P=0.05.

3.4 Results

3.4.1 Soybean Injury

Soybean injury varied among herbicide treatments at the both evaluation dates. At 2 WAE, pyroxasulfone/flumioxazin and *S*-metolachlor/metribuzin caused 13-16 and 21-24% soybean injury, respectively (Table 3.1). Injury was ≤6% for all treatments at 4 WAE. At 4 WAE, soybean injury from pyroxasulfone/flumioxazin was reduced to 5-6% and injury from *S*-metolachlor/metribuzin was 4-6%.

3.4.2 Waterhemp Control

At 2, 4, 8 and 12 WAA, glyphosate/dicamba applied PRE controlled GR waterhemp 79, 77, 45 and 33%, respectively (Table 3.2).

At 2 WAA, a PRE application of pyroxasulfone, pyroxasulfone/sulfentrazone, *S*-metolachlor/metribuzin or pyroxasulfone/flumioxazin controlled GR waterhemp similarly at 86, 92, 94 and 96%, respectively (Table 3.2). Pyroxasulfone was the only treatment that was similar to glyphosate/dicamba. At 4 WAA, pyroxasulfone/flumioxazin provided better control of GR waterhemp than pyroxasulfone. Pyroxasulfone/sulfentrazone, and *S*-metolachlor/metribuzin did not differ in GR waterhemp control from pyroxasulfone or pyroxasulfone/flumioxazin (Table 1.2). At 8 WAA, glyphosate/dicamba, pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone and pyroxasulfone/flumioxazin controlled GR waterhemp 45, 79, 87, 91 and 95%, respectively (Table 1.2). At 12 WAA, pyroxasulfone/flumioxazin controlled GR waterhemp 97%, which was more efficacious than pyroxasulfone or *S*-metolachlor/metribuzin. Pyroxasulfone/flumioxazin was similar to pyroxasulfone/sulfentrazone for the control of GR waterhemp.

At 2, 4, 8 or 12 WAA, there was no increase in GR waterhemp control, with the addition of glyphosate/dicamba to pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone or pyroxasulfone/flumioxazin. At 2 WAA, glyphosate/dicamba + pyroxasulfone, glyphosate/dicamba + *S*-metolachlor/metribuzin, glyphosate/dicamba + pyroxasulfone/sulfentrazone and glyphosate/dicamba + pyroxasulfone/flumioxazin, controlled GR waterhemp 91, 97, 94 and 97%, respectively. Visible control of GR waterhemp was similar to herbicides without the addition of glyphosate/dicamba, although there was a decrease in visible control of 1 to 10%. At 12 WAA, pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone and pyroxasulfone/flumioxazin controlled GR waterhemp 78, 86, 92, and 97, respectively. Comparably, pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone or pyroxasulfone/flumioxazin with the addition of glyphosate/dicamba controlled GR waterhemp 87, 93, 91 and 94%, respectively. A similar trend was observed at 4 and 8 WAA.

3.4.3 Density and Biomass

Glyphosate-resistant waterhemp density and biomass in the weedy control was 169 plants m^{-2} and 157 g m^{-2}, respectively. At 4 WAA, glyphosate/dicamba applied PRE did not reduce GR waterhemp density and biomass relative to the weedy control (Table 3.2). Pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone and pyroxasulfone/flumioxazin alone or tankmixed with glyphosate/dicamba reduced GR waterhemp density and biomass relative to the weedy control.

3.4.4 Soybean yield

Soybean yield in the untreated weedy control was reduced by 52% from GR waterhemp interference. The poor control of GR waterhemp with glyphosate/dicamba applied PRE resulted in a soybean yield loss of 33%. Soybean yields in the remaining herbicide treatments were similar to the weed-free control, due to the reduction in GR waterhemp interference.

3.5 Discussion and Conclusion

Pyroxasulfone/flumioxazin, pyroxasulfone/sulfentrazone and *S*-metolachlor/metribuzin provided the best control of GR waterhemp; pyroxasulfone provided intermediate control and

glyphosate/dicamba was the least efficacious. Sulfentrazone and flumioxazin are two Group 14 herbicides, which have been observed to be efficacious on small-seeded broadleaf weeds (Niekamp and Johnson 2001). The addition of glyphosate/dicamba to pyroxasulfone, *S*-metolachlor/metribuzin, pyroxasulfone/sulfentrazone and pyroxasulfone/flumioxazin did not improve the control of GR waterhemp at any time during the study.

At some locations in this study, the PRE herbicides controlled GR waterhemp 100%. In practice, if the PRE herbicide provides less than 100% waterhemp control, then growers should consider applying a POST herbicide before soybean canopy closure. Other studies have shown that fomesafen, acifluorfen, glyphosate/dicamba, glufosinate and 2,4-D applied POST following a PRE herbicide provided effective control of GR waterhemp (Schryver et al. 2017c; Hedges et al. 2018-submitted).

All of the PRE herbicides evaluated in this study reduced GR waterhemp density and biomass by >95 and 90%, respectively; the exception was glyphosate/dicamba, which reduced the density and biomass 49 and 56%, respectively. Glyphosate provides no residual weed control and dicamba provides short residual control, which explains the poor control observed in this study (Franz et al. 1997; Smith 1973). While short residual control may not be an issue to control biennial, winter annual or summer annual weed species that emerge primarily in the spring, it is an issue for waterhemp since it emerges throughout the growing season in Ontario. Although there was no increase in GR waterhemp control with the addition of glyphosate/dicamba, the use of multiple modes-of-action will delay the evolution of herbicide-resistant weeds (Beckie et al. 2009; Diggle et al. 2003).

All herbicide treatments evaluated, except glyphosate/dicamba, reduced GR waterhemp interference such that the soybean yields were equivalent to the weed-free control; this indicates that there are several good options for managing GR waterhemp. Weed management decision-makers are encouraged to include diverse integrated weed management strategies. In addition to the use of multiple herbicide modes-of-action, reducing the selection intensity for herbicide-resistant weeds should be considered by using multiple crops in the rotation, tillage at strategic points in the crop rotation, competitive hybrids or cultivars, high seeding rates, narrow row widths, proper fertility, cleaning harvest equipment, and control of insect and disease pest (Beckie 2006).

3.6 Tables

Table 3.1 Soybean visible injury at 2 and 4 weeks after application of herbicides in Ontario in 2016 and 2017.

Treatment	Rate (g ai/ae ha^{-1})	[a]Visible injury (%)	
		[b]2 WAE	4 WAE
Weedy control		0	0
Weed-free control		0	0
Glyphosate/dicamba	1800	1.4cd	0.3c
Pyroxasulfone	150	4.3cd	2.2abc
S-metolachlor/metribuzin	1943	16.0ab	3.8abc
Pyroxasulfone/sulfentrazone	300	5.2cd	2.0bc
Pyroxasulfone/flumioxazin	240	23.6a	4.8ab
Pyroxasulfone +	150	6.6bcd	1.6bc
Glyphosate/dicamba	1800		
S-metolachlor/metribuzin +	1943	12.9abc	5.6a
Glyphosate/dicamba	1800		
Pyroxasulfone/sulfentrazone +	300	6.6bcd	1.6bc
Glyphosate/dicamba	1800		
Pyroxasulfone/flumioxazin +	240	21.0a	5.7a
Glyphosate/dicamba	1800		

Abbreviations: WAE, weeks after emergence
[a]Means within each column followed by the same letter are not statistically different according to Fisher's Protected LSD (P=0.05)
[b]Visible injury estimates relative to weedy and weed-free control

Table 3.2 The effect of herbicide treatments on waterhemp control at 2, 4, 8 and 12 WAA, density and biomass at 4 WAA and soybean yield across six location-years.

Treatment	Rate (g ai/ae ha^{-1})	[ab]Visible control (%) 2 WAA	4 WAA	8 WAA	12 WAA	[a]Waterhemp density (plants m^{-2})	[a]Waterhemp biomass (g m^{-2})	[a]Soybean grain yield (t ha^{-1})
Weedy control		0	0	0	0	169a	157a	0.8c
Weed-free control		100	100	100	100	0	0	1.7a
Glyphosate/dicamba	1800	79c	76.7c	45c	33c	86a	69a	1.1bc
Pyroxasulfone	150	86bc	94.1bc	79b	78b	8b	15b	1.4ab
S-metolachlor/metribuzin	1943	94ab	98.5ab	87ab	86b	6b	11b	1.5ab
Pyroxasulfone/sulfentrazone	300	92ab	98.1ab	91ab	93ab	5b	11b	1.6ab
Pyroxasulfone/flumioxazin	240	96ab	99.4a	95a	97a	4b	6b	1.6a
Pyroxasulfone +	150	91ab	98.0ab	84ab	88b	6b	8b	1.6ab
Glyphosate/dicamba	1800							
S-metolachlor/metribuzin +	1943	97a	99.2ab	88ab	93ab	5b	8b	1.4ab
Glyphosate/dicamba	1800							
Pyroxasulfone/sulfentrazone +	300	94ab	99.3a	91ab	92ab	4b	8b	1.7a
Glyphosate/dicamba	1800							
Pyroxasulfone/flumioxazin +	240	97a	99.6a	93a	94ab	4b	6b	1.6a
Glyphosate/dicamba	1800							

Abbreviations: WAA, weeks after application

[a]Means within each column followed by the same letter are not significantly different according to Fisher's Protected LSD (P=0.05)

[b]Visual control estimates based on comparisons made to weedy and weed-free control

Chapter 4: Control of Glyphosate-Resistant Canada Fleabane with Multiple Effective Sites-of-Action in Glyphosate/Dicamba-Resistant Soybean

4.1 Abstract

Canada fleabane is a winter or summer annual that is found throughout North America. Fall-emerged Canada fleabane can fix carbon early in the growing season, giving it a competitive advantage with nearby crop and weed species. Glyphosate-resistant (GR) Canada fleabane was originally found in one county in Ontario, Canada in 2010 and has spread to at least 29 counties within the province by 2016. Previous research with several pre-plant herbicides resulted in variable control of GR Canada fleabane in soybean. The objective of this study was to evaluate the efficacy of glyphosate/dicamba (1800 g ae ha^{-1}) alone or with the addition of a second effective site-of-action for the control of GR Canada fleabane. At 4 WAA, glyphosate/dicamba plus saflufenacil, saflufenacil/dimethenamid-*P*, saflufenacil/imazethapyr, or paraquat controlled GR Canada fleabane 97, 96, 97 and 98%, respectively. All herbicide treatments decreased Canada fleabane density and biomass by 93-99%. When choosing herbicide programs, it is important to consider the use of multiple sites-of-action to decrease the selection pressure for the evolution of herbicide-resistant Canada fleabane.

4.2 Introduction

Canada fleabane (*Conyza canadensis* (L.) Cronq.), also known as horseweed or marestail, is a member of the Asteraceae family and is native to North America (Weaver 2001; Loux et al. 2006). Canada fleabane is a winter or summer annual weed species that produces seeds capable of germinating in the fall after maturation and seed shed due to the absence of seed dormancy (Buhler and Owen 1997). Tozzi and Van Acker (2014) reported that the majority of Canada fleabane emerges in Ontario during the last two weeks of May and a three-week period from the last week in August until the second week of September. Biotypes of Canada fleabane in Ontario can germinate at temperatures as low as 8°C, while biotypes from different areas around the world germinate at higher temperatures (Tozzi et al. 2013). Fall-emerging Canada fleabane are photosynthetically active at low temperatures, and thus are competitive with annual crops seeded in the spring for nutrients, space, water, and light early in the growing season (Main et al. 2006).

In Ontario, Canada fleabane interference caused soybean yield losses of up to 93% when no herbicide was applied (Byker et al. 2013b).

Dispersal of Canada fleabane seed is facilitated by a 2-3 mm pappus, which can efficiently disperse seed hundreds of metres from the source (Royer and Dickenson 1999). Shield et al. (2006) found Canada fleabane seeds in the Planetary Boundary Layer, which is evidence that seed may be dispersed hundreds of kilometers from the parent plant. Canada fleabane can produce up to 200 000 seeds per plant when grown in an environment free from competition from neighbouring plants; Canada fleabane escapes in soybean can produce up to 72 000 seeds per plant (Weaver 2001; Davis and Johnson 2008).

Currently, Canada fleabane is resistant to five known herbicide site-of-actions: Group 2 [acetolactate synthase (ALS) inhibitors], Group 5 and Group 7 [photosystem II (PSII) inhibitors at two different sites-of-action], Group 9 [5-enolpyruvoylshikimate-3-phosphate synthase (EPSPS) inhibitors] and Group 22 [photosystem I electron diverters (PSI) inhibitors] (Heap 2018). Multiple resistance to herbicides with two different sites-of-action have been confirmed in several Canada fleabane biotypes around the world (Heap 2018). Glyphosate-resistant (GR) Canada fleabane was first observed in Essex County, Ontario in 2010 and has since been found in 30 counties in Ontario from the most southern to the most eastern (Budd et al. 2016a). Herbicide resistance is a growing issue around the world with 487 unique cases as of 2017 (Heap 2018). Integrated weed management practices are required to reduce the selection pressure for herbicide-resistant weeds and options include the use of narrow rows, herbicide tankmixes, strategic tillage, cover crops and equipment modifications (Upadhyaya and Blackshaw 2007; Walsh et al. 2013). Herbicides applied together with different sites-of-action delay the evolution of herbicide resistance longer than sequential herbicide applications with different sites- or modes-of-action (Beckie et al. 2009).

Previous research found that residual herbicides applied in the spring provide better control of spring and late-emerging Canada fleabane than herbicides applied in the fall (Loux et al. 2006; Davis et al. 2007). Unacceptable control of Canada fleabane was observed with paraquat (560 g ai ha^{-1}), metribuzin (420 g ai ha^{-1}), saflufenacil/imazethapyr + glyphosate (100 + 900 g ai/ae ha^{-1}), *S*-metolachlor/metribuzin + glyphosate (1943 + 900 g ai/ae ha^{-1}) and saflufenacil/dimethenamid-*P* + glyphosate (245 + 900 g ai/ae ha^{-1}) (Bruce and Kells 1990;

Soltani et al. 2017). Therefore, other herbicides or herbicide combinations should be evaluated for the control of GR Canada fleabane.

Glyphosate/dicamba-resistant soybean (Roundup Ready 2 Xtend® soybean) is one transgenic option growers can use to control GR weeds. Other transgenic options include: glufosinate-resistant crops and glyphosate/2,4-D resistant corn; with more herbicide-resistant hybrids/cultivars projected to come to market within the next decade. Dicamba is a growth regulator herbicide that had to be applied three to four weeks before soybean planting to minimize soybean injury and yield loss (Thompson et al. 2007). Glyphosate/dicamba-resistant soybean contains two transgenes that code for resistance to glyphosate and dicamba, allowing both herbicides to be applied preplant (PP), preemergence (PRE) and POST on the crop. Low volatile formulations of dicamba are required with this technology and are sold as Xtendimax™, Fexapan™, and Engenia™ or with the addition of glyphosate under the trade name Roundup Xtend™.

The objective of this study was to evaluate the efficacy of glyphosate/dicamba with the addition of a second-effective site-of-action for the control of GR Canada fleabane. This research is important to delay the evolution of resistance to additional effective herbicide sites-of-action, sustaining the use of current herbicide sites-of-action for commercial growers in the future.

4.3 Materials and Methods

This study was conducted at four locations in 2016 and three locations in 2017 for a total of seven site-years. Locations were near Harrow, Ontario (42.035582, -82.918173), in 2016 and 2017, near Mull, Ontario (42.401671, -81.991098) in 2016 and 2017, two locations were near Blenheim, Ontario (42.335561, -81.997442) in 2016 and one near Thamesville, Ontario (42.551722, -81.977180) in 2017. All experiments consisted of 10 treatments arranged in a random complete block design (RBCD) with 4 replications. The treatment list included a weedy control, weed-free control and 8 herbicide treatments (Table 1). All herbicides were applied PP when Canada fleabane plants were approximately 10 cm in diameter or height. The weed-free control received a PP application of glyphosate/dicamba (1800 g ae ha^{-1}) + saflufenacil (25 g ai ha^{-1}) + metribuzin (400 g ai ha^{-1}). Any weed escapes in the weed-free control were removed by hand weeding. In season cover sprays of glyphosate (450 g ae ha^{-1}) were applied to the entire experimental area to remove confounding effects of other weed species. Soybean was seeded

with a three row no-till planter at approximately 400 000 seeds per ha^{-1} to a depth of 4 cm. Soybean cultivars DKB14-41 and DKB10-01 (Monsanto, St. Louis, MO), were seeded in 2016 and 2017, respectively. Plots were 2.25 wide (3 soybean rows spaced 0.75 m apart) by 8 m in length with a 2 m walkway between blocks.

Soybean injury was evaluated 2 and 4 weeks after emergence (WAE) on a scale of 0 to 100%, where 0 was no visible injury and 100 was plant death compared to the weedy control. Weed control was evaluated visually at 2, 4, 8 and 12 weeks after application (WAA) on a scale of 0 to 100, where 0 represented no control and 100 was complete GR Canada fleabane control. Canada fleabane density and biomass was determined 4 WAA by counting and cutting the Canada fleabane plants within two, 0.25 m^{-2} quadrats per plot. Plants were cut at the soil surface, placed in a paper bag, dried in a kiln at 60^{o}C for two weeks before the weight was recorded. Soybean was harvested by hand in 2016 by cutting two, 1 m subsamples in a plot and threshing with an Almaco thresher (Almaco, Nevada, IA). In 2017, an Almaco small-plot combine was used to harvest two rows of soybean per plot. Seed moisture was measured at harvest and yields were adjusted to 14.5% moisture content before analysis.

Data were analyzed as a RBCD using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC). Herbicide treatment was the fixed effect, and environment (year x location) and block were the random effects. PROC UNIVARIATE was utilized to assess data for normality, homogeneity and errors independent from one another. All environments were combined for analysis. The weedy and weed-free controls were not included in the analysis of Canada fleabane control, and the weed-free control was not included in the analysis of Canada fleabane density and biomass. Control data at 2 WAA was fit to a beta distribution; the cumulative complementary log-log link was used because the dataset was large (Bowley 2015). Canada fleabane control at 4, 8 and 12 WAA were fit to a normal distribution and identity link. Data from 2 WAA were backtransformed in SAS using the ilink option. The use of different distributions enabled the best-fitting models for analysis. Density and biomass data were analyzed using a lognormal distribution with the identity link and backtransformed within SAS. Yield data were analyzed using a normal distribution. All data were analyzed with a multiple comparison Fisher protected LSD and Tukey-Kramer adjustment, with an accepted significance value of $p=0.05$.

4.4 Results

4.4.1 Soybean Injury

At 2 and 4 WAE, soybean injury was ≤10% at all site-years (data not presented).

4.4.2 Canada Fleabane Control

At 2 WAA, both glyphosate/dicamba applied alone and glyphosate/dicamba + 2,4-D ester were the least efficacious options, controlling 54% of GR Canada fleabane (Table 4.1). Glyphosate/dicamba + metribuzin and glyphosate/dicamba + metribuzin + chlorimuron controlled GR Canada fleabane 68 and 63%, respectively. Glyphosate/dicamba with the addition of either saflufenacil, saflufenacil/dimethenamid-*P*, saflufenacil/imazethapyr or paraquat were the most efficacious herbicide applications and controlled GR Canada fleabane 97-98%.

At 4 WAA, the most efficacious herbicide tankmixes were glyphosate/dicamba with the addition of saflufenacil, saflufenacil/dimethenamid-*P*, saflufenacil/imazethapyr or paraquat, which provided 97, 96, 97, and 98% GR Canada fleabane control, respectively (Table 4.1). Glyphosate/dicamba controlled GR Canada fleabane 87%; there was no increase in control with the addition of 2,4-D ester, metribuzin or metribuzin + chlorimuron. In this study, glyphosate/dicamba + 2,4-D ester was applied PP when GR fleabane plants were up to 10 cm in height and provided 90% of GR Canada fleabane. In contrast, Kruger et al. (2010) reported 97% control of Canada fleabane 7 to 15 cm tall after an application of 2,4-D ester (560 g ae ha^{-1}). Previous research by Waggoner et al. (2011) observed 96 and 65% control of GR Canada fleabane with a PP application of saflufenacil (25 g ai ha^{-1}) at 7 and 30 days after application (DAA), respectively. In the same study, paraquat (702 g ai ha^{-1}) applied PP provided 84 and 70% control of GR Canada fleabane, 7 and 30 DAA, respectively. Less control was observed at approximately 4 WAA with both treatments, which may be due to poor coverage with saflufenacil and paraquat; both are contact herbicides.

At 8 and 12 WAA, glyphosate/dicamba controlled GR Canada fleabane 94 and 92%, respectively (Table 4.1). There was no improvement in GR Canada fleabane control with any of the tankmixes evaluated. At 8 WAA, glyphosate/dicamba + 2,4-D ester provided better control of GR Canada fleabane than glyphosate/dicamba + metribuzin or glyphosate/dicamba + metribuzin + chlorimuron. At 12 WAA, glyphosate/dicamba + paraquat provided better control

of GR Canada fleabane than glyphosate/dicamba + metribuzin + chlorimuron. At 8 WAA, Soltani et al. (2017) observed 70, 77, 68, 61, 91, and 61% control with saflufenacil, saflufenacil/dimethenamid-*P*, saflufenacil/imazethapyr, 2,4-D ester, metribuzin and chlorimuron-ethyl + metribuzin, respectively. In contrast, all treatments in this study controlled GR Canada fleabane 91 to 98%, 8 WAA. Glyphosate/dicamba + metribuzin controlled GR Canada fleabane 92%, which was numerically similar to metribuzin applied alone in previous research by Soltani et al. (2017). Moseley and Hagood (1990) observed similar Canada fleabane control to this study with a PP application chlorimuron + metribuzin (90%), however less control with metribuzin (78%).

4.4.3 Density and Biomass

At 4 WAA, glyphosate/dicamba applied PP reduced GR Canada fleabane density by 95% (Table 4.1). Glyphosate/dicamba + 2,4-D ester or paraquat reduced GR Canada fleabane density by 99%; these were the only tankmixes that reduced GR Canada fleabane density more than glyphosate/dicamba applied alone. In previous research, paraquat reduced Canada fleabane density by 48 to 80% (Eubank et al. 2008).

At 4 WAA, glyphosate/dicamba applied PP reduced GR Canada fleabane biomass 99% (Table 4.1). Glyphosate/dicamba + 2,4-D ester reduced GR Canada fleabane biomass 99.8%, which was the only tankmix that reduced GR Canada fleabane biomass more than glyphosate/dicamba applied alone. Previous research observed an application of 2,4-D ester (560 g ae ha^{-1}) reduced Canada fleabane biomass by 59 to 76% 4 WAA (Kruger et al. 2010). Research by Eubank et al. (2008) observed a 57 to 88% decrease in Canada fleabane biomass with paraquat applied PP.

4.4.4 Soybean Yield

Soybean yield in the weedy control was 67% less than the weed-free control (Table 4.1). Soybean yield was similar to the weed-free control in all the herbicide treatments because of reduced GR Canada fleabane interference. Eubank et al. (2008) observed a 62-97% decrease in yield in the untreated control compared to the most efficacious herbicide treatment. Previous research observed GR Canada fleabane caused a 35-42% reduction in soybean yield when glyphosate (900 g ae ha^{-1}) was applied alone (Byker et al. 2013d).

4.5 Discussion and Conclusion

In previous research by Budd et al. (2016b), glyphosate (900 g ae ha^{-1}) + saflufenacil (25 g ai ha^{-1}) controlled GR Canada fleabane 99% at 4 WAA. In this study, glyphosate/dicamba controlled GR Canada fleabane 54 and 87% at 2 and 4 WAA, respectively, which indicates that dicamba is a slower acting herbicide than saflufenacil. In the same study, glyphosate (900 g ae ha^{-1}) + saflufenacil (25 g ai ha^{-1}) controlled GR Canada fleabane 88% at 8 WAA, while in this study, glyphosate/dicamba controlled GR Canada fleabane 94 and 92% at 8 and 12 WAA, respectively. At 2 and 4 WAA, the addition of saflufenacil, saflufenacil/dimethenamid-*P*, saflufenacil/imazethapyr or paraquat to glyphosate/dicamba improved the control of GR Canada fleabane; however, there was no improvement in GR Canada fleabane control at 8 and 12 WAA with the tankmixes evaluated. This is important since the critical weed-free period for soybean is from V1 to V3 (Green-Tracewicz et al. 2012). The benefit of the addition of a second effective site-of-action to glyphosate/dicamba is important for resistance management, even when an acceptable level of control is achieved.

In previous research, Budd et al. (2016b) found the control of GR Canada fleabane with glyphosate (900 g ae ha^{-1}) + saflufenacil (25 g ai ha^{-1}) was improved with the addition of dicamba at 600 g ae ha^{-1} but there was no improvement when dicamba was added at 300 g ae ha^{-1}. In this study, glyphosate/dicamba was applied at 1800 g ae ha^{-1} (2:1 ratio); this high rate of glyphosate/dicamba may be beneficial based on previous research.

Previous research found that glyphosate with the addition of saflufenacil, saflufenacil/dimethenamid-*P*, saflufenacil/imazethapyr, 2,4-D ester, metribuzin or chloriumuron-ethyl + metribuzin applied at the same rates as in this study controlled GR Canada fleabane 74, 79, 74, 68, 92 and 67% respectively, 4 WAA (Budd et al. 2016b). In this study, tank mixing glyphosate/dicamba with saflufenacil, saflufenacil/dimethenamid-*P*, saflufenacil/imazethapyr, 2,4-D ester, metribuzin or chlorimuron-ethyl + metribuzin controlled GR Canada fleabane 97, 96, 97, 90, 88 and 85%, respectively, 4 WAA. There was a numeric improvement in control 4 WAA with the addition of a second effective site-of-action with the exception of glyphosate/dicamba + metribuzin.

As the season progressed from 2 to 12 WAA, there was a numeric decrease in control of GR Canada fleabane in this study for all treatments with the exception of glyphosate/dicamba + paraquat. Future research should study the difference between herbicides with and without the

addition of glyphosate/dicamba, although there is an additional benefit to a second effective site-of-action. The delay in the evolution of multiple-resistant GR Canada fleabane is essential to ensure the continued use of these herbicides. Although multiple sites-of-action have been mentioned in this study, biotypes can be cross-resistant, meaning that they are resistant to more than one herbicide within the same mode-of-action (Cobb and Reade 2010). In that case, herbicides with multiple modes-of-action may be preferred to reduce selection pressure.

Table 4.1 [a]Means of glyphosate-resistant Canada fleabane control, density, biomass and soybean yield in Ontario in 2016 and 2017.

		[b]GR Canada fleabane control (%)				4 WAA		
Treatment	Rate (g ai/ae ha^{-1})	2 WAA	4 WAA	8 WAA	12 WAA	Density (plants m^{-2})	Biomass (g m^{-2})	Soybean yield (t ha^{-1})
Weedy control		0	0	0	0	113a	161.8a	0.9b
Weed-free control		100	100	100	100	0e	0e	2.7a
Glyphosate/dicamba	1800	54c	87b	94abc	92ab	6bc	2.3bc	2.3a
Glyphosate/dicamba +	1800	97a	97a	94abc	91ab	2bcd	1.2cd	2.5a
Saflufenacil[c]	25							
Glyphosate/dicamba +	1800	98a	96a	94abc	91ab	2cd	1.2cd	2.6a
Saflufenacil/dimethenamid-*P*[c]	245							
Glyphosate/dicamba +	1800	97a	97a	96abc	93ab	2cd	1.1cd	2.5a
Saflufenacil/imazethapyr[c]	100							
Glyphosate/dicamba +	1800	54c	90b	98a	95ab	1de	0.4de	2.3a
2,4-D ester	500							
Glyphosate/dicamba +	1800	68b	88b	92bc	91ab	7b	4.3b	2.6a
Metribuzin	400							
Glyphosate/dicamba +	1800	97a	98a	97ab	97a	1de	0.6cde	2.5a
Paraquat	1100							
Glyphosate/dicamba +	1800	63bc	85b	91c	88b	7b	4.5b	2.4a
Chlorimuron-ethyl +	9							
Metribuzin	412.5							

Abbreviations: WAA, weeks after application; GR, glyphosate-resistant

[a]Means followed by the same letter with a column are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$)

[b]Control estimates based on comparisons made to the weedy control

[c]Merge at a rate of 1 L ha^{-1} was added to the tank

Chapter 5: Influence of Glyphosate/Dicamba Application Rate and Timing on the Control of Glyphosate-Resistant waterhemp in Glyphosate/Dicamba-Resistant Soybean

5.1 Abstract

Glyphosate-resistant (GR) waterhemp was first found in one county in Ontario, Canada in 2014. Since then, it has been found in two additional counties in southwestern Ontario. Glyphosate/dicamba-resistant soybean was first marketed in Canada in 2017, allowing dicamba to be applied pre-plant, preemergence or postemergence. The objective of this study was to determine the effect of glyphosate/dicamba application timing (5, 15 or 25 cm tall waterhemp) and rate (900, 1350 and 1800 g ae ha^{-1}) on GR waterhemp control. There was no interaction between application rate and timing for waterhemp control, density and biomass or soybean yield. There was an effect of application timing on GR waterhemp control. At 2 WAA, glyphosate/dicamba applied to 5, 15 and 25 cm tall plants controlled GR waterhemp 81, 73 and 61%, respectively. The reduced GR waterhemp control with glyphosate/dicamba when applied to waterhemp greater in height is attributed to the slow activity of the herbicide on large weeds. Conversely, at 8 WAA, glyphosate/dicamba applied to 5, 15 and 25 cm tall plants controlled GR waterhemp 61, 68 and 72%, respectively. The improved control with the late application is attributed to the extended emergence pattern of waterhemp with more weeds emerged at the time of application. As the rate of glyphosate/dicamba was increased there was an increase in GR waterhemp control and a decrease in density and biomass. A single POST application of glyphosate/dicamba did not provide commercially acceptable, season-long control of GR waterhemp due to the short residual activity of dicamba and extended emergence pattern of waterhemp.

5.2 Introduction

Herbicides are important for weed control in crop production systems. They are a common choice due to ease, cost, and efficacy (Baylis 2000). Glyphosate is a non-selective, systemic herbicide that can be used as a pre-plant burndown for weed control; however, it has no residual activity (Franz et al. 1997). The introduction of glyphosate-resistant (GR) crops resulted in multiple in-season applications of glyphosate and led to glyphosate becoming the most widely used herbicide globally (Duke and Powles 2008). Repeated use of glyphosate has increased

selection pressure for GR weeds. Currently, there are 41 known GR weed species globally (Heap 2018). Glyphosate-resistant waterhemp [(*Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea and Tardif] was first found in the United States of America in Texas and Kansas in 2006 and Canada in 2014 (Costea and Tardiff 2003; Heap 2018). Glyphosate-resistant waterhemp has been confirmed in three counties in Ontario as of 2017 (Schryver et al. 2017a).

Waterhemp can be distinguished from other Amaranthus species by lanceolate leaves that are smooth and glossy, and a hairless stem (Nordby et al. 2007; Pratt and Clark 2001). Waterhemp has an extended emergence pattern from May until the end of October in Ontario (Vyn et al. 2006; Schryver et al. 2017b). Waterhemp has male and female reproductive structures on separate plants (dioecious), therefore both are needed for populations to establish in a new area (Costea et al. 2005). Waterhemp is a competitive weed that can grow up to 2.5 cm per calendar day (Horak and Loughin 2000). Upon maturity, waterhemp seeds have primary dormancy; there is greater emergence after cold stratification (Leon and Owen 2003). Waterhemp densities of up to 989 plants m^{-2} have been documented in Ontario fields, which have reduced soybean yield up to 73% (Schryver et al. 2017b; Vyn et al. 2007).

At 8 weeks after application (WAA), glyphosate (840 g ae ha^{-1}) controlled glyphosate-susceptible waterhemp 58% when applied to plants 10 cm in height; control of glyphosate-susceptible waterhemp increased to 90% when glyphosate rate was increased to 1120 g ae ha^{-1} and application was delayed to when waterhemp was 15 cm in height (Krausz and Young 2003). Krausz et al. (1996) observed an increase in glyphosate-susceptible waterhemp control with glyphosate as the rate was increased from 560 to 2800 g ae ha^{-1}. Klingman et al. (1992) reported an increase in control of waterhemp when the rate of imazethapyr was increased from 53 to 140 g ai ha^{-1}. Hager et al. (2003) found an increase in waterhemp control when the rate of acifluorfen was increased from 70 to 280 g ai ha^{-1}, lactofen rate was increased from 55 to 218 g ai ha^{-1}, and fomesafen rate was increased from 87 to 350 g ai ha^{-1}. In addition, they reported a decrease in control when the herbicide application timing was delayed from when waterhemp was 5 cm to 10 cm in height. Mayo et al. (1995) observed a decrease in waterhemp control with acifluorfen (420 g ai ha^{-1}) when applied at 30 cm compared to application at 4 -12 cm in height; in contrast there was no decrease in control when lactofen (220 g ai ha^{-1}), chlorimuron (9 g ai ha^{-1}), thifensulfuron (4 g ai ha^{-1}), imazethapyr (70 g ai ha^{-1}) or imazaquin (70 g ai ha^{-1}) was applied to 30 cm compared to 4 -12 cm waterhemp. Robinson et al. (2012) reported an increase in

waterhemp control when 2,4-D was applied at 20 compared to 30 cm in height and an increase in control as 2,4-D rate was increased from 280 to 1120 g ae ha^{-1}. There was no increase in waterhemp control when the aforementioned rates of 2,4-D were added to glyphosate (840 g ae ha^{-1}). In summary, the efficacy of a number of herbicides for the control of waterhemp is influenced by application rate and height of waterhemp at the time of application.

Glyphosate/dicamba-resistant soybean is a herbicide-resistant technology that was introduced in North America in 2017. Dicamba-resistance in glyphosate/dicamba-resistant soybean is conferred by the insertion of a gene that codes for dicamba monoxygenase (DMO) from *Pseudomonas maltophilia* (Behrens et al. 2007). This gene enhances the metabolism of dicamba through the removal of a methyl group, converting dicamba to inactive 3,6-dichlorosalicylic acid (Behrens et al. 2007). Dicamba is a Group 4, synthetic auxin (growth regulator), benzoic acid herbicide (Cobb and Reade 2010). Dicamba provides approximately 2 weeks residual weed control; however, the actual length of residual activity is influenced by soil type, soil organic matter content, rainfall, and weed species sensitivity (Smith 1973; Shaner et al. 2014; Burnside and Lavy 1966). Low-volatile formulations of dicamba were developed in parallel with glyphosate/dicamba resistant soybean to reduce off-site injury to sensitive plants.

The hypothesis for this study was that the rate of glyphosate/dicamba will need to increase as weed height at the time of herbicide application increases for acceptable control of GR waterhemp. Therefore, the objective of this study was to determine the impact of glyphosate/dicamba rates and application timings on the control of GR waterhemp. The results will be used to develop guidelines for optimal glyphosate/dicamba rates and application timings for the control of GR waterhemp in glyphosate/dicamba-resistant soybean.

5.3 Materials and Methods

Six experiments were completed over a two-year period (2016, 2017) in Ontario, Canada. Two experiments were located on Walpole Island (42.592650, -82.476869) and one near Cottam Ontario (42.128549, -82.744135) in each year.

A 3 x 4 factorial experiment was arranged in a randomized complete block design (RBCD) with four replications. Factor one was application timing (when the height of waterhemp was approximately 5, 15 or 25 cm); factor two was glyphosate/dicamba rate (0, 900, 1350 or 1800 g ae ha^{-1}). The seedbed was prepared with one pass of a tandem disc followed by a

second pass with a field cultivator. Soybean cultivars DKB14-41 and DKB10-01 (Monsanto, St. Louis, MO) were planted in 2016 and 2017, respectively, at a rate of 400 000 seeds ha^{-1} to a depth of 4 cm. Each plot was 2.25 m wide (3 soybean rows spaced 75 cm apart) and 8 m in length. Percent visible soybean injury was assessed at 2 and 4 WAA, where 0% was no injury compared to the weedy control and 100% was plant death. Waterhemp control was estimated visually at 2, 4, and 8 weeks after application (WAA), where 0% was no decrease in waterhemp biomass compared to the weedy control and 100% was complete control. Waterhemp density and biomass were determined at 6 WAA. Waterhemp within two 0.25 m^{-2} quadrants was counted, cut at the soil surface, placed in a paper bag, dried in a kiln set to 60°C for two weeks, and then weighed. Soybean was harvested at maturity in 2016 by harvesting a 1 m subsample from 2 rows and threshing in a stationary Almaco threshing machine (Almaco, Nevada, IA). In 2017, two rows of soybean were harvested with a small plot Almaco combine. Soybean weight and moisture content were recorded; seed weights were adjusted to 14.5% moisture content before analysis.

Statistical analysis was completed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). The fixed effect was the herbicide rate-by-application timing combinations, and the random effects were environment and block. In all analyses, residuals were analyzed using the UNIVARIATE procedure to test for normality, homogeneity and errors independence of each other. Weedy controls were not included in the analysis for waterhemp control since they were artificially set to 0% control. Soybean yield and waterhemp control data at 2, 4, and 8 WAA were fit to a normal distribution using the identity link. Waterhemp density and biomass data were analyzed using a lognormal distribution with the identity link and the means were backtransformed for presentation purposes. Waterhemp control and soybean yield data did not need transformation. Treatment means were separated by Fisher's Protected LSD and Tukey-Kramer adjustment with alpha set at P=0.05.

5.4 Results and discussion

5.4.1 Soybean Injury

There was no visible soybean injury detected with the glyphosate/dicamba rates and application timings evaluated (data not presented).

5.4.2 Interactions

There was no interaction between glyphosate/dicamba application timing and rate for waterhemp control, density and biomass or soybean yield; therefore, the simple effects will be presented (Table 5.1).

5.4.3 Application Timing

At 2, 4 and 8 WAA, there was an effect of glyphosate/dicamba rate (900, 1350, 1800 g ae ha^{-1}) on GR waterhemp control. At 2 WAA, was a decrease in GR waterhemp control as application timing was delayed from 5 to 15 to 25 cm GR waterhemp (Table 5.1). The reduced control with the late is application is attributed to slower herbicide activity on weeds with greater biomass at the time of application. At 4 WAA, glyphosate/dicamba provided the same control of GR waterhemp that was 15 and 25 cm in height, but this control was less than when glyphosate/dicamba was applied to 5 cm tall GR waterhemp. At 8 WAA, glyphosate/dicamba was more efficacious when applied to GR waterhemp at 25 cm compared to 5 cm in height, while applications to GR waterhemp that was 15 cm resulted in control that was similar to the 5 and 25 cm treatments. The improved control with the late application can be attributed to the extended emergence pattern of GR waterhemp in Ontario. When glyphosate/dicamba application was delayed until GR waterhemp was 25 cm tall, a greater proportion of the weeds had emerged at the time of application. There was no impact of GR waterhemp height at the time of glyphosate/dicamba application on GR waterhemp density or biomass and soybean yield.

5.4.4 Application Rate

At 2, 4 and 8 WAA there was an increase in GR waterhemp control as the rate of glyphosate/dicamba was increased from 900 to 1350 to 1800 g ae ha^{-1} (Table 5.1). Glyphosate-resistant waterhemp density and biomass decreased with an increase in the rate of glyphosate/dicamba. The weedy control had the highest GR waterhemp density and biomass, and glyphosate/dicamba applied at 1800 g ae ha^{-1} had the lowest GR waterhemp density and biomass. Glyphosate/dicamba applied at 900, 1350 and 1800 g ae ha^{-1} reduced GR waterhemp density by 65, 78 and 86%, redpectively, and biomass by 67, 81 and 90%, respectively. The application of glyphosate/dicamba at 900 g ae ha^{-1} increased soybean yield compared to the

weedy check; there was no difference in soybean yield among the three rates of glyphosate/dicamba evaluated.

5.5 Discussion and Conclusions

The application of glyphosate/dicamba did not cause injury in glyphosate/dicamba-resistant soybean. This was expected since glyphosate/dicamba soybean contains transgenes that confer resistance to both glyphosate and dicamba and acceptable tolerance has been observed up to 2.8 kg ha^{-1} (Behrens et al. 2007).

There was an effect of glyphosate application timing on GR waterhemp control in this study. At 2 WAA, the control of GR waterhemp decreased as the size of GR waterhemp at the time of application increased. The results from this study are consistent with Spaunhorst and Bradley (2013) who found that glyphosate + dicamba (860 + 560 g ae ha^{-1}) applied to GR waterhemp that was at 7.5, 15 and 30 cm in height provided 62, 40 and 30% control, respectively. In this study, the response reversed by 8 WAA, where improved GR waterhemp control was observed with the late application. At 8 WAA, glyphosate/dicamba applied to 5 cm waterhemp decreased to 61%, the reduced control can be attributed to the extended emergence pattern of waterhemp in Ontario and the relatively short residual activity provided by dicamba (Vyn et al. 2006). This is similar to Dalley et al. (2004), who observed that delaying applications of glyphosate reduced weed biomass. Season-long control of GR waterhemp is important to increase ease of harvest and reduce weed seed return to the soil.

In this study, control of GR waterhemp was affected by the rate of glyphosate/dicamba. At 2, 4 and 8 WAA, glyphosate/dicamba applied at 900, 1350 and 1800 g ae ha^{-1} controlled GR waterhemp up to 66, 78 and 87%, respectively. At 2 and 4 WAA, only glyphosate/dicamba (1800 g ae ha^{-1}) provided >80% control of GR waterhemp. At 8 WAA, there was improved control of GR waterhemp with the higher rates of glyphosate/dicamba.

This study concludes that there was no interaction between glyphosate/dicamba application timing and rate. An increase in herbicide rate increased control of GR waterhemp, and early application timings were more efficacious than later application timings early in the growing season but not at the end of the growing season. Sequential applications of glyphosate/dicamba may be needed for full season control of GR waterhemp. However, this strategy is not preferred due to the increased selection pressure for glyphosate + dicamba-

resistant waterhemp. Research on the control of GR waterhemp in narrow row soybean should be conducted to determine if earlier canopy closure will result in improved control. For full season control of GR waterhemp, a two-pass weed control program of an effective soil applied herbicide followed by an effective postemergence may be required.

5.6 Tables

Table 5.1 [a]Interactions between glyphosate/dicamba application timing and rate. Means for glyphosate-resistant waterhemp control, density and biomass and soybean yield for glyphosate/dicamba application timing and herbicide rate from six site-years in 2016 and 2017 in Ontario.

	Waterhemp control (%)					
Main effects	2 WAA	4 WAA	8 WAA	Density (plants m^{-2})	Biomass (g m^{-2})	Yield (t ha^{-1})
Application timing (cm)	*	*	*	NS	NS	NS
5	81a	82a	61b	61	74	1.4
15	73b	77b	68ab	65	71	1.4
25	61c	72b	72a	71	73	1.3
Herbicide rate (g ai ha^{-1})	*	*	*	*	*	*
0	0	0	0	202a	255a	1.0b
900	61c	66c	58c	71b	85b	1.5a
1350	72b	78b	68b	45c	49c	1.5a
1800	82a	87a	77a	28d	26d	1.6a
Timing x rate	NS	NS	NS	NS	NS	NS

Abbreviations: WAA, weeks after application; NS, not significant at a level of P=0.05
[a]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (P=0.05).
[b]Control estimates based on comparisons made to weedy and weed-free control

Chapter 6: Influence of Glyphosate/Dicamba Application Rate and Timing on the Control of Glyphosate-Resistant Canada fleabane in Glyphosate/Dicamba-Resistant Soybean

6.1 Abstract

Dicamba may be an efficacious option for the control of glyphosate-resistant (GR) Canada fleabane in glyphosate/dicamba-resistant soybean; strategies are needed to optimize the application rate based on Canada fleabane height at the time of application. The purpose of this study was to determine the effect of glyphosate/dicamba rate and application timing for the control of GR Canada fleabane. Glyphosate/dicamba was applied at three rates (900, 1350 and 1800 g ae ha^{-1}) at three application timings (5, 15 and 25 cm) in a factorial design. There was no interaction between glyphosate/dicamba rate and timing for GR Canada fleabane control or soybean yield; however, there was an interaction for GR Canada fleabane density and biomass. At 2 and 4 weeks after application (WAA) there was a decrease in GR Canada fleabane control as the height as the time application increased. At 4 WAA the application of glyphosate/dicamba to GR Canada fleabane that was 5, 15 and 25 tall provided 87, 76 and 62%, respectively. There was no impact glyphosate/dicamba application timing on soybean yield. At 2, 4 and 8 WAA there was an increase in GR Canada fleabane control as the rate of glyphosate/dicamba was increased. At 8 WAA, glyphosate/dicamba applied at 900, 1350, and 1800 g ae ha^{-1} controlled GR Canada fleabane 76, 87 and 92%, respectively. Earlier application timings and higher rates of glyphosate/dicamba caused the greatest reduction in GR Canada fleabane density and biomass. Reduced GR Canada fleabane competition with the application of glyphosate/dicamba resulted in a 100 to 144% increase in soybean yield, but there was no difference in soybean yield among glyphosate/dicamba rates tested.

6.2 Introduction

Glyphosate is a non-selective, systemic herbicide that inhibits 5-enolpyruvylshikimate 3-phosphate synthase within plants, bacteria and some fungi (Franz et al. 1997; Dill 2005). Glyphosate is efficacious on annual, biennial and perennial weeds, is relatively inexpensive, and exhibits low toxicity to the environment and mammals (Duke and Powles 2008). Glyphosate translocates within the apoplast and symplast, accumulating in actively growing tissues within

the plant (Franz et al. 1997). Glyphosate readily binds to soil colloids, providing no residual weed control (Franz et al. 1997). The efficacy of glyphosate is affected by weed species and weed size at the time of application; therefore, herbicide rate may need to be adjusted for acceptable weed control (Hartzler et al. 2006). Glyphosate-resistant (GR) crops were first introduced in 1996 in canola and soybean (Dill 2005). The introduction of GR crops has led to increased use of glyphosate within a growing season and in consecutive years, which increases the selection pressure for GR weeds (Duke and Powles 2008).

Canada fleabane (*Conyza canadensis* (L.) Cronq.), also known as horseweed or marestail, is a broadleaf weed in the Asteraceae family (Loux et al. 2006). Canada fleabane can germinate in the fall after seed release from the mother plant, allowing it to overwinter and have a competitive advantage over annual crops and weed species the following growing season (Main et al. 2006). In Ontario, Canada fleabane biotypes have been observed to germinate at 8°C (Tozzi et al. 2013). The majority of Canada fleabane emergence in Ontario has been observed in the month of May and late August (Tozzi and Van Acker 2014). Tillage is an important factor in Canada fleabane management, as the reduction in tillage has led to an increase of Canada fleabane in no-till corn and soybean (Loux et al. 2006). Nandula et al. (2006) observed a decrease in emergence with an increase in seed depth, which may be due to the small Canada fleabane seeds having limited reserves (Tozzi et al. 2014). The dispersion of Canada fleabane seed hundreds of metres from the source is due to a pappus attached to the seed (Dauer et al. 2006).

Canada fleabane has evolved resistance to five different sites-of-action globally: Groups 2, 5, 7, 9, and 22 (Heap 2018). Previously, glyphosate provided excellent control of Canada fleabane, but the intense selection pressure from multiple applications of glyphosate led to the evolution of GR Canada fleabane in Delaware, USA in 2000, and in Ontario, Canada in 2010, along with many other locations in North America (Bruce and Kells 1990; Van Gessel 2001; Byker et al. 2013; Heap 2018). Glyphosate-resistant Canada fleabane has been reported to decrease soybean yield up to 69% in Ontario (Budd et al. 2016b).

The increase in GR weeds, and weeds with resistance to multiple sites-of-action has increased the demand for new herbicide sites-of-action, modes-of-action and biotechnology traits. One solution that became commercially available in Canada in 2017 is glyphosate/dicamba-resistant soybean (Roundup Ready 2 Xtend® soybean).

Glyphosate/dicamba-resistant soybean contains separate genes that confer resistance to glyphosate and dicamba. Glyphosate is an efficacious option for the control of susceptible grass and broadleaf weeds; dicamba has activity on a wide range of broadleaf weeds including GR biotypes and can be a second effective mode-of-action on glyphosate-susceptible broadleaf species. Dicamba is a Group 4, benzoic acid, growth regulator (synthetic auxin) herbicide (Cobb and Reade 2010). Dicamba provides short residual broadleaf weed control. The length of residual activity is dependent on the soil type, rainfall, and soil organic matter (Smith 1973; Shaner et al. 2014; Burnside and Lavy 1966).

Previous research by Budd et al. (2016b) found that saflufenacil (25 g ai ha^{-1}) applied to 25 cm tall GR Canada fleabane provided 95 to 99% control. Other research on the control of GR Canada fleabane observed there was a decrease in efficacy with saflufenacil (25 or 50 g ai ha^{-1}) as plant height increased from 5 to 45 cm; increasing the rate to ≥75 g ai ha^{-1} did not increase control (Mellendorf et al. 2013). Kruger et al. (2010) observed 97 to 98% GR Canada fleabane control across all applications timings (0-7 cm, 7-15 cm, 15-30 cm and >30 cm) with the diglycolamine salt of dicamba (280 g ae ha^{-1}). Dicamba (280 g ae ha^{-1}, dimethylamine salt) controlled Canada fleabane 89 to 99% with ≥97% control of plants 1-30 cm; 2,4-D ester and 2,4-D amine (560 g ae ha^{-1}) controlled Canada fleabane (30 cm tall at application), 94 to 97% and 90 to 93%, respectively (Kruger et al. 2010).

Our hypothesis was that GR fleabane control will decrease as the application timing is delayed and the rate of glyphosate/dicamba is reduced. Therefore, the objective of this study was to evaluate the efficacy of glyphosate/dicamba for the control of GR Canada fleabane at three rates of glyphosate/dicamba and three application timings.

6.3 Materials and Methods

This study was conducted near Mull (42.401671, -81.991098), Blenheim (42.335561, -81.997442) and Harrow, Ontario (42.035582, -82.918173) in 2016 and near Mull, Thamesville (42.551722, -81.977180) and Harrow, Ontario in 2017, for a total of six site-years.

A 3 x 4 factorial was arranged in a randomized complete block design (RBCD) with four replications. Factor one was application timings (Canada fleabane height was approximately 5, 15 or 25 cm); factor two was glyphosate/dicamba rate (0, 900, 1350 or 1800 g ae ha^{-1}). The herbicide applications were based on weed height and not soybean stage, therefore, some

applications made at the 5 cm or 15 cm stage were applied before crop planting or emergence. Soybean cultivars DKB14-41 and DKB10-01 (Monsanto, St. Louis, MO) were planted with a no-till planter in 2016 and 2017, respectively. Each plot was 2.25 m wide (3 soybean rows spaced 75 cm apart) and 8 m in length. Soybean was planted at a rate of approximately 400 000 seeds ha^{-1} to an approximate depth of 4 cm. Soybean injury was assessed visually at 2 and 4 weeks after emergence (WAE), where 0% was no injury compared to soybean in the weedy control and 100% was plant death. Canada fleabane control was assessed visually at 2, 4, and 8 weeks after application (WAA), where 0% was no difference between the treatment and the weedy control and 100% was when there were no visible weeds in the plot. At 6 WAA, density and biomass were determined by counting the number of Canada fleabane plants within two 0.25 m^{-2} quadrants in each plot. The plants were cut at the soil surface, placed in a paper bag, and dried at 60°C. After two weeks, the plant samples were removed and the weight was recorded. In 2016, soybean yield was determined by harvesting two 1 m subsamples by hand, from two rows in the middle of each plot. The soybean plants were threshed with a stationary Almaco thresher (Almaco, Nevada, IA). In 2017, two rows of soybean per plot was harvested at maturity with an Almaco small plot combine. Soybean weight and moisture content were recorded and moisture content was corrected to 14.5%.

The GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC) was used for statistical analysis. The fixed effect was the herbicide rate and application timing, and the random effects were environment and block. Residuals were analyzed individually for each analysis using the UNIVARIATE procedure for normality, homogeneity and errors independent of each other. The weedy controls were not included in the control analysis. Soybean yield and waterhemp control data at 2 and 4 WAA were fit to a normal distribution using the identity link. Waterhemp control data at 8 WAA was fit to a beta distribution and cumulative complementary log-log link was utilized. Waterhemp density and biomass data were analyzed using a lognormal distribution with the identity link and backtransformed within SAS for presentation purposes. Soybean yield data did not need transformation. Treatment means were separated by Fisher's Protected LSD and Tukey-Kramer adjustment with alpha set at P=0.05.

6.4 Results

6.4.1 Injury

Glyphosate/dicamba caused no visible soybean injury at the application rates and timings evaluated (data not presented).

6.4.2 Interactions

There was no interaction between glyphosate/dicamba application timing and rate for GR Canada fleabane control and soybean yield; therefore, the main effects will be presented (Table 6.1). There was an interaction between glyphosate/dicamba application timing and rate for GR Canada fleabane density and biomass; therefore, the simple effects will be presented (Table 6.2).

6.4.3 Application Timing

Glyphosate-resistant Canada fleabane control and soybean yield was affected by glyphosate/dicamba application timing (5, 15 or 25 cm tall GR Canada fleabane at the time of application) (Table 6.1). At 2 and 4 WAA, as the application of glyphosate/dicamba was delayed, there was a decrease in the control of GR Canada fleabane. At 2 WAA, there was a no difference in control when glyphosate/dicamba was applied to GR Canada fleabane that was 5 or 15 cm in height, but control was decreased when application was delayed until GR Canada fleabane was 25 cm in height. At 4 WAA, glyphosate/dicamba applied to 5, 15 and 25 cm tall GR Canada fleabane provided 87, 76 and 62% control, respectively. At 8 WAA, glyphosate/dicamba applied to 15 cm Canada fleabane was the most efficacious, and applications to Canada fleabane 5 and 25 cm in height were similar and less than the 15 cm application timing. There was a trend to reduced soybean yield as the application of glyphosate/dicamba was delayed but differences were not statistically significant.

6.4.4 Application Rate

There was an effect of glyphosate/dicamba rate (900, 1350 and 1800 g ae ha^{-1}) on GR Canada fleabane control. At 2, 4 and 8 WAA, there was an increase in GR Canada fleabane control as the rate of glyphosate/dicamba increased (Table 6.1). At 2, 4 and 8 WAA, glyphosate/dicamba (900 g ae ha^{-1}) controlled GR Canada fleabane 42, 68 and 76%,

respectively; whereas when the rate was increased to 1800 g ae ha^{-1} control increased to 52, 81 and 92%, respectively; there was a 10 to 16% increase in control with the high rate. Reduced GR Canada fleabane interference after application of glyphosate/dicamba (900, 1350 and 1800 g ae ha^{-1}) resulted in an increase in soybean yield of 100 to 144% compared to the weedy control.

6.4.5 Interaction of Glyphosate/Dicamba Application Timing and Rate on GR Canada Fleabane Density and Biomass

Glyphosate/dicamba reduced GR Canada fleabane density 22-95% (Table 6.2). Glyphosate/dicamba (900, 1350 and 1800 g ae ha^{-1}) applied to 5, 15 and 25 cm tall plants reduced in GR Canada fleabane density 78-95%, 71-86% and 36-57%, respectively, indicating that the delayed application resulted in a smaller decrease in density. At 6 WAA, glyphosate/dicamba at 900, 1350 and 1800 g ae ha^{-1} applied at the 5 cm application timing decreased GR Canada fleabane density 78, 92 and 95%, respectively; when the application was delayed until the GR Canada fleabane was 15 cm in height there was a decrease in density at only the 1350 and 1800 g ae ha^{-1} rates and when the application was delayed until the GR Canada fleabane was 25 cm in height there was a no decrease in GR Canada fleabane density. Although, there was a numeric decrease in density as glyphosate/dicamba rate was increased at each application timing, there was a much greater decrease in density with the early application timing.

Glyphosate/dicamba (900, 1350 and 1800 g ae ha^{-1}) reduced Canada fleabane biomass 64-97% (Table 6.2). Glyphosate/dicamba applied to 5, 15 and 25 cm tall plants reduced GR Canada fleabane biomass 87-97%, 90-96% and 64-79%, respectively, indicating that the delayed application resulted in a smaller decrease in biomass. At 6 WAA, glyphosate/dicamba at 900, 1350 and 1800 g ae ha^{-1} applied to 5 or 15 cm decreased GR Canada fleabane biomass 87-90, 95 and 96-97%, respectively. When the glyphosate/dicamba application was delayed until GR Canada fleabane was 25 cm in height there was no decrease in biomass when glyphosate dicamba was a applied at 900 and 1350 g ae ha^{-1}; glyphosate/dicamba at 1800 g ae ha^{-1} decreased GR Canada fleabane biomass 79%. There was a numeric decrease in GR Canada fleabane biomass as glyphosate/dicamba rate was increased at each application timing, and there was a decrease in biomass with all glyphosate/dicamba rates when applied to Canada fleabane that was 5 or 15 cm in height, but there was only a significant decrease in biomass when

glyphosate/dicamba was applied at 1800 g ae ha^{-1} when application was delayed to when fleabane was 25 cm in height.

6.5 Discussion and Conclusions

There was no soybean injury observed in this trial, which was expected with the use of glyphosate/dicamba-resistant soybean cultivars.

There was an increase in GR Canada fleabane control with an increase in glyphosate/dicamba rate and at earlier application timings. Reductions in biomass and density followed the same trend, with the greatest reduction observed when the herbicide was applied at the higher rates to weeds that were 5 or 15 cm tall. Control at 2 WAA was 40-54%, indicating that dicamba is a slow acting herbicide.

Early weed control is important; previous research found a soybean yield loss of 5% or less when soybean was maintained weed-free until the V3 growth stage (Van Acker et al. 1993). Similarly, in this study there was a trend to reduced soybean yield when herbicide application was delayed.

At 6 WAA, the late application timing (25 cm) resulted in a smaller decrease in GR Canada fleabane density and biomass, indicating reduced activity with delayed herbicide applications. At all application timings there was a trend to a greater decrease in density and biomass as the rate of glyphosate/dicamba was increased.

This study found that glyphosate/dicamba should be applied at medium to high rates (1350-1800 g ae ha^{-1}) to weeds <15 cm to ensure adequate GR Canada fleabane control.

6.6 Tables

Table 6.1 [a]Interactions between application timing, rate, and timing x rate. Means for GR Canada fleabane control, density, biomass and soybean yield for application timings and herbicide rates at six site-years locations in 2016 and 2017 in Ontario.

	GR Canada fleabane control (%)					
	2 WAA	4 WAA	12 WAA	Density (plants m^{-2})	Biomass (g m^{-2})	Soybean yield (t ha^{-1})
Application timing (cm)	*	*	*			NS
5	54a	87a	84b	22	18	2.0
15	48a	76b	89a	53	25	1.7
25	40b	62c	83b	81	55	1.5
Herbicide rate (g ae ha^{-1})	*	*	*			*
0	0	0	0	142	163	0.9b
900	42b	68c	76c	48	26	1.8a
1350	47ab	76b	87b	31	16	2.2a
1800	52a	81a	92a	20	10	2.2a
Timing x rate	NS	NS	NS	*	*	NS

Abbreviations: WAA, weeks after application; GR, glyphosate-resistant; NS, not significant
[a]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (P=0.05).
*significant difference between application timings, herbicide rates or timings x rates

Table 6.2 [a]Means for Canada fleabane density, and biomass from six experiments in Ontario in 2016 and 2017.

Height (cm)	Rate (g ae ha^{-1})	Density (# m^{-2})	Biomass (g m^{-2})
5	0	128ab	150ab
	900	27cde	19cde
	1350	10ef	7ef
	1800	6f	4f
15	0	189a	207a
	900	54abc	21cde
	1350	31cde	10def
	1800	25de	8ef
25	0	119ab	140ab
	900	76abc	44bc
	1350	93abc	50bc
	1800	51abc	29cd

Abbreviations: WAA, weeks after application

[a]Means followed by the same letter within a column are not statistically different according to Fisher's Protected LSD (P=0.05).

Chapter 7: General Discussion

7.1 Contributions

The focus of these studies was to understand the effect of application rate and timing and develop a multiple mode-of-action chemical control strategy for GR Canada fleabane and waterhemp in glyphosate/dicamba-resistant soybean. This research increased knowledge of how to manage GR Canada fleabane and waterhemp in glyphosate/dicamba-resistant soybean in Ontario.

Glyphosate/dicamba effectively controlled GR Canada fleabane. At 8 and 12 WAA, glyphosate/dicamba (1800 g ae ha^{-1}) controlled GR Canada fleabane 94 and 92%, respectively. At 12 WAA, glyphosate/dicamba (1800 g ae ha^{-1}) with the addition of the following herbicides provided >90% control of GR Canada fleabane: saflufenacil (25 g ai ha^{-1}), saflufenacil/dimethenamid-*P* (245 g ai ha^{-1}), saflufenacil/imazethapyr (100 g ai ha^{-1}), 2,4-D (500 g ae ha^{-1}), metribuzin (400 g ai ha^{-1}), and paraquat (1100 g ai ha^{-1}). At 4, 8 and 12 WAA, the addition of the aforementioned herbicides maintained or exceeded the level of control provided by the application of glyphosate/dicamba alone.

Glyphosate/dicamba rate influenced the control of GR Canada fleabane. At 4 WAA, glyphosate/dicamba applied at 900, 1350 or 1800 g ae ha^{-1} controlled GR Canada fleabane 81, 89 and 93%, respectively. At 4 and 8 WAA, there was an increase in GR Canada fleabane control as the rate was increased. Biomass and density decreased with an increase in glyphosate/dicamba rate.

Control of GR Canada fleabane with glyphosate/dicamba decreased as weed size at the time of application increased. Overall, there was a trend to decreased control with an increase in Canada fleabane height, but differences were not always statistically different. Biomass and density increased with an increase in weed height at the time of application.

There are a number of PRE herbicides that provide effective control of GR waterhemp in soybean in Ontario. At 12 WAA, the PRE herbicides that provided >90% control of GR waterhemp were: *S*-metolachlor/metribuzin (1943 g ai ha^{-1}), pyroxasulfone/sulfentrazone (300 g ai ha^{-1}) and pyroxasulfone/flumioxazin (240 g ai ha^{-1}). The tank-mixtures of: *S*-metolachlor/metribuzin + glyphosate/dicamba (1943 g ai ha^{-1} + 1800 g ae ha^{-1}), pyroxasulfone/sulfentrazone + glyphosate/dicamba (300 g ai ha^{-1} + 1800 g ae ha^{-1}) and

pyroxasulfone/flumioxazin + glyphosate/dicamba (240 g ai ha^{-1} + 1800 g ae ha^{-1}) controlled GR Canada fleabane 90%. The addition of glyphosate/dicamba to the PRE herbicides evaluated in this study did not increase control GR waterhemp. Glyphosate/dicamba, applied PRE, is not an efficacious option for the control of GR waterhemp due to the short residual activity of dicamba.

Glyphosate/dicamba rate influenced the control of GR waterhemp. At 2 and 4 WAA, there was an increase in GR waterhemp control with an increase in glyphosate/dicamba rate when waterhemp was 5, 15 or 25 cm in height at the time of herbicide application. A decrease in control was observed with an increase in waterhemp height, although differences were not always statistically significant. One application of glyphosate/dicamba did not provide commercially acceptable control of GR waterhemp. At 8 and 12 WAA, glyphosate/dicamba applied PRE or POST controlled GR waterhemp 51 to 85% and 71%, respectively. Although a sequential application of glyphosate/dicamba is needed for full season control of GR waterhemp, this is not recommended due to increased selection pressure for herbicide-resistant weeds.

A two-pass program of an effective PRE herbicide followed by glyphosate/dicamba, applied POST, controlled GR waterhemp 91%. This approach to GR waterhemp management provides excellent full season control and reduces the selection intensity for herbicide-resistant weeds.

7.2 Limitations

As with all research, there are always opportunities to improve. All trials were conducted in soybean seeded in rows spaced 76 cm apart, which is the worst-case scenario for weed control. However, the wide row spacing facilitates efficient weed harvest for density and biomass determinations. Soybean row widths of 50, 37.5 and 17.5 cm are used by Ontario farmers which will close the canopy earlier and may result in improved weed control in narrow compared to wide row widths. This may be especially important for waterhemp since it emerges throughout the growing season and germination is affected by light. The extended emergence pattern of waterhemp influenced the weed control results in this study, at the later weed control evaluation timings there was reduced control with the early herbicide application timings since weeds emerged after application; it is difficult to distinguish between suppressed and newly emerged waterhemp plants.

The application timing of the second POST-only application in the two-pass waterhemp control study was delayed so that it corresponded with the height of the weed escapes in a PRE treatment. This delay would not be an acceptable practice on commercial farms in Ontario. In future studies the timing of the POST herbicides may need to be based on weed height in each individual treatment independent of the PRE treatments.

The injury rating for two-pass systems was right before and after the POST application of glyphosate/dicamba. Glyphosate/dicamba should be safe on glyphosate/dicamba-resistant soybean since it has transgenes that confer resistance to glyphosate and dicamba. In contrast, some of the PRE herbicides do cause soybean injury in stressed environments, so the injury assessment should be based on days after soybean emergence and not the timing of the POST herbicide.

The waterhemp density and percent emerged at the time of herbicide application varied within and between trial locations and years. While this is a common issue with field weed control studies, it does influence control assessment. Higher efficacy may be observed in locations with lower density due to increased herbicide coverage compared to higher densities.

Cover sprays of glyphosate were used to remove the confounding effect of other weed species/biotypes in the trial areas. But, because there was a mixture of glyphosate-susceptible and -resistant waterhemp biotypes some had a higher population of susceptible waterhemp compared to others and would have been controlled by glyphosate. This may have influenced the assessments made because locations with higher ratios of susceptible-to-resistant waterhemp biotypes would have higher efficacy in late data collection points than populations with lower ratios.

An important concern of growers is ease of harvest; therefore, it would be beneficial to include a 12 WAA rating for the GR Canada fleabane in the application timing by rate trials.

7.3 Future Research

All of the studies were completed in soybean seeded in rows spaced 76 cm apart; future research should study the interaction between glyphosate/dicamba rate and row width for the control of GR waterhemp. This would compliment the height by rate study and give more accurate guidelines to growers. A split-plot design with height as an additional factor could also be considered.

Canada fleabane emerges primarily in the fall and spring. There may be a difference in efficacy between the two emergence timings, therefore, future research should evaluate the control of fall-emerged versus spring-emerged Canada fleabane.

All research was conducted using either no-till (Canada fleabane) or conventional tillage (waterhemp) which was based on grower practices in the fields where these studies were conducted. Future research should evaluate the impact of tillage on the control of GR Canada fleabane and waterhemp.

Previous research has found that cover crops reduce GR Canada fleabane density (Cholette et al. 2017). Waterhemp is known to require light for germination, therefore trials with different cover crops may be beneficial to reduce the emergence and establishment of waterhemp and subsequent weed seed return to the soil.

The highest registered herbicide rate was used in all of the trials; therefore, biologically effective rate studies should be conducted to determine the lowest effective rate for each herbicide.

All locations and years were combined for analysis, and while soil type didn't drastically differ between locations in this study, efficacy of soil-applied herbicides is affected by soil type. Future research should study the effect of soil type on herbicide efficacy. Additionally, rainfall is important to dissolve herbicides in soil water solution so that they can be taken up by plants. Drought or excessive rainfall conditions should be studied to better understand the impact of these weather extremes on herbicide efficacy.

Cost-effectiveness of herbicide programs was not evaluated in this study. Future research should include a partial economic analysis to determine the most cost-effective weed management program.

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Chapter 9: Appendices

9.1 Code for analyzing Mean comparisons in Chapter 2

```
title ‘Glyphosate-resistant Waterhemp- two-pass;
data first;
input env$ plot trt block injury_2 injury_4 control_2 control_4 control_8 control_12 density
dry_weight yield_t;

*/For control
if trt=1 then delete;
if trt=2 then delete;

*/For density or biomass
if trt=2 then delete;

*/For control
y=control_2/100;
if y=0 then y=0.0000000001;
if y=1 then y=0.9999999999;
datalines;
;

*/ for distribution see materials and methods

proc glimmix data=first nobound;
nloptions maxiter=200;
class trt block env;
model y = trt / dist=beta link=ccll;
Random block env;
Lsmeans trt / pdiff adjust=tukey lines ilink;
covtest "block=0" 0 . .;
covtest "env=0"  . 0 .;
output out=second predicted=pred residual=resid residual(noblup)=mresid student=studentresid
student(noblup)=smresid;
run;

*/linearity of fixed effects -both scatter & boxplot;
proc sgplot data=second;
scatter y=studentresid x=trt; refline 0;
run;
proc sgplot data=second; vbox studentresid / group=trt datalabel;
run;

*/homegeneity of effects;
proc sgscatter data=second;
plot studentresid*(pred trt block env);
```

```
run;

**Q-Q plot and Shapiro-Wilk for normal distribution;
Proc univariate data=second normal plot;
  var studentresid;
Run;

proc means data=first;
class trt;
var control_2;
run;
```

9.2 Code for analyzing Mean comparisons in Chapter 3

```
title 'Glyphosate-resistant Waterhemp- 2nd effective MOA';
data first;
input env$ plot trt block injury_2 injury_4 control_2 control_4 control_8 control_12 density
dry_weight yield_t;

*/For control
if trt=1 then delete;
if trt=2 then delete;

*/For density or biomass
if trt=2 then delete;

*/For control
y=control_2/100;
if y=0 then y=0.0000000001;
if y=1 then y=0.9999999999;
datalines;
;

*/for distributions see materials and methods

proc glimmix data=first nobound;
nloptions maxiter=200;
class trt block env;
model y = trt / dist=beta link=ccll;
Random block env;
Lsmeans trt / pdiff adjust=tukey lines ilink;
covtest "block=0" 0 . .;
covtest "env=0"  . 0 .;
output out=second predicted=pred residual=resid residual(noblup)=mresid student=studentresid
student(noblup)=smresid;
run;

*/linearity of fixed effects -both scatter & boxplot;
proc sgplot data=second;
scatter y=studentresid x=trt; refline 0;
run;
proc sgplot data=second; vbox studentresid / group=trt datalabel;
run;

*/homegeneity of effects;
proc sgscatter data=second;
plot studentresid*(pred trt block env);
run;
```

```
**Q-Q plot and Shapiro-Wilk for normal distribution;
Proc univariate data=second normal plot;
  var studentresid;
Run;

proc means data=first;
class trt;
var control_2;
run;
```

9.3 Code for analyzing Mean comparisons in Chapter 4

```
title ‘Glyphosate-resistant Canada fleabane- 2nd effective MOA’;
data first;
input env$ plot trt block injury_2 injury_4 control_2 control_4 control_8 control_12 density
dry_weight yield_t;

*/For control
if trt=1 then delete;
if trt=2 then delete;

*/For density or biomass
if trt=2 then delete;

*/For control
y=control_2/100;
if y=0 then y=0.0000000001;
if y=1 then y=0.9999999999;
datalines;
;

*/ for distribution see materials and methods

proc glimmix data=first nobound;
nloptions maxiter=200;
class trt block env;
model y = trt / dist=beta link=ccll;
Random block env;
Lsmeans trt / pdiff adjust=tukey lines ilink;
covtest "block=0" 0 . .;
covtest "env=0"  . 0 .;
output out=second predicted=pred residual=resid residual(noblup)=mresid student=studentresid
student(noblup)=smresid;
run;

*/linearity of fixed effects -both scatter & boxplot;
proc sgplot data=second;
scatter y=studentresid x=trt; refline 0;
run;
proc sgplot data=second; vbox studentresid / group=trt datalabel;
run;

*/homegeneity of effects;
proc sgscatter data=second;
plot studentresid*(pred trt block env);
run;
```

```
**Q-Q plot and Shapiro-Wilk for normal distribution;
Proc univariate data=second normal plot;
  var studentresid;
Run;

proc means data=first;
class trt;
var control_2;
run;
```

9.4 Code for analyzing Factorial interaction in Chapter 5

```
title ‘base code’;
data first;
input env$ plot trt block injury_2 injury_4 control_2 control_4 control_8 density biomass yield;
if trt = 1 then do;
  height = 5;
  rate = 0;
end;
if trt = 2 then do;
  height = 5;
  rate = 900;
end;
if trt = 3 then do;
  height = 5;
  rate = 1350;
end;
if trt = 4 then do;
  height = 5;
  rate = 1800;
end;
if trt = 5 then do;
  height = 15;
  rate = 0;
end;
if trt = 6 then do;
  height = 15;
  rate = 900;
end;
if trt = 7 then do;
  height = 15;
  rate = 1350;
end;
if trt = 8 then do;
  height = 15;
  rate = 1800;
end;
if trt = 9 then do;
  height = 25;
  rate = 0;
end;
if trt = 10 then do;
  height = 25;
  rate = 900;
end;
if trt = 11 then do;
  height = 25;
```

```
  rate = 1350;
end;
if trt = 12 then do;
  height = 25;
  rate = 1800;
end;

*/ include in control data only
y=control_2/100;
if y=0 then y=0.0000000001;
if y=1 then y=0.9999999999;
if trt = 1 then delete;
if trt = 5 then delete;
if trt = 9 then delete;
datalines;

*/for distributions see materials and methods

Proc glimmix data=first;
nloptions maxiter=200;
  class block env height rate;
  model y = height rate height*rate / dist=normal link=identity;
  random block*height*trt env*block*trt;
  covtest "block=0" 0 . .;
  covtest "env=0" . 0 .;
  lsmeans height rate height*rate /pdiff adjust=tukey lines ilink;
  output out=second predicted=pred residual=resid residual(noblup)=mresid student=studentresid
student(noblup)=smresid;
  title "Control 2 WAA";
Run;
Proc freq;
  tables height rate height*rate;
Run;
*/linearity of fixed effects -both scatter & boxplot;
proc sgplot data=second;
scatter y=studentresid x=height; refline 0;
run;
proc sgplot data=second; vbox studentresid / group=height datalabel;
run;
*/linearity of fixed effects -both scatter & boxplot;
proc sgplot data=second;
scatter y=studentresid x=rate; refline 0;
run;
proc sgplot data=second; vbox studentresid / group=rate datalabel;
run;
*/homegeneity of effects;
```

```
proc sgscatter data=second;
plot studentresid*(pred trt block env);
run;

**Q-Q plot and Shapiro-Wilk for normal distribution;
Proc univariate data=second normal plot;
  var studentresid;
Run;

proc means data=first;
class trt;
var control_2;
run;
```

9.5 Code for analyzing Factorial interaction in Chapter 6

```
title ‘Factorial-CF;
data first;
input env$ plot trt block injury_2 injury_4 control_2 control_4 control_8 density biomass yield;
if trt = 1 then do;
  height = 5;
  rate = 0;
end;
if trt = 2 then do;
  height = 5;
  rate = 900;
end;
if trt = 3 then do;
  height = 5;
  rate = 1350;
end;
if trt = 4 then do;
  height = 5;
  rate = 1800;
end;
if trt = 5 then do;
  height = 15;
  rate = 0;
end;
if trt = 6 then do;
  height = 15;
  rate = 900;
end;
if trt = 7 then do;
  height = 15;
  rate = 1350;
end;
if trt = 8 then do;
  height = 15;
  rate = 1800;
end;
if trt = 9 then do;
  height = 25;
  rate = 0;
end;
if trt = 10 then do;
  height = 25;
  rate = 900;
end;
if trt = 11 then do;
  height = 25;
```

```
  rate = 1350;
end;
if trt = 12 then do;
  height = 25;
  rate = 1800;
end;

*/ include in control data only
y=control_2/100;
if y=0 then y=0.0000000001;
if y=1 then y=0.9999999999;
if trt = 1 then delete;
if trt = 5 then delete;
if trt = 9 then delete;
datalines;
;

*/for distributions see materials and methods

Proc glimmix data=first;
nloptions maxiter=200;
  class block env height rate;
  model y = height rate height*rate / dist=normal link=identity;
  random block*height*trt env*block*trt;
  covtest "block=0" 0 . .;
  covtest "env=0" . 0 .;
  lsmeans height rate height*rate /pdiff adjust=tukey lines ilink;
  output out=second predicted=pred residual=resid residual(noblup)=mresid student=studentresid
student(noblup)=smresid;
  title "Control 2 WAA";
Run;
Proc freq;
  tables height rate height*rate;
Run;
*/linearity of fixed effects -both scatter & boxplot;
proc sgplot data=second;
scatter y=studentresid x=height; refline 0;
run;
proc sgplot data=second; vbox studentresid / group=height datalabel;
run;
*/linearity of fixed effects -both scatter & boxplot;
proc sgplot data=second;
scatter y=studentresid x=rate; refline 0;
run;
proc sgplot data=second; vbox studentresid / group=rate datalabel;
run;
```

```
*/homegeneity of effects;
proc sgscatter data=second;
plot studentresid*(pred trt block env);
run;

**Q-Q plot and Shapiro-Wilk for normal distribution;
Proc univariate data=second normal plot;
  var studentresid;
Run;

proc means data=first;
class trt;
var control_2;
run;
```