Control and Distribution of Herbicide Resistant Waterhemp
(Amaranthus tuberculatus) in Ontario.

by

Lauren Benoit

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ABSTRACT

CONTROL AND DISTRIBUTION OF HERBICIDE RESISTANT WATERHEMP
(Amaranthus tuberculatus) IN CORN (Zea mays L.) IN ONTARIO.

Lauren Benoit
University of Guelph, 2019

Advisor(s):
Dr. P. H. Sikkema
Dr. D. E. Robinson

Field surveys were conducted in 2016, 2017 and 2018 to build on previous distribution research. Waterhemp was located at 42 additional locations in seven Ontario counties and the province of Quebec. Greenhouse testing of populations collected between 2014 and 2017 has shown that 100% of the populations are resistant to Group 2 herbicides, 83% to Group 5, 87% to Group 9, and 28% to Group 14. This is the first reported case of a Group 14-resistant weed in Eastern Canada. Thirty-four field studies were conducted at three locations over three years to identify efficacious PRE, POST, and two-pass herbicide programs for control of MR waterhemp in corn. S-metolachlor/mesotrione/bicyclopyrone/ atrazine applied PRE provided 91% control of waterhemp, 8 WAA. POST-applied mesotrione + atrazine provided 92% control 8 WAA. All two-pass programs provided greater than 94% control of waterhemp 12 WAA. Ontario grain farmers have access to multiple effective options for controlling MR waterhemp in corn.
DEDICATION

To Ontario’s farmers, I hope my work makes your job just a little bit easier.

To Mark & Sandi, for inspiring me to be ambitious, be curious, reach higher, and do better.

To Paul & Ginny, for graciously sharing their home, their farm, and their love of sweet, little, brown cows.

To my Dad, who has taught me more about resilience, courage and grace than anyone else.
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“If I have seen further it is by standing on the shoulders of giants”, is one of my favourite quotes, most famously attributed to Issac Newton in 1675. More often than not our accomplishments are not due solely to our own abilities or talents, but because we are lifted up by those who came before us, and those who helped us along the way. The completion of this thesis was made possible because of the contributions and support of many giants, to whom I extend my sincerest appreciation.

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1.1 Waterhemp (*Amaranthus tuberculatus*)

*Amaranthus tuberculatus*, commonly known as waterhemp, is a member of the Amaranth family (OMAFRA 2005). Waterhemp is native to the United States Midwest and has spread to Texas, Maine and Ontario (Olsen et al. 2014). It has been ranked as one of the most troublesome weeds in the US Midwest, particularly since the 1990s when no-till agronomic practices used in conjunction with chemical herbicides were becoming increasingly popular (Nordby et al. 2007). Group 2 resistant waterhemp was first confirmed in Iowa in 1993; since then, populations resistant to five modes of action have been confirmed: acetolactate synthase (ALS) inhibitors, photosystem II (PS II) inhibitors, 5-enolpyruvyl-skikimate-3-phosphate synthase (EPSPS) inhibitors, growth regulators and protoporphoryinogen IX oxidase (PPO) inhibitors (Heap 2016; Bell et al. 2013). In 2002, populations resistant to PSII- and ALS-Inhibitors were confirmed in Ontario; glyphosate resistance was confirmed in Ontario in 2014 (Heap, 2016). Shryver et al. (2017) collected waterhemp seed from 49 fields in southwestern Ontario and reported individuals from within each seed sample were resistant to the Group 2 herbicide imazethapyr. The speed at which herbicide resistance evolves in waterhemp, and the already limited control options, makes waterhemp a serious agronomic threat to Ontario farmers.
1.1.1 Nomenclature
The official botanical name of *Amaranthus tuberculatus* has changed multiple times since it was first classified in 1835. Initially, waterhemp was given two names *A. miamiensis* and *A. altissimus* (Riddell 1835). The species was renamed in 1849 as a single species, with a single name *Acnida tuberculata* (Moquin-Tandon 1849). In 1955, the species was again reclassified into two distinct species, both in the *Amaranthus* family, *A.tuberculatus* and *A.rudis*. *Amaranthus rudis* plants were to be distinguished from *A. tuberculatus* by a single tepal and dehiscent fruits (Sauer 1955). Many scientists now recognize waterhemp as a single, highly variable species, *Amaranthus tuberculatus* var *rudis*, that is subject to geographical differences (Pratt and Clark 2001), although there is still some controversy (Costea 2005). As of 2018, the WSSA has *A. tuberculatus* listed as the appropriate designation for plants formally known as either tall (*A. tuberculatus* var. *tuberculatus*) or common (*A. tuberculatus* var. *rudis*) waterhemp, with the common name listed as ‘waterhemp’ (WSSA 2018). Controversy and inconsistency in the naming and identification of waterhemp remains today and is the source of discrepancies in the literature.

1.1.2 Origin
It was once hypothesized that waterhemp evolved from two separate species found on either side of the Mississippi river in the American midwest, *Amaranthus tuberculatus* var *rudis* to the West and *Amaranthus tuberculatus* var. *tuberculatus* to the East (Sauer 1957; Olsen et al. 2014). The species that presents a problem in agricultural ecosystems is *A. tuberculatus* var. *rudis* and not a hybridization of the two previously defined species (Waselkov et al. 2014). In 2014, it was concluded that the recent
spread of the weedier biotypes is due to the eastern movement of the historically western biotypes (Waselkov et al. 2014). Western biotypes are more morphologically suited to be a competitive agricultural weed. *Amaranthus rudis* is not native to southwestern Ontario and was first recorded in Lambton county in 2002, while *Amaranthus tuberculatus* has been recorded along waterways and beaches since the 1800s (OMAFRA, 2005).

1.1.3 Description

Waterhemp is a small-seeded, annual broadleaf weed belonging to the *Amaranthus* family in the subgenus *Acinda* along with nine other *Amaranth* species (Mosyakin and Robertson 1996). Waterhemp is a highly competitive, C4 weed species (Costea et al. 2005; Lorentz et al. 2014). Waterhemp has an erect growth habit to 200 cm in height. Waterhemp roots have been reported to extend up to 2 m and 70 cm in depth (Horak and Loughin 2000; Costea et al. 2005). A large root structure is advantageous to the plant as it allows the plant to explore a larger soil volume for moisture and nutrients. Waterhemp has been reported to grow up to 0.16 cm per growing degree day, which is considerably faster than other Amaranth species (Horak and Loughin 2000). Waterhemp is difficult to distinguish from other closely related weeds in the Amaranth family. Waterhemp seedlings are hairless and cotyledons are more egg-shaped compared to other species in the Amaranth family, such as redroot pigweed (*Amaranthus retroflexus*) and Palmer amaranth (*Amaranthus palmeri*) (Nordby et al. 2007). Seedlings range from green to reddish, and glabrous to lightly hairy (Bryson)(year). The leaves on mature plants are elongated, lance-shaped and often have a glossy or waxy appearance (Nordby et al. 2007). Seed heads are long and
highly-branched (Anonymous 2005). Biologically, the physical characteristics and rapid growth of waterhemp contribute to the high competitiveness of the species.

1.1.4 Optimal Growing Conditions and Germination

Full sunlight and well-drained soils are optimal for waterhemp growth and development. As sunlight irradiation is reduced, the growth efficiency of waterhemp also decreases (Hand 1993). A waterhemp plant that emerged in late May and grown in full sunlight can produce 720 g of above ground biomass; biomass decreased to 500, 370 and 1 g plant\(^{-1}\) under 40, 68 and 99% shade, respectively (Steckel et al. 2003). In Ontario, waterhemp seedlings emerge over an extended period of time. Refsell and Hartzler (2009) reported that approximately 7% of waterhemp seeds in the seed bank will emerge annually. Waterhemp seed can remain viable in the soil up to 17 years; however most seed will germinate with in the first three years (Egley and Williams 1990).

Emergence begins in early May and continues until September or October (Vyn 2007; Schryver 2017). This extended emergence window contributes to the difficulty farmers face controlling waterhemp (Leon 2006). One of the reasons for an extended emergence window is the wide range of temperatures suitable for germination. The minimum temperature for germination is 10°C (Leon et al. 2004) with an optimal temperature range of 33-35°C, after which germination begins to decrease (Guo and Al-Khatic 2003). With a day/night temperature of 50°C/45°C, germination was reduced to zero (Guo, 2003).

Similarly, light quantity and quality influence germination. Seed depth with in the soil profile influences the availability of stimuli, such as light and oxygen, which promote
germination. Tillage practices impact the fraction of seeds found within the top 5 cm of soil, in a no-till system, 70-85% of seeds are in this zone resulting in a larger percentage of seeds that will germinate (Mulugeta and Stoltenberg 1997). Waterhemp seeds under soil cover need a minimum 1000 µmol m$^{-2}$ of light, unburied seeds require 3 µmol m$^{-2}$ (Gallagher and Cardina 1998). Leon and Owen (2006) reported that there is four times greater waterhemp germination in no-till compared to soil that has been chisel or moldboard plowed (Leon and Owen 2006). The rate and duration of germination is strongly affected by tillage practices, in a no-till system the window for waterhemp seedling emergence was 26 days longer compared to emergence in a chisel-plowed system. Growing conditions in Ontario are suitable for the growth and development of waterhemp, however the shift to no-till production systems has influenced the recent increase of waterhemp in agricultural production fields in the province.

1.1.5 Reproduction and Seed Dispersal

Waterhemp is a dioecious species with male and female reproductive organs on separate plants. Consequently, waterhemp cannot self-pollinate which allows genes to spread quickly across a population contributing to rapid herbicide resistance evolution. Waterhemp does not reproduce vegetatively (Costea et al. 2005). Most populations in Ontario have a 1:1 ratio of male:female plants (Costea et al. 2005). In contrast in Illinois 1:3 population ratios of male:female have been recorded (Lemen 1980). Wind pollination is the predominant method of pollen dispersal; pollen grains are small, spherical (20 µm in diameter) and structured to remain airborne for long distances (Franssen et al. 2001). Once released from the male anther, pollen can remain viable for up to 5 days and travel as far as 800 m (Liu et al. 2012). On average a waterhemp
plant will produce around 300,000 seed, however, under optimal conditions a single female waterhemp plant can produce up to 1.2 million seeds. (Horak et al. 2000). Seed production per plant is determined by environmental factors, emergence date and the presence of competition from other species (Costea et al. 2005). Once a mature plant is pollinated, it takes between 9 to 14 days for seeds to mature and become viable (Bell et al. 2010). Seeds are small, with approximately 3670 seeds per gram, although size and weight vary depending on location, growing conditions and population (Sellers et al. 2003).

Waterhemp seeds float and can be dispersed via waterways, an advantageous biological adaptation considering the plant’s preference to grow in close proximity to water (Costea et al. 2005). Seeds can also be dispersed via human traffic, birds, animals and manure (Costea et al. 2005). Waterhemp is a genetically diverse dioecious species, which produces a large number seeds per plant that are relatively short-lived in the soil.

1.1.6 Dormancy
Seed dormancy influences timing and extent of seed germination and emergence. Seed dormancy benefits the plants by inhibiting seed germination until specific conditions have been met, increasing the probability of successful seedling establishment. Dormancy is established genetically but influenced by many different environmental factors including temperature, moisture, and light (Allen and Meyer 1998). Light quality and temperature have the greatest effect on waterhemp germination (Leon et al. 2003). Germination in waterhemp is triggered by photoreceptors that detect the amount and wavelength of light called phytochromes (Leon et al. 2003). Stratification accelerates
loss of seed dormancy in waterhemp (Leon et al. 2006). In a controlled environment seeds that had been chilled were four times as likely to germinate compared to seeds that had not been chilled (Leon et al. 2003). The ratio of red to far-red light also played an important role in germination, with three times as many waterhemp seeds germinating under red light than far-red light (Leon et al. 2003). Plants with very specific requirements to break dormancy often germinate over a relatively short period of time, while those with less strict requirements germinate over an extended period of time. The environmental requirements needed to break dormancy in waterhemp are relatively general; meaning that they will be met multiple times throughout the average growing season, inducing multiple flushes of waterhemp germination.

1.1.7 Competitiveness of waterhemp

Weed control during the critical weed free period is crucial to minimize crop yield losses due to weed interference (Cerrudi et al. 2012). The greatest impact on crop yield is seen when weeds emerge with the crop (Hall et al. 1992). In general, compared to other Amaranthus species, waterhemp is more competitive than redroot pigweed (Amaranthus retroflexus) and less competitive than Palmer amaranth (Amaranthus palmeri) (Bensch et al. 2003; Horak and Loughin 2000). Waterhemp, if not controlled, can reduce corn yield by as much as 74% (Steckel et al. 2004). In a study conducted in Missouri, there was a positive correlation between waterhemp density and corn yield loss; low densities (35-82 m²) resulted in 8% yield loss, higher densities (369-445m²) resulted in 36% yield loss (Cordes et al. 2004). Waterhemp is highly susceptible to corn competition, when inter-seeded with corn the majority of waterhemp plants which emerged at or before the corn reached V4 survived. There was 90% mortality of
waterhemp which emerged at V6-V8, and no plants that emerged after V10 reached maturity (Steckel and Sprague 2004). Early season control of waterhemp in corn is critical to minimize yield losses due to weed interference.

1.2 Weed Control in Corn

1.2.1 Corn

Globally, corn is the most widely grown crop, in respect to dollar value, and is a major food source in North and South America and Southern and Eastern Africa (FAOStat 2017). In addition, corn is also used as a fuel source in the form of ethanol (Ranum 2014). Corn ranks fifth in seeded acres in Canada, following canola, wheat, soybean and barley (StatsCanada 2017). The majority of Canadian corn production occurs in Ontario and Quebec, representing 61.7 and 30.2% of total production, respectively (StatsCanada 2017). In 2014, corn was grown on just over 1.2 M ha, down from 1.4M in 2013 (FAOStat 2017). Corn is a major source of food calories for more than 4.5 billion people in 94 developing countries, the majority of global corn production occurs in low to middle income countries (Shiferaw 2011). Many different factors can cause a crop to fail to reach its full yield potential; lack of adequate nutrients, heat, light or water or pressure from disease, insect or weed pests. The degree of corn yield loss due to weed interference is influenced, in part, by weed species composition, weed density and the relative of time of weed and crop emergence. Weed interference a can reduce corn yields by over 75% (Zimdahl 2004). In addition to yield loss, the presence of weed seeds in the final grain corn sample may result in a greater dockage at the point of sale, lower grade, and reduced returns to the producer (Canadian Grain Commision 2016). When best agronomic management practices are followed, with the exception of any
weed control tactics, corn yield loss was an average of 51.4%, equivalent to approximately 4,000,000 tonnes of grain (Soltani 2016). Corn yield loss due to weed interference remains the largest, and most economically significant, limitation to crop productivity for most farmers (Page 2012).

1.2.2 Mechanisms of interference
Weed interference induces crop yield loss through resource-dependent and resource-independent interference (Page 2012). Resource dependent interference occurs when weeds are in direct competition with the crop for the available resources; examples would be competition for water, nutrients or sunlight. Resource independent interference has not been widely studied and is not as well understood as resource-dependent interference. Changes in light quality induced by neighbouring plants – specifically an increase in far red-light – and the subsequent accumulation of organic compounds such as ethylene (Afifi and Swanton 2012) illustrates one example of resource-independent interference. The consequences of early season stress on corn plants can include; reduced dry matter accumulation, increased plant-to-plant variability, increased shoot: root ratio and ultimately a decrease in grain yield (Evans, et al. 2003, Tollenaar and Wu 1999). An increase in plant-to-plant variability has a significant impact on corn yield per hectare (Glenn 1974). An early season increase in shoot: root ratio is a shade avoidance response, and can increase the risk of lodging and stalk breakage later in the season (Cerrudi 2012). Both resource-dependent and resource-independent factors, in addition to environmental conditions, determine the eventual corn yield loss due to weed interference.
One of the most commonly used guidelines for managing weeds in crop production is what is known as the critical weed free period. The concept of the CWFP was first introduced by Neito et al. in 1968; since then the concept has been applied to a large variety of crops (Neito 1968). The CWFP is the time during the growth of the crop during which it must be weed free to prevent yield loss greater than 5% (Hall 1992, Van Acker 1993, Knezevic 2002). The presence of weeds outside of this time frame should have negligible impact on yield; however, it is important to keep fields clean throughout the entire growing season in order to prevent weeds from reproducing and adding seed to the soil seed bank. In corn the CWFP is approximately from emergence up to 14th leaf (Hall 1992). However, this statement is not consistent throughout the literature; Cox et al. (2006) found that if weeds were controlled before the corn reached the V3-V4 growth stage there was no significant yield loss; however by delaying weed control until the V5-V6 growth stage the crop experienced delayed silking and a yield reduction (Cox 2006). The CWFP is subject to fluctuations field-to-field and year-to-year because of varying weed species and composition, weed density, relative time of weed and crop emergence, nutrient availability, soil type, weather patterns, planting date and crop hybrid. For example, an increase in available nitrogen early in the growing season improved a corn population’s tolerance to weed pressure and shortened the CWFP (Evans, et al. 2003). This phenomenon is not fully understood although the authors hypothesized that it is partially due to the increased rate of growth and larger leaf size of a more competitive corn crop. When a crop is exposed to more than one plant stress there is a synergistic impact on yield. Therefore, as abiotic and biotic stresses accumulate, the CWFP is extended.
1.2.3 Control Options

Growers have a suite of weed control options available to them in corn. Options for weed control include; chemical control, such as herbicides, mechanical control, such as tillage or scuffling, biological control, such as bio-pesticides and cultural control such as the use of cover crops, row width, seeding rate, and fertilizer rate. Most farmers use integrated weed management strategies to optimize weed control and minimize both cost and off-target environmental impacts.

1.2.3.1 Mechanical Weed Control

Tillage, the physical manipulation of the soil for seedbed preparation or inter-row cultivation, has historically been the most widely used method of weed control. Tilling the seed bed prior to seeding provides a weed-free field for the start of the season eliminating the need for a pre-plant burn down herbicide application. Tillage alone doesn’t provide any residual weed control and is less effective on weeds that propagate via stolons or rhizomes. Early germination of annual broadleaf weed seeds may be promoted in conventional-tillage systems as more weed seeds are brought to the surface (Teasdale and Mirsky 2015; Froud-Williams 1983). The effect of spring tillage varies greatly between weed species; large crabgrass (*Digitaria sanguinalis* L.), giant foxtail (*Setaria faberi* Herrm.), smooth pigweed (*Amaranthus hybridus* L.) and common ragweed (*Ambrosia artemisiifolia* L.) have all shown greater emergence when exposed to spring tillage; however, other small seeded summer annuals, eastern black nightshade (*Solanum ptycanthum* Dun.) and velvetleaf (*Abutilon theophrasti* Medic.) are largely unaffected (Myers et al. 2005). Vencill and Banks (1994) investigated the use of a disk harrow at a depth of 15 cm prior to planting grain sorghum and found no impact
on total weed density or *Amaranthus* sp., treatments with pre-plant tillage and no in-season control experiences yield losses of up to 100% (Vencill and Banks 1994). Buhler (1992) investigated the uses of different tillage systems in conjunction with the use alachlor (1.7 kg ai ha$^{-1}$) and atrazine (1.1 kg ai ha$^{-1}$) applied pre-emergence and found that compared to no-till, chisel plowing, conventional-til (moldboard plowed and disked twice) and ridge-till reduced populations of redroot pigweed (*Amaranthus retroflexus*) by 20%, 92% and 95% respectively. Tillage practises alone are not able to provide broad-spectrum, full-season weed control required to eliminate corn yield loss due to weed interference; however, they have value when used as a part of an integrated weed management strategy.

1.2.3.2 Chemical Weed Control

Globally, herbicides are the most widely used method of crop protection from weed interference. Herbicides were rapidly adopted by farmers in the late 1950’s after the registration of 2,4-D in 1946 (Pest Management Regulatory Agency 2009). Herbicides are economical, easy to apply, efficient and government-regulated to minimize any risk to human health or the environment. A study prepared for CropLife Canada in 2015 determined that 22.8% of corn yields in Canada can be attributed to pest control with the use of pest control products; including herbicides, fungicides and insecticides (RAIS 2015). When used in combination with no-till practises, the use of herbicides reduces the risk of erosion and benefits soil health. There are a broad range of herbicide options for farmers to choose from, this range in choice is partially what makes chemical control so attractive for farmers. The efficacy of herbicides is influenced by a wide variety of factors including; soil characteristics, precipitation, temperature, weed species
composition, weed density, and weed size and stage of development. The specific weeds controlled and duration of residual control is dependent on the herbicide used, weather and soil characteristics. Each herbicide has a different spectrum of weeds controlled, therefore, tank-mixtures of herbicides are often recommended to provide more consistent and wide spectrum weed control. In Ontario there are 18 herbicide products registered for use in corn pre- or early-post emergence and 18 herbicide products registered for use in corn post-emergence (OMAFRA 2016). When selecting a herbicide farmers take into account the weed spectrum, the time of the application, and the price of the product.

Herbicides can be either applied directly to the soil or to the foliage of emerged weeds. Soil-applied herbicides are applied prior to crop seeding or emergence, these herbicides may provide control of weeds that have emerged in the early spring, or provide residual control into the growing season. Soil-applied herbicides require rainfall to dissolve the herbicide into the soil water solution so that they can be taken up by the weed roots or shoots, for most pre-emergence herbicides precipitation is required 7-14 days after application for optimal performance (Stewart 2012). Foliar-applied (or POST) herbicides are applied after the crop and weeds have emerged and provide in-season control of weeds or weeds that have escaped following a soil applied residual herbicide; some foliar herbicides also provide residual control. POST herbicides have a period of time after application, termed the ‘rain-fast period’, where precipitation is undesirable and can result in reduced efficacy (Stewart 2012).

To ensure that corn fields remain weed free for the complete growing season, many farmers will implement a two-pass weed control program. A two-pass system
involves the use of a soil applied residual herbicide followed by a POST, in-crop herbicide, usually with a different mode of action. Two-pass weed control programs consistently provide a longer period of weed control compared to single-pass programs (Owen et al. 2015). Herbicides continue to be an integral component of weed management in corn in Ontario. Corn producers are moving toward integrated weed management strategies with practises that are best for both the environment and their farming operations.

1.2.3.3 Cultural and biological weed control

Cultural practises, such as crop rotation, row spacing and the use of cover crops, augment weed control efforts. There are numerous benefits to crop rotation including: disease prevention, insect prevention, improved soil health, and weed control. Weeds and crops that have similar life cycles tend to associate together; for example, winter annual grasses tend to be a larger problem in winter wheat because seedling emergence and flowering periods occur at the same time (Moyer et al. 1994). Compared to monoculture, crop rotations reduced weed densities in 19 of the 25 cases summarized by Liebman and Ohno (1998). It is commonly accepted that a longer, more diverse crop rotation will have a greater impact on reduction of weed densities and the weed-seed bank. However, even a two crop corn-soybean rotation consistently lowered Canada fleabane densities compared to a continuous soybean system (Davis 2009). In rotation, forage and silage crops are particularly effective at suppressing weeds, and reducing the weed seed bank, because they are often harvested before germinated weeds can reach maturity and produce viable seed (Harker et al. 2003).
Improved crop competitiveness aids in weed management and reduces the potential impact for weeds to have on yield. Velvetleaf seed production is reduced by 99% when corn is planted at 128,000 plants ha\(^{-1}\) compared to the standard population of 64,000 plants ha\(^{-1}\) (Teasdale 1998). Earlier maturing hybrids with higher leaf area index, and greater leaf distribution influence the time to complete canopy closure of the crop. A crop that reaches canopy closure more quickly is more competitive with weeds and often results in reduced weed survival and reduced weed seed return (Jha et al. 2017). Narrower row spacing and increased seeding density both increase the speed of canopy closure, reducing the amount of light available for weed seeds to germinate and compete with a crop (Jha et al. 2017).

The use of cover crops as a weed management tool in grain crop production is a relatively new concept; until recently, maintaining pristine fields was the primary objective for farmers. Originally developed as an organic practice, cover cropping has been adopted by both organic and conventional farmers for both the soil health and weed suppression benefits. It is evident from the literature that a living cover crop will suppress weeds; a dessicated cover crop can also suppress weeds, although this effect is limited to the earlier portion of the growing season (Blackshaw 2001, Teasdale and Daughtry 1993). The presence of a cover crop, either as a living mulch or a dead residue, on a soil surface; decreases the light quality and quantity required for weeds to germinate, lowers the daily temperature change and physically impedes the progress of weed seedlings (Teasdale and Mohler 1993). Cover crops that produce significant amounts of biomass and are able to over-winter are the best suited for weed suppression (Teasdale and Mohler 2000). Cover crops are terminated with the use of a
non-selective herbicide, or the use of a roller-crimper, before they become detrimental to the yield of the crop. In conclusion, the use of cover crops for weed suppression is a feasible option for farmers. When used in conjunction with other weed control methods and cultural practices they can be a significant component of an integrated weed management program.

1.2.4 Herbicide-Resistant Crops

The introduction of herbicide-resistant crops caused an industry paradigm shift; and began a new era of weed control for farmers. Through genetic engineering, scientists developed crops that were near isolines of conventional varieties with the exception that they were resistant to previously lethal, broad-spectrum, non-selective herbicides. The introduction of herbicide-resistant technology largely changed the way weeds were controlled. The first herbicide-resistant crop commercialized was glyphosate-resistant soybean in 1996; since then glyphosate-resistant cotton, corn, canola and sugar beet have also been introduced (Shaner 2000). These crops provided farmers with an easy and economical solution for in-crop weed control and were quickly adopted. Farmers were now able to seed a glyphosate-resistant hybrid/cultivar, apply glyphosate post-emergence and have complete broad spectrum weed control. Two years after the launch of glyphosate-resistant soybeans, 40% of US soybean acres were seeded with the GE crop; today 98% of corn produced in Canada is seeded to herbicide-resistant hybrids (RAIS Inc. 2015).

The adoption of herbicide-resistant crops brought with it an increase in the use of glyphosate, and a decrease in the use of alternative mode-of-action herbicides. One of the consequences of the over dependence on a single mode-of-action herbicide is an
increase in selection pressure for weeds resistant to that herbicide. The adoption of herbicide-resistant crops and the subsequent increase in glyphosate-only applications accelerated the evolution of glyphosate resistant weeds. As glyphosate resistance becomes more prevalent, farmers are again required to change their weed management practices; in recent years growers have moved toward a better understanding of the relationship between single mode-of-action herbicide use and the evolution of herbicide-resistant weeds (Johnson 2009).

1.3 Glyphosate

Glyphosate [N-(phosphonomethyl) glycine] is the most commonly used herbicide globally. First commercialized by Monsanto in 1974, glyphosate quickly became popular because it is an efficacious, non-selective, systemic herbicide with minimal residual or toxicity concerns. Originally, glyphosate was used as a pre-plant burn down herbicide or pre-harvest desiccant; however, after the introduction of glyphosate-resistant crops, glyphosate could also be used as a postemergence in-crop herbicide. Glyphosate is registered for use in food and non-food field crops as well as for industrial, commercial or residential uses (Monsanto Canada Inc., 2015). As the agriculture industry develops new technologies and best management practises, glyphosate continues to be the most widely used herbicide for weed management.

1.3.1 Chemical Classification and Formulation

Technical glyphosate, the compound in its purest form before being formulated into a commercial product, is classified as a weak organic acid; the herbicidally active compound used in commercial formulations is a salt of glyphosate. The chemical formula for technical glyphosate is C₃H₈NO₅P with a molecular mass of 169.1. As a free
acid, technical glyphosate has low water solubility (1.16 g L\(^{-1}\) at 25\(^{\circ}\)C). When formulated as a salt, water-solubility of glyphosate increases (Knuutila & Knuuttila, 1985). The solubility of glyphosate acid is too low for it to be an effective herbicide which is why the majority of commercial glyphosate products are formulated as salts of glyphosate (Dill, et al., 2010). Glyphosate has a low volatility (2.59 x 10\(^{-5}\) Pa at 25\(^{\circ}\) C) and a high density (1.75 g cm\(^{-3}\)). These two characteristics suggest that evaporation from the treated foliar surface or non-target injury due to lingering spray droplets in the air after application is highly unlikely (Dill et al. 2010). Commercial formulations also include a surfactant, most commonly polyoxethylene amine (Takacs, et al., 2002). The surfactant improves retention on the leaf surface so it can be absorbed as opposed to rolling off of the waxy cuticle. The most common trade name for glyphosate sold by Monsanto is Roundup™. The early glyphosate formulations contained 360 g ae L\(^{-1}\) of the isopropyl amine salt of glyphosate, which has transitioned to 540 g ae L\(^{-1}\) potassium salt of glyphosate in recent years; although, some products are still available in the 360 g ae L\(^{-1}\) formulation (Abraham, 2003). Other commercial formulations include sodium, tetramethylsulfonium (TMS), potassium, ammonium, monoethanolamine and dimethylamine salts (Dill et al. 2010).

### 3.2 Antagonism and Synergy

To reduce the number of agrochemical applications made in a growing season, many farmers will co-apply crop protection products in a single pass. Depending on the physiochemical properties of the products being mixed, this can result in an antagonistic, additive or synergistic response. Glyphosate efficacy is reduced when tank-mixed with positively-charged micronutrients such calcium, magnesium, zinc and
manganese (Bailey et al. 2002; Bernards et al. 2005; Scroggs et al. 2009). For instance, common lambsquarters (*Chenopodium album* L.) control was reduced by 14% and 36% when glyphosate was applied with zinc and manganese, respectively (Chahal et al. 2012). This antagonism is attributed to positively charged cations associating with negatively charged glyphosate anions, which effectively deactivates the herbicidal molecule (Thelen et al. 1995). This antagonism can be reversed by the addition of ammonium sulphate (AMS) to the spray solution prior to adding glyphosate (Thelen et al. 1995).

Weed control efficacy with glyphosate when tank-mixed with other herbicides or fertilizers is weed species specific. When glyphosate was tank-mixed with either chloriumuron of cloransulam-methyl control of pitted morningglory (*Ipomoea lacunose* L.) and hemp sesbania (*Sesbania exaltata* (Raf.) Cory) was reduced (Shaw and Arnold 2002); conversely, there was no antagonism of the two products when applied to barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) (Jordan et al. 1997). Reduced glyphosate efficacy when tank-mixed with manganese fertilizer has been reported has by Bernards et al. (2005). When tested in Ontario, tank-mixing glyphosate with commercial manganese formulations resulted in up to a 100% reduction in efficacy (Soltani, et al., 2011). Similarly, glyphosate antagonism with clay-based herbicides, such as simazine and atrazine, has also been observed. However, the antagonism can be overcome by increasing the rate of glyphosate (Appleby & Somabhi, 1978; Selleck & Bailey, 1981). Antagonistic effects of herbicide tank-mixes with glyphosate are herbicide and weed species specific.
1.3.3 Glyphosate Uptake and Mobility

Glyphosate is absorbed through the foliage and is a phloem mobile or symplastically translocated herbicide. In corn, droplet size has a significant impact on the amount of glyphosate retained after application and eventually absorbed through the leaf cuticle. A finer droplet achieves higher initial retention, however; the highest adsorption occurred when coarse droplets were applied. Coarse droplets proved to be the most efficacious even though they did not have the highest initial retention rates (Feng et al. 2003). Due to glyphosate’s strong affinity for soil particles and rapid deactivation once in contact with soil, there is very little entry into plants via the root system (Giesy et al. 2000). After glyphosate is absorbed through the foliar tissue, it follows a typical source-sink pattern and is transported to the meristematic tissue (Gougler and Geiger 1981). Within a few hours after application, glyphosate is transported through the waxy cuticle of the leaf and begins moving throughout the plant. Glyphosate uptake, translocation and efficacy are influenced by several interdependent elements, including glyphosate rate, water carrier volume, droplet size, plant cuticle thickness, weed species, weed size at the time of application, and weather conditions prior to and after spraying (Kirkwood and McKay 1994; Jordan 1997). The efficacy of glyphosate is largely determined by the amount of glyphosate transported to the growing point (Feng et al. 2003).

1.3.4 Glyphosate Degradation and Metabolites

The primary metabolite of glyphosate, both in plants and in the environment, is aminomethylphosphonic acid (AMPA) (Stock, 1991; Gout, 1992). In plants, glyphosate is degraded very slowly and the primary compound found is glyphosate itself. The levels
of AMPA measured in plant tissue after a glyphosate application are approximately 10% those of glyphosate. The specific ratio of glyphosate:AMPA is subject to fluctuation depending on the crop or weed species being studied; however, the conclusion that the primary residue found in plant material is glyphosate itself, is constant across the literature (Duke 2011).

In soil the glyphosate molecule is readily broken down by soil microbes. There are two major processes that effectively inactivate the glyphosate molecule: breakage of the C-P bond or breakage of the C-N bond (Duke, 2011). The C-P bond can be broken by the enzyme C-P lyase, found in Pseudomonas sp., Rhizobium spp. and Streptomycyes sp., or by the presence of manganese oxide in the soil (Barrett and McBride 2005, Duke 2011, Pizzulm et al. 2009). The resulting metabolites from this reaction are sarcosine and inorganic phosphate (Barrett and McBride 2005). The C-N bond can be broken by glyphosate oxidoreductase (GOX), found in Arthrobacter atrocyaneus and Pseudomanas sp., resulting in formation the metabolites AMPA and glyoxylate. The half-life of glyphosate in soil can range anywhere from <1 to 151 days; however, a meta-analysis found that the average half-life is approximately 38 days and always under a year (Giesy et al. 2000). The degradation of glyphosate in soil is a function of soil type, soil microbe population and weather conditions.

1.3.5 Mechanism of Action

Glyphosate stops the production of aromatic amino acids, tyrosine (Tyr), tryptophan (Trp) and phenylalanine (Phe), through inhibition of the 5-enolpyruvateshikimic-3-phosphate synthase (EPSPS) enzyme in the shikimate acid pathway. Amino acids are essential for life in all living organisms; plants, fungi and
bacteria obtain the necessary amino acids through synthesizing them themselves; humans and animals cannot synthesize amino acids and must rely on various components of their diets to obtain them. The aromatic amino acids are precursors to a variety of compounds that are crucial to plant development, including alkaloids, flavonoids, lignans and lignin (Maeda and Dudareva 2012). Of all the aromatic amino acids, Phe derived compounds contribute up to 30% of the organic matter in some plant species. Herbicidal symptoms and growth inhibition can be reversed when the three aromatic amino acids are applied to plant tissue previously treated with glyphosate; this reversal method is effective for both microorganisms and higher plants (Jaworski, 1972). Glyphosate is only effective on species with the shikimate acid pathway including; plants, fungi and bacteria

1.3.6 The Shikimate Acid Pathway and the EPSPS enzyme

The shikimate acid pathway, located in the plastids, is responsible for the formation of aromatic amino acids in plants. There are seven enzymatic processes that transform phosphoenolpyruvate (PEP) and erthrose-4-phosphate (E4P) into chorismate (Amrhein, et al., 1980). Chorismate is then transformed into tryptophan, tyrosine and phenylalanine (Bentley, 1990; Herrmann, 1995). 5-enolpyruvateshikimic-3-phosphate (EPSP) synthase is the penultimate step in the shikimate acid pathway. The enzyme triggers the transfer of the enolpyruvate moiety from PEP to shikimate-3-phosphate, an anabolite of the previous steps in the shikimate acid pathway (Schronbrunn et al. 2001). Glyphosate blocks the binding site for PEP on the EPSP synthase enzyme, effectively preventing the reaction to continue (Schronbrunn et al. 2001).
1.3.7 Safety and Regulatory Approval

To be sold as a pest control product to Canadian farmers, glyphosate was reviewed by the Pest Management Regulatory Agency (PMRA), a division of Health Canada. The role of PMRA is to determine “if proposed pesticides can be used safely when label directions are followed and will be effective for intended use”. A pesticide must not pose reasonable risk to human health or the environment, in order to be approved for use in Canada. When reviewing the safety of agrichemicals it is important to understand the difference between risk and hazard. A hazard is any product or process that under imaginable conditions has the potential to cause harm to a person or the environment. A risk is calculated as hazard multiplied by exposure; meaning that the product in question must be present in a sufficient quantity to cause harm under conditions that permit it to do so (Health Canada PMRA, 2018). The PMRA basis its regulatory decision on whether the Canadian consumer will be exposed to the substance under conditions that create reasonable risk.

Glyphosate has been registered by the PMRA, the US Environmental Protection Agency (USEPA) and the European Commission (EC). Regardless, glyphosate still faces heavy public opposition. The large amount of public opposition has generated numerous studies investigating the relationship between glyphosate and human health. Toxicological studies have determined that the acute oral LD$\text{50}$ of glyphosate, when tested on rodents is in the 2000 to 5000 mg kg$^{-1}$ range (Williams et al. 2000), which is significantly higher than common substances such as caffeine (192 mg kg$^{-1}$) or aspirin (1240 mg kg$^{-1}$). After thorough review, and over 90 000 individual studies, there is no
evidence of a correlation between glyphosate use and any deleterious effects on human health (Mink et al. 2012, Mink et al. 2011).

1.4 Herbicide Resistance

Herbicide resistance is defined as “the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type” (WSSA 1998). In contrast, herbicide tolerance is the natural ability of a plant to survive a herbicide application (WSSA 1998). Resistance is induced, tolerance is inherent. Eleven years after the introduction of modern herbicides in 1946, a population of 2,4-D resistant wild carrot (*Daucus carota* (L.) in Ontario, Canada became the first recorded case of herbicide resistance globally (Switzer, 1957). Since then, 252 weed species worldwide have been confirmed with herbicide resistance (Heap, 2017).

Plant species can evolve resistance to one or multiple herbicide families. The latter includes cross resistance, involving a single mechanism conferring resistance to more than one herbicide family, and multiple resistance which involves more than one mechanism of herbicide resistance. In Canada, there are currently 65 unique cases of herbicide resistance across 37 weed species (Heap, 2017). Of these, five species have confirmed resistance to glyphosate: common ragweed (*Ambrosia artemisiifolia* L.) (Van Wely et al. 2015), giant ragweed (*Ambrosia trifida* L.) (Sikkema et al. 2009), Canada fleabane (*Conyza canadensis* (L.) Cronq) (Byker et al. 2013), kochia (*Kochia scoparia* (L.) Schrad.) (Hall et al. 2014) and common waterhemp (*Amaranthus tuberculatus* (Moq.) Saur var. *rudis* (Costea Tardif)) (Schryver et al. 2017). Additionally, all of these weed species have populations with resistance to Group 2 (ALS-inhibitor) herbicides, and populations with multiple resistance to both glyphosate and Group 2 herbicides.
Farmers will need to implement best practices for herbicide stewardship to reduce the development of herbicide resistance. in order to maintain the value and efficacy of products currently available.

1.4.1 Evolution of Herbicide Resistance

Herbicide resistance is a product of genetic mutations combined with high selection pressure (Dinelli et al. 2006). Repeated applications of herbicides with the same mode of action effectively control all susceptible biotypes in a population, leaving only resistant plants to reproduce seed for the next generation. In a weed population, the individuals least susceptible to herbicide applications are selected for, effectively killing all susceptible plants and leaving only resistant biotypes to reproduce seed (Delye et al. 2013). Glyphosate resistance can be coded by either one or more dominant nuclear genes, one or more incompletely dominant genes or a recessive gene (Powels, 2006). Resistance produced by a single major gene mutation can spread across a population much more quickly than resistance produced by multiple genetic mutations (Mithila and Godar 2013). In the case of common waterhemp, a resistant population can vary in its susceptibility. This is attributable to different plants containing all, or a portion of, multiple incompletely dominant genes that code for resistance (Zelaya and Owen 2005). Herbicide resistance coded for by a recessive allele moves much more slowly through a population, following Mendel’s third law of dominance. Plants with a heterozygous or homozygous-dominant genotype will still be susceptible to the herbicide in question and only the homozygous-recessive genotype will survive, greatly reducing the population (Jasieniuk 1996). Almost all cases of herbicide resistance are transmitted via nuclear inheritance, through pollen and ovules of resistant plants.
Many factors influence the rate at which a weed population will develop resistance. Factors such as incidence of resistant individuals in the initial population, weed biology and dominance of the resistant gene are outside of a farmer’s control (Powles and Yu 2010). The greater the number and dominance of resistant alleles in the initial population, the higher the likelihood that resistance will be selected for (Mithila and Godar 2013). Reproductive biology of the weed in question also impacts resistance development. A dioecious species will have greater gene flow between generations, facilitating the movement of resistance compared to a predominately self-pollinating species (Loureiro et al. 2016).

Management factors such as tank-mixing and rotating herbicides with different modes-of-action, applying herbicides according to label directions and proper scouting habits are practices that farmers can use to slow the development of resistance. Tank-mixing herbicides with different modes of action often reduces the development of resistance because the likelihood that a single plant has a genetic mutation for resistance to both modes of action is low. Rotating herbicides throughout a growing season and year-to-year will also decrease the selection pressure for single mode of action resistance (Boerboom, 1999). Using a lower than registered rate or spraying a herbicide when weed size exceeds the label directions are both practices that result in reduced herbicide efficacy, ultimately leaving the most resilient plants in the population to produce seed the following year (Manalil et al. 2011). When using herbicides, the development of resistance is always a possibility, but the process can be slowed by using proper management practices and herbicide stewardship.
1.4.2 Mechanisms of Resistance

There are several possible mechanisms which confer herbicide resistance in a plant, and they can be classified into one of two groups: target site and non-target site resistance. Herbicides effectively inhibit various enzymes and critical processes within the plant. For example, glyphosate inhibits the EPSPS enzyme in the shikimate aromatic amino acid pathway (Steinruck and Amrhein 1980). Target site resistance involves either a change in a specific enzyme that alters the herbicide bonding site, rendering the herbicide incapable of occupying the site, or gene amplification, which increases the expression of the gene coding for the enzyme, producing more of the enzyme than the herbicide can inhibit (Nandula et al. 2010). Target site resistance is well-understood and testing for it is relatively straightforward (Nandula et al. 2010). Non-target site resistance includes any change within the plant that is not at a target site. This can include anything that inhibits the herbicide from reaching the target site or detoxifying it before it arrives (Yuan, 2007). Testing for non-target site resistance is not as well understood and much more complex. Of the two groups of herbicide resistance mechanisms, target site resistance is better understood.

1.4.2.1 Target Site Resistance

Target site resistance involves a mutation to the gene encoding the target site that renders the herbicide molecule ineffective in inhibiting the enzyme (Mithila and Godar 2013). Different mutations to a target site can result in resistance. For example, 16 distinct mutations have been identified that result in modification of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, resulting in resistance to
glyphosate (Gaines and Heap 2017). An altered target site was reported as a mechanism for glyphosate resistance in common waterhemp populations in Mississippi and Missouri (Nandula et al. 2013; Schultz et al. 2015). Target site mutations were also reported to confer acetyl-CoA synthase (ALS) and protoporphyrinogen oxidase (PPO) inhibitor resistance in waterhemp (Tranel et al. 2017). The more common mechanism of target site resistance to glyphosate is over-expression of the EPSPS enzyme (Nandula et al. 2010). This was reported as the cause of glyphosate resistance in Canada fleabane and Palmer amaranth (Amaranthus palmeri S. Wats.) (Dinelli et al. 2006; Gaines et al. 2010). A 2015 Illinois study identified EPSPS gene amplification as the primary resistance mechanism in 20 of 22 confirmed glyphosate resistant (GR) waterhemp populations collected throughout the state (Chatham, 2015). A mutation resulting in target site resistance can manifest as either an altered target site or an over expression of the targeted enzyme.

1.4.2.2 Non-Target Site Resistance

Non-target site resistance is any mechanism that prevents the herbicide from reaching the target site at a lethal dose; this includes enhanced metabolism, sequestration away from the target site, and inhibition (Yuan et al. 2007). Examples of non-target site resistance include reduced uptake, retention, absorption or translocation and increased metabolism (Sammons and Gaines 2014). Increased herbicide metabolism is the primary mechanism of non-target site resistance (Chatham, 2015). Currently, four gene families have been identified as major contributors to non-target site resistance: P450s, glutathione S-transferases (GSTs), glycosyltransferases and ABC transporters (Yuan et al. 2007). Although not confirmed as a source of evolved
resistance in wild species, P450s are the main enzymes responsible for detoxification of herbicides and used in the production of herbicide resistant crops (Yuan et al. 2007; Schuler and Werck-Reichhart 2003). When CYP2B6, a gene in the P450 family, was inserted into common rice (*Oryza sativa*), the resulting transgenic crop displayed a high tolerance to thirteen of seventeen herbicides including metolachlor, pendimethalin and chlorotoluron (Hirose et al. 2005). Similar to P450s, GSTs and glycosyltransferases are plant enzymes that naturally play a role in herbicide metabolism and detoxification (Yuan et al. 2007). The GSTs were first associated with atrazine and ACCase inhibitor resistance in numerous grass species (Jensen et al. 1977, Cummins et al. 2013). In contrast to the other three families, ABC transporters are primarily responsible for elimination of toxic substances and their metabolites from the cell. When upregulated in a plant, they are able to more quickly move a herbicide away from its target site and into a vacuole (Yuan, 2007). Glyphosate needs to be translocated to meristematic tissue to be effective, the region of the plant where the majority of the EPSPS enzyme is synthesized. By redirecting glyphosate to older tissue away from the growing point, a plant effectively achieves resistance without truly metabolizing or detoxifying the herbicide (Shaner, 2009). In Canada fleabane, sequestration of glyphosate away from the growing point and into a vacuole is responsible for reduced glyphosate translocation, resulting in resistance (Ge et al. 2010). This version of non-target site resistance is relatively rare and has only been recorded as a mechanism of resistance for one other herbicide, paraquat (Preston et al. 2005). As our understanding of the relationship between herbicide resistance and genetics evolves, we are better able to identify non-target site resistance mechanisms.
1.4.3 Resistance in waterhemp

There are waterhemp populations with confirmed resistance to six different herbicide Groups (synthetic auxins and ALS, PPO, Photosystem II, HPPD and EPSPS inhibitors) and many of these have multiple resistances to 2, 3, or 4 modes of action (Heap, 2017). In 2016, an Illinois waterhemp population was confirmed to have multiple resistances to five herbicide Groups: synthetic auxins and ALS, Photosystem II, PPO and HPPD inhibitors (Heap 2017, Patzoldt et al. 2005). First discovered in Missouri, US in 2005, GR waterhemp is now considered one of the most troublesome weeds in the American mid-west and is estimated to cover over 1.2 million hectares (Light et al. 2011). GR waterhemp was first confirmed in 2014 in Lambton County, Ontario; since then, populations resistant to ALS inhibitors, PPO inhibitors and triazine herbicides have also been confirmed (Schryver et al. 2017). The occurrence of PPO and HPPD resistance in common waterhemp is particularly notable. Waterhemp is the first known case of HPPD inhibitor resistance globally (McMullan and Green 2011) and PPO resistant waterhemp represents a new resistance mechanism. PPOs are responsible for the production of heme and chlorophyll in a plant, coded by two distinct genes; a codon deletion in one of these genes results in resistance to PPO inhibitors (Lee et al. 2008). This mechanism of resistance is novel and to the best of our knowledge has not been recorded for any other species or herbicide (Patzoldt et al. 2006). Considering the number of herbicide options that have become ineffective, the job of controlling common waterhemp is a challenging one for farmers and researchers alike. Farmers facing 5- and 6-way resistance are forced to either heavily rely on the few effective modes of action left or adopt non-chemical control options.
1.4.4 Herbicide Best Management Practices

There are production strategies available to farmers to alleviate the development of herbicide resistance. Acknowledging that best management practices will vary among farms, all reviews suggested similar practices to reduce the development of herbicide resistance. Maintaining quality data and records year to year are crucial to making informed decisions. Records on herbicides applied in previous years, their efficacy and weed species present allow for proper monitoring and identification in the event that a farmer suspects a resistant population. Collecting appropriate data on all fields permits farmers to make the best possible weed management decisions on individual fields. Integrated weed management solutions involve using herbicides alongside non-chemical options for the control of weeds, thus reducing selection pressure for single-mode of action resistance (Harker, 2013). Proper sanitation habits and environment control (cleaning of equipment between fields, control of weeds around field borders and planting weed-free seed) all decrease the spread of seed geographically and reduces the weed seed bank (Beckie and Harker 2017). The other core practice in effectively managing resistance is the use of multiple effective modes of action and rotating herbicides; it is also the most costly. When recommending herbicide best management practices, it is important to recognize that the effectiveness and feasibility of most options vary depending on climate, geographic area, availability of equipment and biology of the weed in question (Beckie and Harker 2017).
1.4.5 Economics of resistance

Economics influence decisions made on the farm. Herbicide resistance can cause crop yield loss from uncontrolled weeds or increased costs through multiple applications of herbicides. Alternative methods such as tillage and/or cover cropping provide value in an integrated weed management strategy but are generally less effective and less economical than their chemical counterparts (Blackshaw et al. 2007). Due to the complexity and diversity of weed management systems, determining the true cost of resistance management and the economic benefits of such practices is difficult. Orson (1999) concluded that the cost of proper tank-mixing habits to prevent resistance is less than the cost to manage resistance once it develops. However, the author did note that many external factors influence resistance and preventative measures do not guarantee that resistance will not develop nor can they accurately predict the speed at which resistance will develop (Orson, 1999). These results are consistent with Edwards et al. (2014), who concluded that although the costs associated with implementing best herbicide management practices were higher, on average so was the profitable yield and ultimately net returns. Environmental and economic sustainability much both be taken into consideration when determining crop production practises.

1.5 Photosystem II Inhibitors

Photosystem II inhibitors are a widely used class of herbicides comprised of many chemical families and active ingredients. Photosystem II inhibitors offer flexible application timing, broadspectrum weed control, and crop safety. The photosystem II inhibitors are further divided by the WSSA mode-of-action classification system into three separate Groups (5, 6 and 7) because the sites of action are different and there is
no cross resistance between Groups (Shaner, 2014). The triazine herbicides: atrazine, simazine and metribuzin are classified as Group 5; the benzonitrile and benzothiadiazoles chemical families are classified as Group 6; and the substituted ureas and amides are classified as Group 7. For the purpose of this literature review, the focus will be on the triazine herbicide and atrazine.

Atrazine is primarily used for broadleaf weed control in corn production and can be applied preplant (PP), preemergence (PRE) or early-postemergence (POST), and works well in conservation-and no-till systems (Fuglie, 1999). As of 2013/14, atrazine was the third most widely used herbicide in Ontario, with 293 208 kg active ingredient (ai) applied annually, ahead of glyphosate and S-metolachlor at 1 151 051 kg ai and 547 774 kg ai applied per year, respectively (Farm & Food Care Ontario, 2015). The amount of atrazine used in Ontario has decreased by 83% since 1983, which can be attributed to the registration of new herbicides in corn, a decrease in the maximum labelled rate for atrazine, and an increase in use of glyphosate (Farm & Food Care Ontario, 2015). However, atrazine remains an important, low-cost herbicide for weed management in corn in Ontario.

1.5.1 Registration

Atrazine, 2-chloro-4-ethylamino-6-isopropylamino-s-triazine, was invented in 1958 for use as a PRE and POST herbicide in corn production (Neighbours, 1970; Ulrich, 2012). Atrazine was registered with the Canadian PMRA by Syngenta Canada Inc. under the trade name Aatrex Liquid 480 Herbicide with a formulation of 480 g ai L⁻¹ (Pest Management Regulatory Agency, 2017). Atrazine is currently registered for the control of annual broadleaf and grass weeds in corn (field, sweet, and seed) and
sorghum (Eastern Canada only). As of 2015, thirteen crop protection products are registered with PMRA that list atrazine as an active ingredient, either alone or in combination (Pest Management Regulatory Agency, 2015). Following a ban on the use of atrazine in Europe by the European Commission due to increasing environmental concerns, the Canadian PMRA initiated a special review of atrazine. Currently, there is an annual maximum use rate of 1.5 kg ai ha\(^{-1}\) year\(^{-1}\) for atrazine and ground equipment must be used for application (Pest Management Regulatory Agency, 2015). The re-evaluation of atrazine is still in progress.

1.5.2 Absorption and Translocation

Atrazine is absorbed by roots and foliage (McCloskey and Bayer 1990; Price and Balke 1983). Root absorption of atrazine occurs through simple diffusion across the tonoplast and plasma membrane (Price and Balke 1983). Atrazine absorption through root structures of corn, oats and tubers of potato occurs relatively quickly; the rate of absorption levels off in all three species after 10 to 20 minutes (Price and Balke 1983). Root uptake is influenced by climatic conditions; warmer soil temperatures and lower relative humidity positively influence the rate of root absorption (Wax and Behrens 1965).

The efficacy of atrazine is affected by foliar interception, retention on the leaf surface, absorption, and translocation. Postemergence atrazine applications need to reach the meristem of target weed species to be controlled (Thompson and Slife 1970). The meristem of emerged grasses is protected by the leaf sheath and not exposed to the herbicide; the protected meristem greatly reduces the impact of the herbicide. As a result, the efficacy of atrazine applied POST on grasses is greatly reduced (Burnside
and Wicks 1964; Thompson and Slife 1970). Foliar applications are also affected by weather conditions at and shortly after application. For instance, a rainfall event too soon after application will wash the herbicide off of the leaf surface. This reduces foliar uptake but the herbicide may be taken up by the roots assuming it remains dissolved in soil-water solution (Thompson and Slife 1970).

Atrazine is apoplastically translocated. Once absorbed into the plant, atrazine moves readily though the xylem (Roeth, 1971). Roeth and Lavy (1971) conducted studies on the translocation using radio-labelled atrazine following root uptake. They observed that 4 weeks after application the majority of the atrazine had been translocated from the roots into the leaves of sudangrass (Sorghum x drummondii), sorghum [Sorghum bicolor (L.) Moench] and corn (Roeth, 1971). This pattern was seen in all three crops; however, the corn roots retained larger amounts of atrazine than either the sudangrass or the sorghum (Roeth, 1971).

1.5.3 Metabolism in Plants

Differential metabolism is the primary basis of selectivity with atrazine. Degradation occurs through three separate mechanisms, which is species and location dependent. Wheat (Triticum aestivum L.) converts atrazine into a 2-hydroxy derivative, hydroxyatrazine, via 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoazoxazine (benzoxazinone) (Shimabukuro, 1967). In grain sorghum and field pea (Pisum sativum L.), atrazine is metabolized through N-dealkynation into its isopropyl derivative, with minor traces of it's ethyl-derivative (Shimabukuro, 1967). Atrazine can be metabolized via conjugation via glutathione s-transferase (GST), which occurs exclusively in the aboveground portion of the plant (Shimabukuro et al. 1970). The dealkynated derivatives of atrazine retain their
phytotoxic properties; in contrast, the hydroxyatrazine is nontoxic in plants (Gysin and Knulsi 1960).

Benzoxazinone, N-dealkynation and GST are all present in corn (Roeth, 1971). The method of atrazine degradation in corn is dependent on the location of atrazine in the plant. When atrazine is applied to the soil, it is taken up through the roots, and benzoxazinone is the primary detoxification mechanism (Mathew et al. 1996). When atrazine absorbed through corn foliage, atrazine detoxification by benzoxazinone is insignificant and detoxification is primarily through conjugation with GST (Raveton et al. 1997; Shimabukuro et al. 1970). Multiple mechanisms of atrazine metabolism confer a high level of tolerance in corn.

1.5.4 Degradation in Soil

Atrazine and its metabolites are persistent in the soil and have been recorded in groundwater across North America (Nousiainen et al. 2014). The half-life of atrazine in subsurface soil is up to 433 days (Vonberg et al. 2014). The half-life of atrazine in soil depends on soil characteristics, environmental conditions, and microbial activity (Krutz et al. 2009). Atrazine, and its metabolites, have been measured in soil cores 21 years after the last application (Vonberg et al. 2014). Atrazine is broken down via chemical hydrolysis and microbial degradation in the soil. As the depth of atrazine in the soil profile increases, the activity of soil microorganisms decreases, resulting in slower atrazine degradation (Shapir and Mandelbaum 1997). When soil was inoculated with *Pseudomonas* spp., atrazine degradation was enhanced; within 15 days of microbial inoculation, 90 to 100% of radio-labelled atrazine had been broken down (Shapir and Mandelbaum 1997). The soil microorganisms responsible for the breakdown of atrazine
are more prevalent in soils that have been exposed to repeated applications of atrazine (Krutz et al. 2009). The increased microbial activity with repeated applications of atrazine has resulted in reduced length of residual weed control (Krutz et al. 2009).

1.5.5 Mode of Action

Atrazine is a potent inhibitor of photosystem II, a vital component of the photosynthetic pathway (Shaner et al. 2014). Triazine herbicides inhibit the $Q_B$ binding site on the D1 protein of photosystem II, effectively displacing plastiquinone and blocking electron transport from $Q_A$ to $Q_B$ (Shaner et al. 2014; Trebst 2008). Electron transport is necessary for the reduction of $CO_2$ into ATP, NADPH and carbohydrates (Cobb and Reade 2010). Inhibition of photosynthesis is the ultimate outcome of a triazine herbicide; however, plant death occurs much more rapidly through other means. The inability to reoxidize plastiquinone results in the formation of free radicals; triplet chlorophyll and singlet oxygen (Shaner et al. 2014). Free radicals damage cell membranes and initiate lipid peroxidation which results in rapid plant death (Koh et al. 2016).

1.5.6 Symptomology

The symptoms caused by POST applications of atrazine are interveinal chlorosis followed by necrosis and plant death. The injury occurs on older leaves first, since atrazine is an apoplasticly translocated (xylem mobile) herbicide. The injury symptoms are more pronounced around the leaf margins and at the leaf tips, as the herbicide is translocated to those areas of the plant. Sub-lethal doses of atrazine cause the same injury symptoms; however, plant death does not occur (Sarangi, 2015).
1.5.7 Synergy

Synergism is when herbicides used in combination result in greater weed control than the expected additive control when they are applied alone (Blouin et al. 2004). There are synergistic effects between photosystem II inhibiting herbicides and hydroxy-phenyl-pyruvate dioxygenase (HPPD-) inhibiting herbicides (Hugie et al. 2008; Woodyard et al. 2009; Walsh et al. 2012). Hydroxy-phenyl-pyruvate dioxygenase-inhibiting herbicides prevent the formation of plastoquinone and α-tocopherols which secondarily inhibits the formation of carotenoids (Abendroth et al. 2006). In plant tissues, α-tocopherols quench free radicals, such as singlet oxygen, to reduce the amount of oxidative damage to the cells (Kim et al. 2006). The modes of action of HPPD-inhibitors and photosystem II inhibitors are complementary to one another, accelerating free radical damage which in combination results in improved weed control.

In field trials, Kohrt and Sprague (2017) investigated the use of four different HPPD-inhibiting herbicides for control of multiple resistant Palmer amaranth (*Amaranthus palmeri* L.) with and without the addition of atrazine. Atrazine provided 61% control 21 days after treatment. Mesotrione, tembotrione, tolpyralate and topramezone alone provided 69, 91, 96 and 77% control, respectively. The addition of atrazine to mesotrione and topramezone increased Palmer amaranth control by 28 and 20%, respectively (Kohrt and Sprague 2017). Similar results were recorded when atrazine and mesotrione were used in combination for control of wild radish (*Raphanus raphanistrum* L.), redroot pigweed (*Amaranthus retroflexus*) and velvetleaf (*Abutilion theophrasti*) (Walsh et al. 2012; Woodyard et al. 2009).
1.5.8 Resistance

Globally, there are 73 weed species that are resistant to the triazine herbicides (Heap, 2017). The first weed species that was confirmed to be resistant to photosystem II inhibitors was common groundsel (*Senicio vulgaris* L.) in 1970. A population of common groundsel insensitive to atrazine and simazine was found in a nursery where the herbicides had been used annually for over a decade (Ryan, 1970). In Canada, there are 11 species that are resistant to the triazine herbicides, including competitive agronomic weeds: common lambsquarters, redroot pigweed, common ragweed, and common waterhemp (*Amaranthus tuberculatus* Moq. Sauer). Of those 11 species, six have confirmed resistance to other herbicide modes-of-action: common waterhemp (Groups 2, 5, and 9), common lambsquarters (Groups 2 and 5), common ragweed (Groups 2, 5, 7, and 9), redroot pigweed (Groups 2, 5, and 7), Powell amaranth (*Amaranthus powellii* S. Wats.) (Groups 2, 5, and 7), and wild mustard (*Sinapis arvensis* L.) (Groups 2, 4, and 5) (Heap, 2017). Considering the prevalence of atrazine in Ontario’s corn production system, the evolution and spread of photosystem II inhibiting herbicide resistance poses a large problem for grain farmers.

1.6 4-hydroxyphenylpyruvate-dioxygenase Inhibitors

The 4-hydroxyphenylpyruvate-dioxygenase (HPPD) inhibiting herbicides are the newest commercial mode of action available to Ontario grain farmers. Research on this mode of action began after scientists noticed the growth of several grass species was inhibited by natural leptospermone 1 secretions from *Callistemon citrinus* L. (Dayan et al. 2007). Pyrazolynate was released in 1980 as the first HPPD-inhibitor, followed by pyrazoxyfen in 1985; both herbicides were registered for broadleaf weed control in rice (Ndikuryayo
et al. 2017). The first wide spectrum HPPD-inhibitor offering control of both grass and broadleaf species was isoxaflutole, registered in 1996 (Ndikuryayo et al. 2017). Currently, three chemical families are classified as HPPD-inhibitors and used in Ontario: isoxazoles (isoxaflutole), triketones (mesotrione and tembotrione) and pyrazolones (topramezone, tolpyralate and pyrasulfatole). Broad-spectrum weed control, crop safety and flexible application timing make HPPD-inhibiting herbicides an attractive herbicide option for Ontario farmers. Additionally, HPPD-inhibiting herbicides have proven to be an efficacious control option for weeds that have developed resistance to ALS-inhibitors, triazine herbicides and glyphosate (Schryver et al., 2017).

1.6.1 Registration and Use Patterns

There are currently seven HPPD-inhibiting herbicides registered for use by the PMRA (OMAFRA 2016). Isoxaflutole, mesotrione and bicyclopyrone are registered for use on field, sweet and seed corn at pre-plant (PP), preemergence (PRE) and postemergence (POST) application timings (OMAFRA 2016). Topramezone, tolpyralate and tembotrione are registered for POST use on field, seed and sweet corn (OMAFRA 2016). Pyrasulfotole, in a premix formulation with bromoxynil, is the only HPPD-inhibiting herbicide registered for use POST in wheat, barley, triticale and timothy (OMAFRA 2016). HPPD-inhibiting herbicides generally provide control of only broadleaf weeds, the exception being mesotrione which is also labelled for control of crabgrass (OMAFRA 2016). Regardless of the application timing, it is common practice to apply an HPPD-inhibiting herbicide with atrazine as a tank-mix partner. The addition of atrazine improves the consistency of weed control and broadens the weed control spectrum.
(Woodyard et al. 2009). The flexible application timings and diversity of options for Ontario growers make HPPD-inhibiting herbicides a popular choice for weed control.

### 1.6.2 Mode of Action

The enzyme 4-hydroxyphenylpyruvate dioxygenase is the target site for HPPD-inhibiting herbicides (Mitchell et al. 2001). HPPD transforms hydroxyphenylpyruvate to homogentisate in the carotenoid biosynthesis pathway, an essential step in the production of plastoquinone and alpha-tocopherols (Lee et al. 1997). HPPD is also involved with the metabolism and break down of the amino acid tyrosine (Mitchell et al. 2001). Logically, the inhibition of HPPD should result in an accumulation of tyrosine and a reduction of plastoquinone in the growing point; both of these outcomes were confirmed when tested in ivyleaf morningglory (*Ipomoea hederacea* L.) (Prisbylla et al. 1993). Carotenoids are essential for the protection of chlorophyll from damage due to reactive oxygen species (Ndikuryayo et al. 2017). In the absence of carotenoids, photosynthetic membranes are destroyed and lipid peroxidation occurs (Meazze et al. 2002). The resulting symptomology is bleaching of the foliage, starting at the growing point and spreading through the newest growth first, followed by necrosis and plant death (Meazze et al. 2002). Injury symptoms usually occur 3-5 days after application (Senseman 2007). In addition to bleaching, growth is inhibited and leaf elongation is repressed (Grossman and Ehrhardt 2007). Larger plants tend to be more difficult to control with a single mesotrione application; sequential applications provide more consistent control (Yu and McCollough 2016). Although herbicides in other chemical families can produce similar symptomology, inhibition of the HPPD enzyme is a unique mode of action.
1.6.3 Absorption and Translocation

HPPD-inhibiting herbicides are absorbed through both foliage and root structures and therefore can be soil- or foliar-applied (Abit and Al-Khatib 2009). Foliar-applied herbicides must remain on the leaf surface long enough to be absorbed through the cuticle to be effective. This process can be affected by a variety of factors including leaf cuticle thickness, humidity, rainfall and the addition of an adjuvant (Abit and Al-Khatib 2009; Goddard et al. 2010; Young and Hart 1998). Smooth crabgrass exhibited increased injury at 90% relative humidity (RH) compared to applications at only 50% RH, strongly suggesting that more herbicide is absorbed at a higher humidity (Goddard et al. 2010). Greenhouse and lab work have shown that approximately 80% of foliar-applied topramezone is absorbed into the plant, although no more than 25% is translocated to the lower shoots (Grossman and Ehrhardt 2007). The addition of methylated seed oil, an adjuvant that reduces the surface tension of the herbicide-water solution and increases speed of adsorption, improved topramezone efficacy when used POST on both grass and broadleaf weeds (Zhang et al. 2013). The addition of a water conditioner, ammonium sulphate, to mesotrione also increased control of giant ragweed (*Ambrosia trifida* L.), Canada fleabane (*Conyza canadensis* L. Cronquist) and Palmer amaranth (*Amaranthus palmeri* L.) by 9, 6 and 9% respectively (Devkota et al. 2016). Mesotrione and tembotrione are both weak acids and absorption through the roots from a soil application is largely pH dependent (EFSA 2013; Mitchell et al. 2001). Similarly, soil activity of isoxaflutole is strongly correlated to pH with higher activity seen in basic soils (Beltran et al. 2003). In general, HPPD-inhibitors are more soluble at higher pH levels, the ideal pH range being from 7-9 (Damalas et al. 2018). Once absorbed into the...
plant, HPPD-inhibitors move both basipetally and acropetally through the phloem (Mitchell et al. 2001). An advantage of HPPD-inhibitors is their quick translocation to metabolic sinks, such as the growing point, where they are the most effective.

1.6.4 Metabolism and Crop Selectivity

HPPD-inhibiting herbicides are registered for use on corn, sorghum and cereal crops. Corn plants have slower uptake and improved metabolism compared to weed species, resulting in crop tolerance (Damalas et al. 2018; Grossman and Ehrhardt 2007). Crop response to an HPPD-inhibitor can be hybrid specific (O'Sullivan et al. 2002). In some scenarios, slight injury in the form of leaf bleaching can be seen on corn plants; however, this injury is rarely greater than 10% and doesn’t result in crop yield loss (Armel et al. 2003). In sweet corn, plants that have been injured due to a mesotrione application may start producing higher levels of carotenoids than their non-injured counterparts, a result that is nutritionally beneficial (Kopsell et al. 2009).

The primary metabolite of the HPPD-inhibitor topramezone is topramezone-desmethyl, which exhibits approximately 50 times less herbicidal activity compared to the parent compound and is generated by desmethylation at the pyrazole ring (Grossman and Ehrhardt 2007). The major metabolites of mesotrione are MNBA [4-(methylsulfonyl)-2-nitrobenzoic acid] in plants, and AMBA [2-amino-4-(methylsulfonyl) benzoic acid] in soil (Alferness and Wiebe 2002). Hybrid sensitivity and environmental conditions such as high temperatures or other stresses which slow down plant metabolism, can result in crop injury symptoms (Williams and Pataky 2010; Gunsolus and Curran 1999). For both foliar- and soil-applied treatments, no herbicide residue or metabolite can be detected in the grain corn kernel at harvest (Alferness and Wiebe 2002).
Isoxaflutole is the only HPPD-inhibitor in the isoxazole family and is characterized by an isoxazole ring. It is considered a pro-herbicide, meaning the chemical in the spray tank is not biologically active until it has undergone the first step of metabolism in soil or plants (Jeschke 2016). Once in the soil or plant, isoxaflutole is quickly metabolized into its herbicidally active metabolite, diketonitrile (DKN) (Jeschke 2016). The main metabolite of DKN is a benzoic acid derivative 2-methanesulfonyl-4-trifluoromethylbenzoic acid (Beltran et al. 2003). Many factors affect crop tolerance to HPPD-inhibiting herbicides, including hybrid sensitivity and stress-inducing environmental conditions.

1.6.5 Soil Degradation and Carry Over

Soil adsorption is influenced by soil moisture, texture and pH. Adequate soil moisture is necessary for the herbicide to be dissolved in soil-water solution and taken up by roots. Rouchard et al. (2001) found that the half-life of mesotrione in soil was greater with the addition of an organic amendment to the soil, and that dissipation occurred more rapidly in sandy soils. As the sand content of a soil increases, the half-life of mesotrione decreases; when tested under field conditions the half-life declined from 50 days in a loam soil to 34 days in a sandy soil (Felix et al. 2007). Regardless of the soil type (sand, silt, clay or loam), mesotrione does not appear to dissipate farther than 15cm into the soil profile (Rouchard et al. 2001). Mesotrione degradation in soil is largely influenced by temperature. When equal rates of mesotrione were applied to soils under identical conditions and only temperature was varied, 80.6% was recovered at 15C while only 25.3% was recovered from the 35C treatment, after 21 days (Su et al. 2017). More rapid degradation under higher temperatures could be attributed to an increase in the activity
of micro-organisms in the soil (Su et al. 2017). Although the re-crop interval for soybeans is labelled as 11 months, injury can occur if specific weather conditions are met. Injury to fall-planted cover crops such as oilseed radish, crimson clover and Austrian winter pea, is also a risk and needs to be considered if applying mesotrione, tembotrione or topramezone (Cornelius and Bradley 2017). Injury to rotational vegetable crops, including snap beans, cabbage and cucumber, planted 12 months after a mesotrione application was 75, 34 and 20%, respectively, resulting in unacceptable yield loss (Felix et al. 2007). Soil degradation of HPPD-inhibitors is influenced by environmental factors and soil characteristics and could impact subsequent crops or cover crops.

1.6.6 Volatility
Triketone herbicides have a very low risk of volatility due to a very low vapour pressure; the risk associated with vapour release into the atmosphere is negligible (Dumas et al. 2017).

1.6.7 Environmental and Human Safety
When HPPD-Inhibiting herbicides were developed, they were regarded as a more environmentally responsible herbicide because of their relationship to natural allelochemicals. However, the status of a compound as ‘naturally-derived’ doesn’t absolve it from any potential environmental consequences and the impact that HPPD-inhibiting herbicides and their metabolites have in the environment and on non-target organisms has been fully reviewed. A review compiled by Carles et al. (2017) concluded that mesotrione posed no risk to humans or non-target organisms, including soil microorganisms. Rapid break-down of mesotrione in the soil results in very low risk of
mesotrione being captured in ground or surface water. Ultimately, no mesotrione was found in corn grain at the time of harvest, assuming that the pre-harvest interval of 20 days was followed (Carles et al. 2017). There is consensus among the US EPA, the Canadian PMRA and the European Commission that HPPD-inhibitors have a relatively low impact on the environment.

1.6.8 Antagonism and Synergy

It is common practice in Ontario corn production to tank-mix HPPD-inhibiting herbicides with the photosystem II herbicide, atrazine. The two herbicide families have complimentary modes of action: photosystem II inhibitors increase the amount of reactive oxygen species and HPPD-inhibitors stop the production of tocopherols (antioxidants that quench reactive oxygen species) and carotenoids (which protect photosynthetic material from damage) (Abendroth et al. 2006). When paired together, the result is expedited injury (Robinson et al., 2006; Robinson, 2008) and increased weed control (Walsh et al. 2012).

The opposite result is seen when HPPD-inhibiting herbicides are tank-mixed with ALS-inhibiting herbicides. Given their flexible application timing and broad spectrum, ALS-inhibiting herbicides are logical tank-mix partners for HPPD-inhibiting herbicides. Kaastra et al. (2008) showed that topramezone or mesotrione tankmixed with nicosulfuron antagonized the control of large crabgrass \([Digitaria sanguinalis (L.) Scop.]\) and barnyardgrass \([Echinochloa crus-galli (L.) P.Beauv.]\), but control of foxtail species was not antagonized. Schuster et al. (2007) also saw a reduction in grass control with mesotrione plus sulfonylurea herbicides compared to sulfonylurea herbicides alone. The mechanism of antagonism in grass species has been attributed to reduced absorption
of the sulfonylurea herbicide when it is tankmixed with mesotrione and atrazine. Translocation and metabolism were comparable (Schuster et al. 2007). When making herbicide choices, farmers need to consider the potential for antagonism to occur.

1.7 PPO-Resistance in Waterhemp

PPO resistant waterhemp was first confirmed in a Kansas soybean field in 2001 (Heap, 2018). The field in question had been continuous soybean for 15 years, with multiple applications of acifluorfen (Shoup, et al., 2003). Survey work confirmed 19 additional sites within 16km of the initial population (Falk et al., 2005). Resistant biotypes since been confirmed in Illinois, Missouri, Indiana, Minnesota, Wisconsin and Nebraska (Heap, 2018). Most documented cases of PPO resistance describe necrosis on leaf tissue immediately after treatment (maximum injury seen 5-10 DAT); however, plants start to regrow from terminal or auxiliary buds around 14 DAT (Falk et al., 2005).

The initial population conferred cross-resistance to acifluorfen, fomesafen, lactofen and sulfentrazone (Shoup 2003). The level of resistance varies depends on the active ingredient in question. The population had the highest resistance to lactofen (82x), followed by acifluorfen (34x), fomesafen (8x) and sulfentrazone (4x) (Shoup et al., 2003). The LD$_{50}$ for acifluorfen, domesafen and lactofen from a susceptible population was 49, 165 and 108 g.a.i ha$^{-1}$, respectively. Compared to the resistant population where the LD$_{50}$ was 308, 406, and 285 g.a.i. ha$^{-1}$, respectively. Greenhouse studies comparing leaf area, plant biomass and photosynthesis of susceptible and resistant biotype in competitive and non-competitive environments found that there is no fitness penalty associated with PPO-resistance (Duff et al., 2009).
1.7.1 Mechanism of Resistance

Patzoldt et al. (2003) first suggested that PPO-resistance in waterhemp was conferred by a single major gene. In 2008 it was confirmed that resistance is a dominant trait conferred by a single major gene, although modifying genes may influence the level of resistance (Shoup et al., 2008). PPO-resistance is a nuclear-encoded gene and can be transferred through pollen or seed distribution (Shoup et al., 2008). This research was done on a Kansas waterhemp population. PPO-resistance is waterhemp is very unique.

The PPO enzyme is the last common step in the formation of chlorophyll and heme (Beale & Weinstein 1990). Most plants have two PPO-enzymes, coded for by nuclear genes: PPX1 and PPX2, that function in two different locations in the plant cell (the plastids and the mitochondria) (Smith et al., 1993). This means that there are effectively two target sites for PPO-inhibiting herbicides and a target site mutation is rather unlikely. Waterhemp is different than most plants and only has a single gene (PPX2L) that codes enzymes for both locations (Lee et al., 2008). A mutation on the PPX2L gene is responsible for PPO-resistance in waterhemp (Lee et al., 2008). A codon deletion, resulting in the removal of one amino acid (G210) on the PPO enzyme, causes PPO-inhibiting herbicides to be unable to bond to the PPO enzyme and renders them ineffective (Lee et al., 2008). Farther research and testing has concluded that the G210 mutation is the most prevalent resistance mechanism in waterhemp (Lee et al. 2008; Thinglum et al. 2011). Mutations conferring target-site resistance to ALS- and PPO-inhibitors in waterhemp are closely located on the waterhemp genome and analysis with molecular markers confirmed the two genes are physically linked (Tranel et al., 2017).
1.7.2 Effect of size and application timing on resistance

Populations resistant to foliar-applied PPO-herbicides seem to still be susceptible to soil-applied applications (Falk et al., 2006). Through greenhouse and field studies Falk established that preemergence applications of fomesafen, lactofen, acifluorfen, oxyflurofen and sulfentrazone all provided greater than 90% control of PPO-resistant waterhemp. In greenhouse trials, POST applications of acifluorfen at the 2-leaf stage gave near-perfect control of PPO-resistant waterhemp, although the level of control decreased as the application timing was delayed and the herbicides were applied to larger plants (Falk et al., 2006). The same pattern was not seen when acifluorfen was applied in combination with bentazon, strongly suggesting that there is a relationship between susceptibility of a waterhemp population and the size at which a POST application is applied (Falk et al., 2006). Wuerffel et al. (2015) continued this work by investigating the influence that the use of PPO-inhibitors pre-emergence has on the development and frequency of resistance. There are a variety of factors that influence soil-applied herbicide efficacy including soil type and precipitation. As a herbicide is broken down in the soil over time the amount of herbicide active ingredient in the soil decreases and efficacy is reduced. Wuerffel et al. (2015) found that resistant biotypes have a selective advantage over susceptible biotypes at low soil-herbicide concentrations. Tank-mixing another effective mode-of-action, such as s-metolachlor, will delay waterhemp emergence, improve consistency and mitigate the development and spread of resistant biotypes (Wuerffel et al., 2015).
1.8 Hypothesis and Objectives

Waterhemp is consistently ranked as one of the most troublesome weeds for grain farmers across the American mid-west. In 2014 the first glyphosate resistant waterhemp population was confirmed in Ontario. Since then, waterhemp has spread to three Ontario counties and resistance to three herbicide modes of action has been confirmed; ALS-inhibitors, photosystem II inhibitors and EPSPS inhibitors. Multiple resistant waterhemp poses a considerable threat to Ontario grain farms. Extensive research has been conducted on control options for waterhemp in soybean. However, there has been little research on options for control in corn. This research broadens our understanding of the distribution of multiple resistant waterhemp across the province and will investigate currently registered herbicides to identify the most efficacious control option for Ontario farmers.

Hypothesis

The hypotheses for this research include:

1. Multiple resistant waterhemp will be found at additional sites in Ontario.
2. Multiple resistant waterhemp can be controlled with currently registered herbicides.
3. Waterhemp collected in Ontario will test positive for resistance to Groups 2, 5, 9 and 14.

Objectives

The objectives of this research are:
1. To build on our knowledge of the distribution of glyphosate and multiple waterhemp in Ontario.

2. To identify the most efficacious herbicide strategies for control of multiple resistant waterhemp in corn.
Chapter 2: Control of multiple-resistant waterhemp (Amaranthus tuberculatus) with pre-emergence and post-emergence herbicides in corn in Ontario.


2.1 Abstract:

Waterhemp is a competitive, summer annual, broadleaf weed that poses a considerable threat to Ontario grain farmers. Populations with multiple-resistance of Group 2 (ALS-inhibitors), Group 5 (photosystem II inhibitors), and Group 9 (EPSPS inhibitors) have been confirmed in Ontario. If left uncontrolled, waterhemp competition can result in corn yield losses of up to 74%. The objective of this research was to evaluate preemergence (PRE) and post-emergence (POST) herbicides for control of multiple herbicide-resistant (MR) waterhemp. Two field studies at two locations (Cottam and Walpole Island) were conducted in 2016 and 2017. Fifteen PRE and twelve POST herbicides were evaluated for waterhemp control, density, and aboveground biomass, and corn yield. At 8 weeks after application (WAA), S-metolachlor/mesotrione/atrazine (1393/139/524 g ai ha\(^{-1}\)) and S-metolachlor/mesotrione/bicyclopyrone/atrazine (1259/140/35/588 g ai ha\(^{-1}\)), applied PRE were the most efficacious, controlling MR waterhemp 87 and 91%, respectively. At 8 WAA, the most efficacious POST herbicides were mesotrione+atrazine, and dicamba/atrazine, controlling MR waterhemp 92, and 87%, respectively. Reduced waterhemp interference with all of the herbicides evaluated resulted in corn yield that was similar to the weed-free control.
2.2 Introduction:

Waterhemp is a highly competitive C4, summer annual, broadleaf weed in the same family (Costea et al. 2005) as other problematic weeds such as redroot pigweed (*Amaranthus retroflexus* L.) and Palmer amaranth (*Amaranthus palmeri* S.Wats.) (Costea et al. 2005). Certain biological characteristics of waterhemp make it particularly problematic for growers and highly susceptible to developing herbicide resistance. Waterhemp in Ontario emerges from early spring until late-October (Schryver et al. 2017b). The extended emergence pattern of waterhemp requires the use of soil-applied herbicides that provide season long residual control of late emerging flushes. Spring tillage can be an effective option for control of early emerging plants; however, late emerging flushes can result in yield loss if not controlled (Cordes et al. 2004). Waterhemp is diecious; therefore, plants are not able to self-pollinate (Liu et al. 2012). Separate male and female plants must cross-pollinate to reproduce, resulting in wide genetic diversity. Cross pollination results in a large range of phenotypic traits, rapid spread of resistance genes through pollen movement, and the ability to adapt to many soil types and environmental conditions (Sarangi et al. 2017). Waterhemp is a prolific seed producer and a single female waterhemp plant has been recorded to produce up to 4.8 million seeds under optimal conditions (Hartzler et al. 2004). A more conservative estimate would be approximately 300,000 seeds (Hartzler et al. 2004). In combination, these traits contribute to waterhemp’s ability to quickly evolve resistance and cause significant yield loss in field crops.
Weed interference in corn is one of the largest contributors to yield loss (Soltani et al. 2016). The use of herbicides is often considered the most efficacious and economical weed control option for grain producers. However, if the same site of action is used repeatedly, weeds can evolve resistance, rendering many herbicides ineffective (Powles and Yu 2010). In Ontario, there are currently four weed species with confirmed resistance to glyphosate: common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), Canada fleabane [*Conyza canadensis* (L.) Cronquist] and waterhemp [*Amaranthus tuberculatus* (Moq. Sauer) var. *rudis* (Costea & Tardif)] (Cowbrough 2017). Waterhemp is native to the American midwest and was first confirmed in Ontario in Lambton County in 2002 (Vyn et al. 2006). Often reported as one of the most difficult weeds to control, crop yield loss due to waterhemp interference depends on the weed density, relative time of weed and crop emergence, soil nutrient status, and weather conditions. For example, when waterhemp densities ranged between 35-82 plants per m², corn yield loss was 8%, compared to 36% yield loss when densities reached 369-445 plants per m² (Cordes et al. 2004). Season-long waterhemp competition can reduce corn yields up to 74% (Steckel and Sprague 2004).

Waterhemp biotypes with multiple resistance are found in North America. In the United States there is confirmed resistance to six different modes-of-action: Group 2, 4 (synthetic auxins), 5, 9, 14 (PPO-inhibitors) and 27 (HPPD-inhibitors) (Hausman et al. 2011; McMullan and Green 2011; Bell et al. 2013). In Missouri, previous reports have confirmed a waterhemp biotype exhibiting resistance to six modes-of-action: Groups 2, 4, 5, 9, 14, and 27 (Shergill et al. 2018). In Ontario, there is confirmed resistance to Groups 2, 5 and 9 (Schryver et al. 2017a). As effective herbicide options for controlling
waterhemp with multiple resistance continues to shrink, it is imperative that farmers implement resistance management practices to prevent the continued evolution and spread of resistance. The use of multiple-effective modes of action limits the development and spread of herbicide resistance. The objective of this study was to identify pre-emergence (PRE) and post-emergence (POST) herbicide options for control of MR waterhemp in corn in Ontario.

2.3 Materials and Methods:

Separate PRE- and POST-applied herbicide trials were established at two locations in Ontario [near Cottam (42.148929°N; -82.50402°) and on Walpole Island (42.56159°N; -82.682538°)] in 2016 and 2017. There are a total of four site-years per application timing trial. Treatments within each trial were arranged in a randomized complete block design with four blocks. Treatments, rates and timings are listed in Tables 1 and 2. Herbicide trade names and manufacturers are listed in Table 2.3. The Cottam location had a sandy-loam soil (66% sand, 24% silt, 10% clay), with 2.2% organic matter and a pH of 6.4. The Walpole Island soil is a loamy sand (78% sand, 14% silt, 8% clay), with 2.3% organic matter and a pH of 8.3. Previous research by Schryver et al. (2017a) confirmed that the waterhemp populations at both sites were resistant to Group 2, 5, and 9 herbicides. Soil was tilled and corn (DKC 53-56) was planted in mid-May to early-June at the rate of 83,000 seeds ha⁻¹. Planting dates are presented in Table 4. The corn variety chosen was resistant to glyphosate and glufosinate. Plots were 2.25 m wide (3 corn rows spaced 0.76 m apart) and 8 m long. Weed-free controls were produced and maintained with two herbicide applications: S-metolachlor/mesotrione/bicyclopyrone/atrazine (SMBA) (1259/140/35/588 g ai ha⁻¹)
applied PRE, followed by dicamba/ atrazine (1800 g ai ha\(^{-1}\)) applied POST if needed, and periodic hand-weeding throughout the growing season. Glyphosate (900 g ae ha\(^{-1}\)) was applied to the entire trial area, including weedy checks, to remove the confounding effect of other weed species and susceptible waterhemp biotypes.

Herbicides were applied using a CO\(_2\) pressurized backpack sprayer equipped with a hand-held boom with four ULD 12002 nozzles (Pentair, New Brighton, MN, USA) spaced 50 cm apart. Water volume and pressure were calibrated to 200 L ha\(^{-1}\) and 260 kPa, respectively. The PRE herbicide treatments were applied 1-3 days after planting; POST herbicide treatments were applied when waterhemp populations reached approximately 10 cm in height. All trials received an activating rainfall. PRE and POST application dates are presented in Table 2.4.

Data collected included visible crop injury, waterhemp density, and waterhemp dry biomass. Crop injury was assessed visually at 2 and 4 weeks after emergence (WAE) for PRE herbicides and 1 and 4 weeks after application (WAA) for POST herbicides. Corn injury was assessed on a percent scale where 0 indicated no visible injury and 100 indicated complete plant death. For both the PRE and POST herbicides, percent waterhemp control was assessed visually at 4 and 8 WAA, relative to the untreated control. Waterhemp density and aboveground biomass were determined at 8 WAA for the PRE study and 4 WAA for the POST study. Waterhemp density within each plot was estimated by counting the waterhemp plants in two 0.25 m\(^2\) quadrats per plot. Aboveground biomass of the waterhemp within the quadrats was determined by cutting the plants at the soil surface, placing the cut plants in paper bags appropriately
sized to the amount of biomass, and dried in a kiln at 49°C for three weeks to constant moisture. At maturity, the grain corn was harvested from the center two rows with a small plot combine and the weight and moisture content were recorded. Due to technical difficulties yield data was not collected in 2016. Grain yields were adjusted to 15.5% moisture, using ARM 8 software (Gylling Data Management, Inc. Brookings, SD), before analysis.

Data were analyzed using PROC GLIMMIX in SAS (version 9.4, SAS Institute Inc., Cary, NC). Environment (year and location combinations) and replication (block within Environment) were random effects. The herbicide treatment was the fixed effect. The objective of this study is to identify the most consistent herbicide options for control of MR waterhemp across Ontario’s varying environments. To achieve this objective data across all four site-years was pooled for each study. To confirm normality assumptions had been met residuals were plotted against, predicted, treatment, block, and trial for each variable. The Shapiro-Wilk statistic was generated using SAS UNIVARIATE to test for normality. To meet the normality assumptions, a natural log transformation was applied to both waterhemp density and aboveground biomass. Treatment means for these variables were back transformed to the original scale for presentation. Means were separated (P=0.05) using the Tukey’s LSD test.

2.4 Results and Discussion:

2.4.1 Pre-emergence herbicide study

At 4 WAA, dicamba, pethoxamid, flumetsulam, and atrazine, applied PRE, controlled MR waterhemp 54, 55, 55, and 73%, respectively (Table 2.1). All the other
herbicides applied PRE controlled waterhemp ≥79% and did not differ from the weed-free control. A similar trend was observed at 8 WAA. Flumetsulam, dicamba, pethoxamid and atrazine controlled waterhemp ≤65%. The results from this study are similar to Vyn et al. (2006) who reported 60% waterhemp control with dicamba at 10 WAE. Previous research at these locations by Schryver et al. (2017a) confirmed that waterhemp at both sites was resistant to the Group 2 and 5 herbicides, which explains the poor control with flumetsulam and atrazine. At 8 WAA, pyroxasulfone and S-metolachlor controlled waterhemp 74 and 73% in this study, this was lower than Schryver et al. (2017b) who reported 87 and 82%, respectively. At 4 and 8 WAA, dimethenamid-P controlled MR waterhemp 79 and 71%, which is similar to Schryver et al. (2017b) who reported 85 and 74% control, respectively. The Schryver et al. (2017b) populations were multiple resistant to Groups 2, 5, and 9 herbicides. At 8 WAA, dicamba/ atrazine controlled waterhemp 76% in this study which is similar to Vyn et al. (2006) who reported 80% control, populations in both studies were resistant to triazine herbicides. At both 4 and 8 WAA, S-metolachlor/mesotrione/bicyclopyrone/atrazine provided the greatest control of MR waterhemp of 94 and 91%, respectively. This finding is consistent with previous research using this premix. Research across Ohio, Illinois, and Indiana showed S-metolachlor/mesotrione/bicyclopyrone/atrazine mixtures, applied PRE, controlled waterhemp 99% when corn was 30 cm tall (Loux et al. 2011). The waterhemp population in this study, however, had no confirmed herbicide resistance. At 8 WAA, the PRE herbicides evaluated reduced MR waterhemp density 57 to 98% compared to the weedy check. Average waterhemp density in the weedy check was 109 plants m⁻². Dicamba, flumetsulam and pethoxamid reduced MR waterhemp density the least at 57,
65 and 68%, respectively. Multiple-resistant waterhemp density with the remaining PRE herbicide treatments was similar to the weed-free control. Saflufenacil/dimethenamid-P, S-metolachlor/atorazine, S-metolachlor/mesotrione/atorazine and S-metolachlor/mesotrione/bicyclopyrone/atorazine, applied PRE reduced MR waterhemp density ≥96%. At 8 WAA, the PRE herbicides evaluated reduced MR waterhemp biomass 52-98%. Pethoxamid and flumetsulam reduced MR waterhemp density the least at 52 and 56%, respectively. Multiple-resistant waterhemp biomass with the remaining PRE herbicides evaluated was similar to the weed-free control. S-metolachlor/atorazine, S-metolachlor/mesotrione/atorazine and S-metolachlor/mesotrione/bicyclopyrone/atorazine, applied PRE reduced MR waterhemp density ≥96%. In this study waterhemp interference reduced corn yield from 10.0 tonnes in the weed-free control to 7.8 tonnes in the weedy check. Reduced MR waterhemp competition from isoxaflutole + atrazine, saflufenacil/dimethenamid-P, S-metolachlor/atorazine or S-metolachlor/mesotrione/ bicyclopyrone/ atrazine, applied PRE, resulted in corn yields that were 0.1, 0.2, 0.4 and 0.5 t greater than the weed-free control, respectively. However, the yield values from these treatments did not differ from the other herbicide treatments evaluated. There was no crop injury with any the PRE herbicides evaluated (data are not presented).

2.4.2 Post-emergence herbicide study

At 4 WAA, mesotrione + atrazine, dicamba/atorazine, 2,4-D ester, and topramezone + atrazine controlled MR waterhemp 90, 84, 83 and 83% respectively (Table 2.2). At 8 WAA, waterhemp control increased 2-8% for all treatments with the exception of halosulfuron which decreased 3%. At 8 WAA, mesotrione + atrazine,
dicamba/ atrazine, 2,4-D ester, topramezone + atrazine, and dicamba, controlled MR waterhemp 92, 87, 85, 83 and 82%, respectively and were similar to the weed-free control. AT 4 WAA, mesotrione + atrazine, dicamba, dicamba/ atrazine, 2,4-D reduced MR waterhemp density 92, 92, 89 and 83%, respectively, compared to the weedy control. The remaining herbicides evaluated had waterhemp density similar to the weedy control. AT 4 WAA, all the POST herbicides evaluated reduced MR waterhemp biomass except for bromoxynil + atrazine and halosulfuron. Poor control of waterhemp with bromoxynil in this study is consistent with results from Woodyard et al. (2009), who reported 27 and 23% waterhemp control with bromoxynil (280 g ai ha$^{-1}$) at 10 and 30 days after treatment (DAT), respectively. At 4 and 8 WAA, glufosinate controlled MR waterhemp 58 and 61%, respectively in this study which is consistent with an Illinois study that showed 68% suppression of waterhemp with glufosinate 2 WAA (Hausman et al. 2016).

At 4 WAA, 2,4-D ester, dicamba, dicamba/ diflufenzopyr, dicamba/ atrazine and dicamba/prosulfuron, applied POST, resulted in 9.7% corn injury (Table 2). Herbicide injury symptoms included brace root and stem malformation, consistent with Group 4 injury symptoms in corn (Gunsolus and Curran 1999). Corn yield with 2,4-D, applied POST, was lower than the highest yielding treatments. All other herbicides applied POST resulted in corn yield that was similar to the weedy and weed-free controls.

2.5 Conclusions and Implications

This study shows that Ontario farmers have PRE and POST herbicide options that provide >90% control of for MR waterhemp in corn. Herbicide tank-mixes with more than one effective site of action provided the highest control with both PRE and POST
herbicides. Multiple effective modes-of-action tank mixtures are not only the most efficacious options; they are also the preferred option to reduce the selection intensity for additional herbicide resistant weeds. Based on this research the most efficacious pre-emergence option for Canadian growers to control multiple resistant waterhemp is S-metolachlor/ atrazine/mesotrione/bicyclopyrone and the most efficacious post-emergence option is dicamba/atrazine. Multiple sources showed >96% control of waterhemp with multiple effective site of action tank-mixes: S-metolachlor + mesotrione, S-metolachlor + atrazine + mesotrione and S-metolachlor + atrazine + isoxaflutole (Shoup and Al-Khatib 2004; Vyn et al. 2006; Legleiter and Bradley 2009). The PRE herbicides provided greater control than the POST herbicides evaluated in this study, which may reflect the extended emergence pattern of waterhemp and the need for herbicides with long residual activity in the soil. Crop safety is an important consideration when selecting a POST herbicide; the Group 4 herbicides including dicamba and 2,4-D ester can cause unacceptable corn injury, especially if applied too late in the growing season (VanGessel et al. 2016). This limits MR waterhemp control options for Ontario grain farmers if the corn is past the V3 growth stage. Considering the biology of waterhemp, a prolific seed producer with a predisposition to developing herbicide resistance, it is important that farmers implement integrated weed management strategies to control of waterhemp effectively and minimize weed seed return and minimize the spread of this weed biotype across Ontario.
### 2.6 Figures and Tables

**Table 2.1** Waterhemp control (4 and 8 WAA), density, and aboveground biomass (8 WAA), and corn yield as impacted by the PRE – applied corn herbicide treatments across three locations in Ontario in 2016 and 2017.

<table>
<thead>
<tr>
<th>Treatments&lt;sup&gt;ab&lt;/sup&gt;</th>
<th>Rate&lt;sup&gt;a&lt;/sup&gt; (g a.i. ha&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Waterhemp control&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Waterhemp control&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Density&lt;sup&gt;c&lt;/sup&gt; (no. m&lt;sup&gt;-2&lt;/sup&gt;)</th>
<th>Biomass&lt;sup&gt;d&lt;/sup&gt; (g m&lt;sup&gt;-2&lt;/sup&gt;)</th>
<th>Yield&lt;sup&gt;ef&lt;/sup&gt; (t ha&lt;sup&gt;-1&lt;/sup&gt;)</th>
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<td>54 a</td>
<td>7.8 b</td>
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<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>73&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>10&lt;sup&gt;bcd&lt;/sup&gt;</td>
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<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>S-metolachlor/atrazine</td>
<td>1600/1280</td>
<td>86</td>
<td>78</td>
<td>4</td>
<td>2</td>
<td>10.4a</td>
</tr>
<tr>
<td>S-metolachlor/mesotrione/atrazine</td>
<td>1393/139/524</td>
<td>93</td>
<td>87</td>
<td>2</td>
<td>1</td>
<td>9.9ab</td>
</tr>
<tr>
<td>S-metolachlor/mesotrione/bicylopyrone/atrazine</td>
<td>1259/140/35/58</td>
<td>94</td>
<td>91</td>
<td>3</td>
<td>2</td>
<td>10.5a</td>
</tr>
<tr>
<td>Mesotrione+rimsulfuron</td>
<td>144+15</td>
<td>84</td>
<td>72</td>
<td>13</td>
<td>17</td>
<td>9.6ab</td>
</tr>
</tbody>
</table>

| SE     | 9.8   | 12.4 | 31.0 | 8.5 | 0.6 |

^a All treatments applied with glyphosate at 450 g a.e. ha⁻¹.

^b Treatments with multiple a.i.'s listed with a "/" indicate pre-mixed formulations, treatments listed with a "+" indicate separate products

^c Standardized to 15.5% moisture.

^d Yield data presented for 2017 site-years only. Due to technical difficulties 2016 data could not be collected.

^e SE values calculated from non-transformed data.

a-e Means followed by the same letter within a column are not significantly different according to Tukey's LSD (P<0.05).
Visual crop injury (1 and 4 WAA), waterhemp control (4 and 8 WAA), density and aboveground biomass (4 WAA) and corn yield as impacted by the POST herbicide treatments across three locations in Ontario in 2016 and 2017.

<table>
<thead>
<tr>
<th>Treatment\textsuperscript{ab}</th>
<th>Rate (g a.i. ha\textsuperscript{-2})</th>
<th>Corn Injury</th>
<th>Waterhemp Control</th>
<th>Waterhemp Density</th>
<th>Waterhemp Biomass</th>
<th>Corn Yield\textsuperscript{cd}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 WAA (%)</td>
<td>4 WAA (%)</td>
<td>4 WAA (%)</td>
<td>8 WAA (%)</td>
<td>(no. m\textsuperscript{-2})</td>
<td>(g m\textsuperscript{-2})</td>
</tr>
<tr>
<td>Weedy control</td>
<td>0 c</td>
<td>0 c</td>
<td>0</td>
<td>0</td>
<td>177 a</td>
<td>99 a</td>
</tr>
<tr>
<td>Weed free control</td>
<td>0 c</td>
<td>0 c</td>
<td>100 a</td>
<td>100 a</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2,4-D Ester</td>
<td>560</td>
<td>11 a</td>
<td>9.7 a</td>
<td>83 ab</td>
<td>85 ab</td>
<td>30 b</td>
</tr>
<tr>
<td>Atrazine</td>
<td>1000</td>
<td>0 c</td>
<td>0 c</td>
<td>58 de</td>
<td>60 ed</td>
<td>76 ab</td>
</tr>
<tr>
<td>Dicamba</td>
<td>600</td>
<td>4 b</td>
<td>5 ab</td>
<td>74 bc</td>
<td>82 abc</td>
<td>14 b</td>
</tr>
<tr>
<td>Dicamba/difluazopyr</td>
<td>57/143</td>
<td>0 c</td>
<td>2.8 bc</td>
<td>67 cd</td>
<td>74 bc</td>
<td>58 ab</td>
</tr>
<tr>
<td>Dicamba/atrazine</td>
<td>504/997</td>
<td>3 bc</td>
<td>1.2 bc</td>
<td>84 ab</td>
<td>87 ab</td>
<td>20 b</td>
</tr>
<tr>
<td>Bromoxynil + atrazine</td>
<td>280+1500</td>
<td>3 bc</td>
<td>0.6 bc</td>
<td>64 cd</td>
<td>68 dc</td>
<td>59 ab</td>
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<tr>
<td>Prosulfuron + dicamba</td>
<td>10+140</td>
<td>0 c</td>
<td>0.3 bc</td>
<td>49 e</td>
<td>54 ed</td>
<td>98 ab</td>
</tr>
<tr>
<td>Mesotrione + atrazine</td>
<td>100+280</td>
<td>0 c</td>
<td>0 c</td>
<td>90 a</td>
<td>92 a</td>
<td>15 b</td>
</tr>
<tr>
<td>Topramezone + atrazine</td>
<td>12.5+500</td>
<td>1 bc</td>
<td>0 c</td>
<td>83 ab</td>
<td>83 ab</td>
<td>104 ab</td>
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<td>Tembotrione/thiencarbazone-methyl</td>
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<td>0 c</td>
<td>64 cd</td>
<td>69 cd</td>
<td>86 ab</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>500</td>
<td>1 bc</td>
<td>0 c</td>
<td>58 de</td>
<td>62 ed</td>
<td>61 ab</td>
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<tr>
<td>Halosulfuron</td>
<td>70</td>
<td>1 bc</td>
<td>0.6 bc</td>
<td>34 f</td>
<td>31 f</td>
<td>137 ab</td>
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</table>

\textsuperscript{SE} 0.7 1.4 8.7 6.2 50.8 22.4 0.2
aAll treatments applied with glyphosate at 900 g a.e. ha⁻¹.
bTreatments with multiple a.i.'s listed with a "/" indicate pre-mixed formulations, treatments listed with a "+" indicate separate products standardized to 15.5% moisture.
cYield data presented for 2017 site-years only. Due to technical difficulties 2016 data could not be collected.
dSE values calculated from non-transformed data.
a-f Means followed by the same letter within a column are not significantly different.
Table 2.3 PRE- and POST-herbicide active ingredients, trade names, and manufacturers for multiple resistant waterhemp control at three locations in Ontario in 2016 and 2017

<table>
<thead>
<tr>
<th>Herbicide Name</th>
<th>Application Timing</th>
<th>Trade Name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-metolachlor</td>
<td>PRE</td>
<td>Dual II Magnum</td>
<td>Syngenta Canada Inc. 140 Research Ln, Guelph, ON.</td>
</tr>
<tr>
<td>Dimethenamid-P</td>
<td>PRE</td>
<td>Frontier</td>
<td>BASF Canada Inc. 100 Milverton Dr., Mississauga, ON.</td>
</tr>
<tr>
<td>Pyroxasulfone</td>
<td>PRE</td>
<td>Zidua</td>
<td>BASF Canada Inc. 100 Milverton Dr., Mississauga, ON.</td>
</tr>
<tr>
<td>Pethoxamid</td>
<td>PRE</td>
<td>Anthem</td>
<td>FMC Corp. 2015 Windsor Place E., Regina, SK</td>
</tr>
<tr>
<td>Atrazine</td>
<td>PRE</td>
<td>Aatrex 480</td>
<td>Syngenta Canada Inc. 140 Research Ln, Guelph, ON.</td>
</tr>
<tr>
<td>Dicamba</td>
<td>PRE</td>
<td>Banvell</td>
<td>BASF Canada Inc. 100 Milverton Dr., Mississauga, ON.</td>
</tr>
<tr>
<td>Dicamba/ atrazine</td>
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<td>Marksman</td>
<td>BASF Canada Inc. 100 Milverton Dr., Mississauga, ON.</td>
</tr>
<tr>
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<td>Callisto</td>
<td>Syngenta Canada Inc. 140 Research Ln, Guelph, ON.</td>
</tr>
<tr>
<td>Flumetsulam</td>
<td>PRE</td>
<td>Broadstrike RC</td>
<td>Dow Chemical Suite 2400 215-2nd Street SW, Calgary, AB.</td>
</tr>
<tr>
<td>Isoxaflutole</td>
<td>PRE</td>
<td>Converge</td>
<td>Bayer CropScience Inc. 160 Quarry Park Blvd, Calgary AB.</td>
</tr>
<tr>
<td>Saflufenacil/dimethenamid-P</td>
<td>PRE</td>
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<td>Syngenta Canada Inc. 140 Research Ln, Guelph, ON.</td>
</tr>
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<td>Herbicide</td>
<td>Application Type</td>
<td>Product Name</td>
<td>Company</td>
</tr>
<tr>
<td>-----------------------------------</td>
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<td>PRE</td>
<td>Lumax EZ</td>
<td>Syngenta Canada Inc.</td>
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<tr>
<td>atrazine</td>
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<tr>
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<td>Acuron</td>
<td>Syngenta Canada Inc.</td>
</tr>
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<td>atrazine/bicyclopyrone</td>
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<td>Mesotrione+rimsulfuron</td>
<td>PRE</td>
<td>Engarde</td>
<td>DuPont Crop Protection Canada Inc.</td>
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<td>2,4-D Ester</td>
<td>POST</td>
<td>-</td>
<td>NuFarm Agriculture Inc.</td>
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<td>Distinct</td>
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<td>POST</td>
<td>Pardner</td>
<td>Bayer CropScience Inc.</td>
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<td>Syngenta Canada Inc.</td>
</tr>
<tr>
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<td>POST</td>
<td>Vios 3G</td>
<td>Bayer CropScience Inc.</td>
</tr>
<tr>
<td>methyl</td>
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<td></td>
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<tr>
<td>Glufosinate</td>
<td>POST</td>
<td>Liberty</td>
<td>Bayer CropScience Inc.</td>
</tr>
<tr>
<td>Halosulfuron</td>
<td>POST</td>
<td>Permit</td>
<td>Gowan Canada</td>
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Table 2.4. Planting date, PRE treatment application, and POST treatment application date for control of multiple resistant waterhemp in Ontario in 2016 and 2017.

<table>
<thead>
<tr>
<th>Year/location</th>
<th>Planting date</th>
<th>PRE Application</th>
<th>POST Application</th>
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<tr>
<td>2016</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cottam</td>
<td>May 23, 2016</td>
<td>May 24, 2016</td>
<td>June 17, 2016</td>
</tr>
<tr>
<td>Walpole Island</td>
<td>May 30, 2016</td>
<td>June 1, 2016</td>
<td>July 5, 2016</td>
</tr>
<tr>
<td>2017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cottam</td>
<td>May 19, 2017</td>
<td>May 22, 2017</td>
<td>June 21, 2017</td>
</tr>
<tr>
<td>Walpole Island</td>
<td>June 8, 2017</td>
<td>June 9, 2017</td>
<td>July 3, 2017</td>
</tr>
</tbody>
</table>
Chapter 3: Occurrence and distribution of waterhemp (*Amaranthus tuberculatus*) from Ontario and Quebec resistant to herbicides spanning up to four modes-of-action.

Lauren Benoit, Brittany Hedges, Mike Schryver, Nader Soltani, David C. Hooker, Darren E. Robinson, Patrick J. Tranel, Darci Giacomini, Peter H. Sikkema.

3.1 Abstract

This is the first record of PPO-inhibitor resistance in eastern Canada, the first record of a weed biotype that is resistant to herbicides spanning four modes of action (Group 2, 5, 9 and 14) in Canada, and the second record of a glyphosate-resistant weed in Quebec. The first herbicide-resistant waterhemp population in Canada was found in Lambton County in 2002; resistant to Group 2 and 5 herbicides. The first case of glyphosate-resistant waterhemp in Canada was confirmed in 2014, also in Lambton County, in a population with multiple resistances to Group 2, 5 and 9 herbicides. In 2016 and 2017, waterhemp seed was collected from 22 locations in Ontario and 3 locations in Quebec, and screened for resistance to imazethapyr, atrazine, and glyphosate, representing Groups 2, 5, and 9, respectively. Visible injury was recorded 1, 3 and 5 weeks after application (WAA) and at 5 WAA, the plants were scored as alive or dead. Of the 25 seed lots tested, 100% had individual plants that were resistant to imazethapyr, 88% with individuals resistant to atrazine, and 84% with individuals resistant to glyphosate. Multiple-resistance to imazethapyr, atrazine, and glyphosate was confirmed in 80% of the 25 seed lots screened. In 2015, 2016, and 2017, waterhemp seed was collected from 74 locations in Ontario or Quebec and screened for
resistance to lactofen representing Group 14. Plant tissue from surviving 2017 plants was analyzed for the presence of the codon deletion that confers resistance to PPO-inhibiting (Group 14) herbicides. Of the 74 seed lots screened in the greenhouse, 28% had individuals that showed some regrowth following the application of lactofen. Of the 11 surviving 2017 populations that were tested for the codon deletion, four were positive. This survey emphasizes the threat of multiple herbicide resistance for Canadian grain farmers and the need to implement long-term, diversified, integrated weed management programs.

3.2 Introduction

Weed control using chemical herbicides has been an effective and economical option since they were first brought to market in the late 1940s. With the commercialization of glyphosate in 1974 and the subsequent introduction of glyphosate-resistant crops in 1996, modern, commercial-scale, agriculture has relied heavily on glyphosate for weed control. Through repeated application of a single herbicide mode-of-action, weeds have evolved resistance to some of the most widely used herbicides, including Group 2, 5 and 9 herbicides (WSSA classification)(Delye et al. 2013).

Waterhemp is a highly competitive, summer annual weed that is often listed as one of the most troublesome weeds in the American midwest (Nordby et al. 2007). Waterhemp interference in corn and soybean production can reduce yields up to 74 and 73%, respectively (Steckel and Sprague 2004; Vyn et al. 2007). Waterhemp is a C4 species that grows more quickly than most other Amaranthus spp.; it can grow up to 0.16 cm in height per growing degree day (Horak and Loughin 2000). Waterhemp is a dioecious (Costea et al. 2005). Obligatory cross-pollination increases genetic diversity
and facilitates the transfer of resistance genes from one population to the next, leading to an increase in the variation of phenotypes that, collectively, are adaptable to a wide range of environments (Costea et al. 2005).

The first case of glyphosate-resistant waterhemp was reported by Legleiter and Bradley (2008) in Missouri, USA. A waterhemp population with resistance to Group 2, 5, and 9 herbicides was confirmed in Ontario from seed collected in 2014 after a grower reported inadequate control in a soybean field (Schyrver et al., 2017). Globally, waterhemp is resistant to herbicides from six mode-of-action Groups: ALS-inhibitors (Group 2), synthetic auxins (Group 4), photosystem II inhibitors (Group 5), EPSPS inhibitors (Group 9), PPO inhibitors (Group 14) and HPPD inhibitors (Group 27) (Heap 2018).

PPO-inhibitor-resistant waterhemp has been confirmed in seven USA states: Illinois, Indiana, Kansas, Minnesota, Missouri, Nebraska, and Wisconsin (Heap 2018). PPO-inhibitor-resistance is a dominant trait, conferred by a single codon deletion resulting in an insensitive PPO-enzyme (Shoup et al. 2008). This codon deletion confers varying levels of resistance to all PPO-inhibiting herbicides in the N-phenylthalamides, diphenylethers, pyrimidinediones, triazolinones and phenylpyrazoles herbicide families (Shoup et al. 2003). Multiple-resistant waterhemp poses a larger challenge to Ontario soybean growers as Group 27 herbicides are not registered for use in soybean. The objective of this research was to document the continued spread of herbicide-resistant waterhemp in Ontario and Quebec, as well as establish the existence and distribution of waterhemp resistant to PPO-inhibiting herbicides in Ontario.
3.3 Materials and Methods

3.3.1 Waterhemp Seed Collection

Waterhemp seed was collected in 2016 and 2017 from fields in Ontario and Quebec. The number of samples collected from each county in each year is presented in Table 2. Samples were collected in the fall of each year prior to soybean harvest when waterhemp seed was mature. Sites were located through communication with farmers, agronomists, and personnel at ag-retail points in the province and random road-side scouting consistent with other herbicide-resistant weed surveys conducted in Ontario (Vink et al., 2012; Budd et al., 2017). Seed was primarily collected from soybean fields because waterhemp grows above the soybean canopy and therefore is more easily seen. Each seed sample consisted of weeds collected from 5-15 female plants. GPS coordinates were recorded for each location and displayed on Figures 1-5.

3.3.2 Resistance Screening

Waterhemp seed was cleaned, labelled, and placed in nylon bags. Waterhemp requires a dormancy period prior to germination (Leon & Owen, 2003). To meet the germination requirements, the nylon bags containing the waterhemp seed were stored in trays filled with sand, watered, and chilled for 10 weeks at 4°C. The sand was watered as needed. After 10 weeks, waterhemp seeds were scattered in germination trays filled with potting soil, the seed was covered with 0.5 cm of potting soil, and the trays were placed on benches in a greenhouse with a 16 hr photoperiod and 23°/18° C day/night temperature. When waterhemp seedlings reached the cotyledon stage, they were transplanted into individual pots (10 cm in diameter) with one plant per pot. Forty-two seedlings were transplanted from each seed lot except seed lot AMATA 1704, due
to low seed availability and poor germination. Only 20 seedlings were transplanted for this seed lot. Waterhemp plants were watered as required. When waterhemp reached an average of 10 cm in height, the most uniform 36 plants were selected from each seed lot and divided into three groups of 12 plants. Ten plants from each group of 12 were sprayed with one of the following three herbicides: glyphosate (900 g a.e. ha$^{-1}$), atrazine (1000 g a.i. ha$^{-1}$) + COC (Assist, 1% v/v), or imazethapyr (75 g a.i. ha$^{-1}$) + NIC (Agral 90, 0.2% v/v) + UAN 28% (1% v/v). Herbicides, herbicide trade names, adjuvants and manufacturers are listed in Table 3.1. The remaining 2 plants were the untreated controls. Herbicides were applied using an enclosed chamber sprayer (Generation II Research Sprayer, DeVries Manufacturing, Hollandale, MN) equipped with a single 8002E brass flat fan nozzle (TeeJet Technologies, Springfield, IL) calibrated to apply 200 L ha$^{-1}$ at 280 kPa. Herbicide dose and surfactant selection was consistent with a previous herbicide-resistant waterhemp survey (Schryver et al. 2017). The plants were left to dry in the spray area for one day and then placed in the greenhouse.

Waterhemp injury was assessed 1, 3, and 5 weeks after application (WAA). Each plant was individually evaluated on a scale of 0 to 100 with 0 representing no injury and 100 representing complete plant death. At 5 WAA, the plants were scored as dead or alive, and any seed lot with at least one surviving plant was considered resistant (Beckie et al. 2000).

### 3.3.3 Verification of PPO-Resistance

Seed samples were collected in 2014 and 2015 from the locations listed by Schryver et al. (2017) and in 2016 and 2017 from the locations presented above, for a total of 74 samples tested. Waterhemp seeds were stored, germinated and transplanted...
following the same methodology as described above. When waterhemp plants were 10 cm in height they were sprayed with lactofen (110 g a.i. ha\(^{-1}\)) + COC (1% v/v). Plants were evaluated for injury at 1, 3 and 5 WAA (data not shown). At 5 WAA, plants were scored as dead or alive; plants that had new growth were recorded as ‘alive’. Populations with at least one surviving plant were considered resistant.

In 2017, waterhemp leaf samples were taken from plants that survived an application of lactofen to confirm the presence of the codon deletion on the PPX2L gene that confers resistance to PPO-inhibiting herbicides using the methodology described by Lee et al. (2008).

3.4 Results and Discussion

3.4.1 Distribution

In 2015, 2016, and 2017, 25 waterhemp seed lots were collected from Ontario and Quebec in 2016 and 2017. Of the seed lots evaluated, 100, 88, 84, and 44% had individuals that were resistant to imazethapyr, atrazine, glyphosate, and lactofen, respectively (Table 3.3). All of the seed lots that had individual plants that were resistant to lactofen, also had resistance to imazethapyr, atrazine, and glyphosate. All of the sites had individual plants that were resistant to at least one herbicide (Table 3.3), 92% of the sites had resistance to two or more modes of action. Waterhemp with multiple-resistance to imazethapyr, atrazine and glyphosate is now confirmed in the southwest corner of Middlesex County; this is the first site with multiple-resistant waterhemp in Middlesex County (Figure 3.4). This population is 50 km farther east than the previous survey on herbicide-resistant waterhemp in Ontario. A population of waterhemp in
Haldimand County was resistant to imazethapyr and atrazine. Of the 74 seed lots screened for resistance to Group 14 herbicides from 2014 to 2017, 21 (28%) showed regrowth after an application of lactofen.

### 3.4.2 PPO-Resistance Verification

Four locations in Ontario tested positive for the ΔG210 codon deletion that confers resistance to PPO-inhibiting herbicides (Table 3.4). Plants that survived a greenhouse application of lactofen had 60-100% injury and necrosis 1 WAA; by 3 WAA regrowth from auxiliary buds was visible, and by 5 WAA growth had resumed. These observations are consistent with other resistant populations (Falk et al., 2005). Susceptible populations were controlled by lactofen and showed no regrowth. We speculate that plants that survived following an application of lactofen but tested negative for the codon deletion may have a different mechanism of resistance.

### 3.5 Conclusions and Implications

The evolution of multiple herbicide-resistant waterhemp limits control options for Ontario grain producers and puts more selection pressure on the remaining effective herbicides. We report here on waterhemp with resistance to 4 herbicide Groups, namely 2, 5, 9 and 14. This is the first record of PPO-inhibitor-resistant waterhemp in Ontario. The Group 27 (HPPD-inhibiting) herbicides are the most effective control option for use in corn, and continue to be effective; however, it is important to note that Group 27, HPPD-resistant waterhemp populations have been confirmed in the midwestern United States (Hausman 2011). Controlling waterhemp with a single tillage pass is ineffective because of the extended emergence pattern. Multiple-resistant waterhemp poses a very serious agronomic threat to Ontario grain farmers.
Waterhemp seeds are small and can easily be moved from one location to another via farm equipment or human traffic. Proper cleaning of equipment and awareness of the locations with herbicide-resistant waterhemp is needed to reduce the spread of herbicide-resistant waterhemp seed from one location to another. The use of mechanical, cultural, biological, and chemical weed control strategies should be used in a long-term, diversified, integrated weed management strategy to minimize the selection and spread of multiple-resistant waterhemp.

3.6 Acknowledgements

We thank Chris Kramer for his greenhouse expertise, Nicole Langdon, Brittany Hedges and Mike Schryver for their work surveying and the Grain Farmers of Ontario for providing the funding for this research.
### 3.7 Tables and Figures

**Table 3.1** Herbicides, herbicides trade names, adjuvants, manufacturers and manufacturer addresses for greenhouse screening of herbicide resistance in Ontario, 2014-2017.

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<tr>
<th>Product</th>
<th>Trade Name</th>
<th>Manufacturer</th>
<th>Manufacturer address</th>
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</thead>
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<td>Herbicides</td>
<td>Glyphosate</td>
<td>Roundup WeatherMAX</td>
<td>Monsanto Canada Inc. 900- One Research Road Winnipeg, Manitoba R3T 6E3</td>
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<tr>
<td></td>
<td>Imazethapyr</td>
<td>Pursuit</td>
<td>BASF Canada Inc. 100 Milverton Drive 5th Floor Mississauga, Ontario L5R 4H1</td>
</tr>
<tr>
<td></td>
<td>Atrazine</td>
<td>Aatrex 480</td>
<td>Syngenta Crop Protection Canada Inc. 140 Research Lance, Research Park, Guelph, ON. N1G 4Z3</td>
</tr>
<tr>
<td></td>
<td>Lactofen</td>
<td>Cobra</td>
<td>Valent USA Corp. P.O. Box 8025 1600 Riviera Avenue, Suite 200 Walnut Creek, CA 94596-8025</td>
</tr>
<tr>
<td>Adjuvants</td>
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<td>Agral 90</td>
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<td></td>
<td>UAN 28%</td>
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Table 3.2 Number of waterhemp (*Amaranthus tuberculatus*) samples collected from counties in Ontario and Quebec in 2016 and 2017.

<table>
<thead>
<tr>
<th>County (Province)</th>
<th>2016</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essex (ON)</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Kent (ON)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lambton (ON)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Middlesex (ON)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Haldimand (ON)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Les Jardins-de-Napierville, (QC)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>
**Table 3.3** Sample locations and number of waterhemp (*Amaranthus tuberculatus*) plants surviving 5 WAA of imazethapyr (75 g a.i. ha$^{-1}$), atrazine (1000 g a.i. ha$^{-1}$), glyphosate (900 g a.i. ha$^{-1}$), or lactofen (110 g a.i. ha$^{-1}$).

<table>
<thead>
<tr>
<th>County (Province)</th>
<th>#/10 surviving 5 WAA</th>
<th>Imazethapyr</th>
<th>Atrazine</th>
<th>Glyphosate</th>
<th>Lactofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essex (ON)</td>
<td>10</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>9</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>10</td>
<td>3</td>
<td>9</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>10</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Walpole Island (ON)</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Walpole Island (ON)</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lambton (ON)</td>
<td>10</td>
<td>0</td>
<td>4</td>
<td>1</td>
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</tr>
<tr>
<td>Essex (ON)</td>
<td>10</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>10</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)$^a$</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>10</td>
<td>4</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>9</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Haldimand (ON)</td>
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<td>7</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lambton (ON)</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Middlesex (ON)</td>
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<td>9</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>10</td>
<td>6</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Essex (ON)</td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Les Jardins-de-Napierville (QC)</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Les Jardins-de-Napierville (QC)</td>
<td>9</td>
<td>4</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Les Jardins-de-Napierville (QC)</td>
<td>9</td>
<td>6</td>
<td>9</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>No. of resistant populations $^b$</td>
<td>25/25</td>
<td>22/25</td>
<td>21/25</td>
<td>11/25</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Due to low seed availability only 5 plants from this sample were sprayed

$^b$ Population was considered resistant if one or more plants survived the herbicide application 5 WAA. (Beckie et al. 2000)
Table 3.4 Identification and status of waterhemp (*Amaranthus tuberculatus*) samples collected in 2017 from Ontario with the ΔG210 codon deletion conferring resistance to PPO-inhibiting herbicides. Plants homozygous for the wild type allele are indicated by S/S, homozygous for the ΔG210 allele by R/R, and heterozygous by S/R.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Genotype Call</th>
</tr>
</thead>
<tbody>
<tr>
<td>1701A</td>
<td>S/S</td>
</tr>
<tr>
<td>1702A</td>
<td>S/R</td>
</tr>
<tr>
<td>1702B</td>
<td>S/R</td>
</tr>
<tr>
<td>1703B</td>
<td>S/R</td>
</tr>
<tr>
<td>1703C</td>
<td>R/R</td>
</tr>
<tr>
<td>1703D</td>
<td>S/R</td>
</tr>
<tr>
<td>1703E</td>
<td>S/R</td>
</tr>
<tr>
<td>1703F</td>
<td>S/R</td>
</tr>
<tr>
<td>1703G</td>
<td>S/R</td>
</tr>
<tr>
<td>1704A</td>
<td>S/S</td>
</tr>
<tr>
<td>1704B</td>
<td>S/S</td>
</tr>
<tr>
<td>1705A</td>
<td>S/R</td>
</tr>
<tr>
<td>1705B</td>
<td>S/S</td>
</tr>
<tr>
<td>1708A</td>
<td>S/S</td>
</tr>
<tr>
<td>1712A</td>
<td>S/R</td>
</tr>
<tr>
<td>1713A</td>
<td>S/S</td>
</tr>
<tr>
<td>1715A</td>
<td>S/S</td>
</tr>
<tr>
<td>1715B</td>
<td>S/S</td>
</tr>
<tr>
<td>1716A</td>
<td>S/S</td>
</tr>
<tr>
<td>1717A</td>
<td>S/S</td>
</tr>
</tbody>
</table>
Figure 3.1 Distribution of waterhemp (*Amaranthus tuberculatus*) populations with single-resistance to ALS-inhibiting herbicides across southwestern Ontario in 2014/15 and 2016/17.
Figure 3.2 Distribution of waterhemp (*Amaranthus tuberculatus*) populations multiple resistant to only atrazine and ALS-inhibiting herbicides across southwestern Ontario in 2014/15 and 2016/17.
Figure 3.3 Distribution of waterhemp (*Amaranthus tuberculatus*) populations multiple-resistant to only glyphosate and ALS-inhibiting herbicides across southwestern Ontario in 2014/15.
Figure 3.4 Distribution of waterhemp (Amaranthus tuberculatus var. rudis) populations multiple-resistant to only glyphosate, atrazine and ALS-inhibiting herbicides across southwestern Ontario in 2014/15 and 2016/17.
Figure 3.5 Distribution of waterhemp (Amaranthus tuberculatus var.rudis) multiple resistant to glyphosate, atrazine, ALS-inhibitors and PPO-inhibitors across southwestern Ontario over 2015, 2016 and 2017.
Chapter 4: Efficacy of HPPD-inhibiting herbicides applied preemergence or postemergence for control of multiple resistant waterhemp (*Amaranthus tuberculatus*)

Lauren Benoit, David C. Hooker, Darren E. Robinson, Peter H. Sikkema.

4.1 Abstract

Waterhemp is a highly competitive summer annual and one of the most problematic weeds in field crop production across the American mid-west and Southwestern Ontario. Populations of waterhemp resistant to four modes-of-action (Groups 2, 5, 9 and 14) have been confirmed in Ontario. Hydroxyphenylpyruvate-dioxygenase inhibitors are one of the few effective herbicide modes-of-action available to Ontario farmers for control of waterhemp both PRE and POST. Research was conducted in 2017 and 2018 to determine the relative efficacy of five HPPD-inhibitors, tank mixed with atrazine, for the control of multiple-resistant waterhemp. At 12 weeks after application (WAA), isoxaflutole + atrazine, mesotrione + atrazine and tembotrione + atrazine, applied PRE, controlled waterhemp 90, 87 and 81%, respectively. None of the HPPD-inhibiting herbicides applied PRE controlled waterhemp equivalent to the weed-free check 12 WAA. Applied POST, topramezone + atrazine, mesotrione + atrazine, tolpyralate + atrazine, and tembotrione + atrazine controlled waterhemp 87, 94, 97, and 98% 12 WAA, respectively, and were all equivalent to the weed-free control.

4.2 Introduction

Waterhemp is native to the American mid-west and was first confirmed in Ontario in 2002 (Vyn et al. 2006). In 2014, waterhemp was the fourth glyphosate-resistant weed
that was documented in the province, after common ragweed \textit{(Ambrosia artemisiifolia} L.), giant ragweed \textit{(Ambrosia trifida} L.) and Canada fleabane \textit{(Conyza canadensis} L. Cronq.) (Sikkema et al. 2009; Byker et al. 2013; Van Wely et al. 2015). Many biological traits contribute to the highly aggressive nature of waterhemp. Waterhemp emerges from May to October; therefore, herbicides must provide full season residual control of both the initial population and subsequent flushes (Schryver et al. 2017). Waterhemp is dioecious, housing male and female reproductive organs on separate plants, this increases gene mixing from one generation to the next and facilitates the movement of resistance genes from one population to another (Liu et al. 2012).

Waterhemp has been identified at 81 locations in southwestern Ontario (Benoit et al. 2018). Schryver (2017) collected seedlots of waterhemp populations in 2015, 2016 and 2017, and tested them for resistance to the Group 2 (ALS-inhibitors), Group 5 (photosystem II-inhibitors) and Group 9 (EPSPS-inhibitors) herbicides. Of the seedlots collected, 100% were resistant to the ALS-inhibitors, 82% resistant to glyphosate, and 76% resistant to atrazine; 74% of the populations were resistant to all three modes-of-action. Multiple resistances to these groups of herbicides, particularly atrazine and glyphosate, which have been cornerstones in corn weed control programs, poses a significant challenge for Ontario grain farmers. Hydroxyphenylpyruvate dioxygenase- (HPPD) inhibiting herbicides, WSSA classification Group 27, have proven to be effective for the control of waterhemp in corn in Ontario (Vyn et al. 2006). The HPPD chemistries are an attractive herbicide option for Ontario grain farmers, since they have a flexible application window and control a wide spectrum of broadleaf weeds (OMAFRA 2018). Bicyclopyrone, isoxaflutole and mesotrione are registered for use preplant (PP),
preemergence (PRE) and early postemergence (POST) in corn. Tembotrione,
tolpyralate and topramezone are registered as POST options in corn (OMAFRA 2018).
Tank-mixing HPPD-inhibitors with atrazine can result in a synergistic effect, broadening
the weed control spectrum and improving consistency of control (Woodyard et al. 2009).
The objective of this study was to determine the efficacy of the currently available
HPPD-inhibiting herbicides applied PRE or POST for the control of multiple-resistant
waterhemp in Ontario. This study investigated each HPPD inhibitor at both PRE- and
POST application timings.

4.3 Materials and Methods

Two field studies were completed in 2017 and 2018 at three locations in Ontario
for a total of six site-years per study. One of the locations was near Cottam, Ontario and
two locations were on Walpole Island, Ontario. Waterhemp populations at all three
locations are multiple-resistant to three herbicide modes-of-action Groups: 2, 5 and 9
(Schryver 2017). The first study investigated the efficacy of HPPD-inhibitors applied
PRE, and the second study investigated their efficacy applied POST. The studies were
established as a randomized complete block design with four replications. Each
replication included a weedy - and a weed-free control. The trials were conducted on
commercial fields and no additional waterhemp seed was added. Soil characteristics
are presented in Table 4.1.

The soil at each site was cultivated in the fall and disked twice in the spring to
prepare the seedbed prior to planting, the previous crop at all location was soybean.
Glyphosate- and glufosinate- resistant corn (DKC 46-82RIB) was planted at a rate of 83,
000 seeds ha⁻¹ in mid-May to early-June, depending on the field location. Each plot was
2.25m wide (3 rows spaced 76 cm apart) and 8-m long. Weed-free controls were maintained with S-metolachlor/mesotrione/ atrazine/bicyclopyrone (2022 g ai ha\(^{-1}\)) applied PRE, followed by dicamba/atrazine (1800 g ai ha\(^{-1}\)) applied POST and subsequent hand weeding throughout the growing season as needed. Glyphosate (450 g ae ha\(^{-1}\)) was applied early POST to the entire experimental area to control susceptible biotypes and all other weed species.

Herbicide treatments were applied using a CO\(_2\) pressurized backpack sprayer calibrated to deliver 200 L ha\(^{-1}\) at 250 kPa. The sprayer was equipped with a 1.5-m handheld boom and four ULD 12002 nozzles spaced 50 cm apart (Pentair, New Brighton, MN USA) producing a spray width of 2.0 m. Recommended adjuvants were added to the herbicides and are presented in Table 4.2. The PRE herbicides were applied 1-5 days after corn planting and POST treatments were applied when waterhemp plants reached approximately 10 cm in height. Herbicide treatments and timings are presented in Tables 4 and 5.

In the PRE study, corn injury was assessed 2 and 4 weeks after emergence (WAE) and in the POST study corn injury was assessed 1 and 4 WAA. Crop injury was assessed visually on a scale of 0 to 100; 0 indicated no injury and 100% indicated complete plant death. Weed control was assessed visually 4, 8 and 12 WAA in both studies on a scale of 0 to 100% reduction in perceived biomass compared to the weedy control. Waterhemp density and biomass were determined by counting plants in two 0.25 m\(^2\) quadrats per plot, the same plants were cut at the soil surface, placed in paper bags, dried at 49°C for three weeks to consistent moisture, and weighed. Corn was
harvested from two rows in the fall with a small-plot combine, yield and moisture were recorded. Yield data was converted to 15.5% moisture before statistical analysis.

Data from the PRE- and POST- studies were analyzed separately in PROC GLIMMIX in SAS v. 9.4 (SAS Institute Inc., Cary, NC). Random effects were environment and replication within environment. Herbicide treatment was the only fixed effect. The objective of these studies is to determine the most consistent herbicide option across varying Ontario environments, for this reason data from all sites for each study were pooled. The Shapiro-Wilk test was applied to test for normality using SAS PROC UNIVARIATE. Residuals were plotted for, predicted, treatment, block and trial in order to confirm assumptions.

4.4 Results

4.4.1 Preemergence

There was no visual evidence that the HPPD + atrazine herbicide tankmixes applied PRE caused any corn injury in this study (data not presented).

At 4 WAA, waterhemp control by HPPD-inhibiting herbicides + atrazine varied between 69 and 93% depending on the herbicide (Table 4.3). The herbicide treatments isoxaflutole + atrazine and mesotrione + atrazine had the highest waterhemp control of 90 and 87%, respectively. This result is consistent with an earlier study that reported 97% control at 10 WAA of triazine-resistant waterhemp with isoxaflutole + atrazine (105 + 1063 g ha⁻¹) (Vyn et al. 2006). Previous field research from Ontario reported 82% waterhemp control 8 WAA for both isoxaflutole + atrazine (105 + 1063 g a.i. ha⁻¹) and mesotrione + atrazine (140 + 1500 g ha⁻¹) applied pre-emergence (Benoit et al. 2017).
Of the treatments tested, topramezone + atrazine had the lowest control of 69%; other HPPD + atrazine treatments provided greater than 83% control. Waterhemp control decreased 3-10 percentage points between the 4 WAA and 8 WAA. This downward trend in waterhemp control was not observed between 8 and 12 WAA. The 12 WAA waterhemp control rating for atrazine, topramezone + atrazine, tembotrione + atrazine and tolpyralate + atrazine increased 5-10 percentage points compared to the 8 WAA rating. It is noteworthy that these four treatments provided the least control 8 WAA. The authors suggest the perceived increase in control could be due to the natural thinning of waterhemp populations as the season progresses, a phenomenon previously reported by Heneghan and Johnson (2017). At 12 WAA no herbicide treatment provided waterhemp control that was equivalent to the weed-free control. Waterhemp interference in the weedy control reduced corn yield up to 14% compared to the highest yielding treatments in this study, mesotrione plus atrazine, and tolpyralate plus atrazine. Although the minimal, late-season, waterhemp competition had no impact on final yield in any treatment, it is still necessary that farmers target perfect, full-season weed control to reduce weed-seed return to the soil. This was not achieved with the soil-applied herbicides evaluated in this study.

4.4.2 Postemergence

At 2 and 4 WAA, the HPPD + atrazine herbicide tankmixes, applied POST, caused up to 3 and 1% corn injury, primarily bleaching, respectively (data not presented).

At 4 WAA, all HPPD + atrazine treatments provided ≥ 86% control (Table 4.4). At 12 WAA, tembotrione + atrazine, tolpyralate + atrazine, and mesotrione + atrazine all
provided greater than 94% waterhemp control. Atrazine provided 64% control; reduced control can be attributed to triazine resistance at all three trial locations. The trend was similar throughout the remainder of the season; at 8 WAA all HPPD + atrazine treatments provided ≥ 88% control. Mesotrione + atrazine provided 91 and 93 % control 4 and 8 WAA, respectively. Results similar to Soltani et al. (2009) who reported 91 and 86% control of a triazine-resistant Ontario waterhemp population with mesotrione + atrazine (100 + 280 g ha\(^{-1}\)) 4 and 10 WAA, respectively. Waterhemp control varied 86-87% with topramezone + atrazine at 4, 8 and 12 WAA. Variable control of *Amaranthus* spp. with topramezone has been previously reported. Topramezone + atrazine (18 + 560 g ha\(^{-1}\)) applied to 15 cm Palmer Amaranth (*Amaranthus palmeri*) resistant to Groups 2, 5 and 9, provided 70% control 3 WAA (Kohrt and Sprague 2017). Isoxaflutole + atrazine was the weakest POST-applied herbicide treatment, providing 86% control 12 WAA. There was no other effect on yield for any treatment.

**4.5 Conclusions and Implications**

This study concludes that isoxaflutole + atrazine, mesotrione + atrazine and tolpyralate + atrazine are the most effective HPPD-inhibiting herbicides applied PRE, and mesotrione + atrazine, tembotrione + atrazine and tolpyralate + atrazine are the most effective HPPD-inhibiting herbicides applied POST. Effective weed control in field crops is influenced by a large variety of factors including: herbicide choice, application timing, rainfall and crop stage; however, relying on a single in-crop application for weed control introduces the risk of weather delays and weeds growing larger than the recommended application size, which can reduce control using any POST treatments (Eure et al. 2013). Although HPPD-inhibitors plus atrazine, applied PRE, provide up to
90% control of waterhemp and up to 98% control when applied POST, in practice it is important for farmers to apply multiple effective modes of action to reduce the likelihood of evolving resistances to these currently effective chemistries (Jansieniuk et al. 1996).
### 4.6 Figures and Tables

**Table 4.1** Soil classification and composition at each field site with HPPD plus atrazine tankmix research conducted on multiple-resistant waterhemp in Ontario in 2017 and 2018.

<table>
<thead>
<tr>
<th>Location</th>
<th>Classification</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>pH</th>
<th>Organic Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottam</td>
<td>Sandy-loam</td>
<td>66</td>
<td>24</td>
<td>10</td>
<td>6.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Walpole 1</td>
<td>Loamy-sand</td>
<td>78</td>
<td>14</td>
<td>8</td>
<td>8.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Walpole 2</td>
<td>Loamy-sand</td>
<td>76</td>
<td>18</td>
<td>6</td>
<td>8.0</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Table 3.2 Adjuvants and surfactants used with HPPD plus atrazine tankmixes applied POST at three locations in Ontario in 2017 and 2018.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rate (g a.i. ha(^{-1}))</th>
<th>Adjuvant(s)</th>
<th>Rate (Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine</td>
<td>1063</td>
<td>Assist oil</td>
<td>1.0 (% v/v)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>concentrate</td>
</tr>
<tr>
<td>Mesotrione + atrazine</td>
<td>100 + 280</td>
<td>Agra 90</td>
<td>0.2 (% v/v)</td>
</tr>
<tr>
<td>Topramezone + atrazine</td>
<td>12.5 + 500</td>
<td>Merge</td>
<td>0.5 (% v/v)</td>
</tr>
<tr>
<td>Tembotrione + atrazine</td>
<td>90 + 1000</td>
<td>Hasten</td>
<td>1.75 (l ha(^{-1}))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28% UAN</td>
<td>3.5 (l ha(^{-1}))</td>
</tr>
<tr>
<td>Tolpyralate + atrazine</td>
<td>30 + 1000</td>
<td>MSO concentrate</td>
<td>0.5 (% v/v)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28% UAN</td>
<td>2.5 (% v/v)</td>
</tr>
</tbody>
</table>
Table 4.3 Means for visual waterhemp control (4, 8 and 12 WAA), waterhemp density and dry biomass (8 WAA), corn yield with PRE-applied HPPD plus atrazine herbicide treatments in Ontario in 2017 and 2018.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rate (g a.i. ha(^{-1}))</th>
<th>4 WAA (%)</th>
<th>8 WAA (%)</th>
<th>12 WAA (%)</th>
<th>Density No. m(^{-2})</th>
<th>Dry Weight g m(^{-2})</th>
<th>Yield t ha(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weedy Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>486 a</td>
<td>248 a</td>
<td>10.3 b</td>
</tr>
<tr>
<td>Weed-Free Control</td>
<td>100 a</td>
<td>100 a</td>
<td>100 a</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
<td>11.4 ab</td>
</tr>
<tr>
<td>Atrazine</td>
<td>1063</td>
<td>75 cd</td>
<td>65 c</td>
<td>71 d</td>
<td>97 b</td>
<td>52 b</td>
<td>11.5 a</td>
</tr>
<tr>
<td>Isoxaflutole + atrazine</td>
<td>105 + 1063</td>
<td>93 ab</td>
<td>90 ab</td>
<td>90 b</td>
<td>13 b</td>
<td>11 b</td>
<td>11.4 ab</td>
</tr>
<tr>
<td>Mesotrione + atrazine</td>
<td>140 + 1490</td>
<td>91 ab</td>
<td>88 ab</td>
<td>87 b</td>
<td>28 b</td>
<td>13 b</td>
<td>12.0 a</td>
</tr>
<tr>
<td>Topramezone + atrazine</td>
<td>12.5 + 500</td>
<td>69 d</td>
<td>65 c</td>
<td>74 cd</td>
<td>133 b</td>
<td>78 b</td>
<td>11.8 a</td>
</tr>
<tr>
<td>Tempotrione + atrazine</td>
<td>90 + 1000</td>
<td>83 bc</td>
<td>76 bc</td>
<td>81 bcd</td>
<td>53 b</td>
<td>36 b</td>
<td>11.9 a</td>
</tr>
<tr>
<td>Tolpyralate + atrazine</td>
<td>30 + 1000</td>
<td>84 bc</td>
<td>79 ab</td>
<td>84 cb</td>
<td>48 b</td>
<td>54 b</td>
<td>12.0 a</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>5.0</td>
<td>7.8</td>
<td>6.1</td>
<td>65.5</td>
<td>39.4</td>
<td>0.8</td>
</tr>
</tbody>
</table>

\(a\)standardized to 15.5% moisture

\(b\)\(n=4\)

\(a-d\) Means followed by the same letter within a column are not significantly different using Tukeys LSD (P>0.05).
Table 4.4 Means for visual waterhemp control (4, 8 and 12 WAA), waterhemp density and dry biomass (4 WAA), corn yield with POST-applied HPPD plus atrazine treatments in Ontario in 2017 and 2018.

| Treatments	a | Rate (g a.i. ha⁻¹) | Crop Injury (%) | Waterhemp control (%)
|-------------|-------------------|----------------|------------------|
|             | 2 WAA  | 4 WAA  | 4 WAA  | 8 WAA  | 12 WAA  | Density | Dry Weight | Yield
| Weedy Control | 0 c     | 0 c     | 0 c     | 0       | 0       | 757 a   | 189 a      | 10.0 a
| Weed-Free Control | 0 c     | 0 b     | 100 a   | 100     | 100     | 0 b     | 0 b        | 10.7 a
| Atrazine     | 1063    | 0 c     | 0 b     | 64 c    | 67 c    | 65 c    | 330 ab    | 35 b     | 10.7 a
| Isoxaflutole + atrazine | 105 + 1063 | 1 bc | 0.3 ab | 88 b   | 90 ab   | 86 b    | 67 b     | 7 b       | 10.7 a
| Mesotrione + atrazine | 100 + 280 | 0 c | 0 b    | 91 ab   | 93 ab   | 94 ab   | 35 b     | 1 b       | 10.7 a
| Topramezone + atrazine | 12.5 + 500 | 0 c | 0 b    | 86 b   | 88 b   | 87 ab   | 279 b    | 9 b       | 10.9 a
| Tembotrione + atrazine | 90 + 1000 | 2 ab | 0 b    | 98 a   | 97 a   | 98 a    | 4 b      | 0 b       | 10.8 a
| Tolpyralate + atrazine | 30 + 1000 | 3 a | 1 a    | 94 ab   | 96 ab   | 97 ab   | 25 b     | 0 b       | 10.0 a
| SE    | 0.6    | 0.2    | 6.0    | 4.5    | 6.2    | 165.6   | 17.8      | 0.3      |

*all treatments applied with glyphosate (900 g a.i. ha⁻¹)

bstandardized to 15.5% moisture

cn=5

a-c Means followed by the same letter within a column are not significantly different using Tukeys LSD (P>0.05).
Chapter 5: Investigating the efficacy of single and two-pass herbicide programs for control of multiple resistant waterhemp (*Amaranthus tuberculatus*) in corn.

Lauren Benoit, David C. Hooker, Darren E. Robinson, Peter H. Sikkema.

5.1 Abstract

Effective control of waterhemp is becoming increasingly difficult in Ontario as biotypes have evolved resistance to four herbicide modes-of-action: Groups 2, 5, 9, and 14. Two studies were conducted at three locations in 2018 to determine if two-pass strategies provide superior control of MR waterhemp compared with single-pass strategies in corn. The first study evaluated MR waterhemp control with three HPPD-inhibitor based herbicides applied PRE: isoxaflutole/atrazine (79 + 800 g ai ha\(^{-1}\)), S-metolachlor/mesotrione/ atrazine/bicyclopyrone (1259/140/35/588 g ai ha\(^{-1}\)) and tolpyralate + atrazine (30 + 560 g ai ha\(^{-1}\)); these were applied with and without a POST application of glufosinate (500 g ai ha\(^{-1}\)). The second study evaluated MR waterhemp control with three non-HPPD inhibitor herbicides applied PRE: S-metolachlor/atrazine (1200/960 g ai ha\(^{-1}\)), saflufenacil/dimethenamid-\(P\) (75/660 g ai ha\(^{-1}\)), and dicamba/atrazine (604/1195 g ai ha\(^{-1}\)); these were applied with and without a POST application of mesotrione + atrazine (100 + 280 g ai ha\(^{-1}\)). Single-pass PRE applications of S-metolachlor/mesotrione/ bicyclopyrone/atrazine (1259/140/35/588 g ai ha\(^{-1}\)) and saflufenacil/dimethenamid-\(P\) (75/660 g ai ha\(^{-1}\)) controlled MR waterhemp 96% 12 WAA and control was not improved with a POST herbicides; however, with all other PRE herbicides, control was improved when followed by a POST application of either mesotrione + atrazine or glufosinate. There was no improvement in corn yield when any
PRE herbicide was followed by a POST application of mesotrione + atrazine or glufosinate. Ontario farmers have multiple effective single- and two-pass weed management strategies that provide effective MR waterhemp control in corn.

5.2 Introduction

Waterhemp (*Amaranthus tuberculatus*) is a dioecious, summer-annual weed, considered one of the most troublesome weeds by grain farmers in the American Mid-West. Waterhemp is difficult to control for multiple reasons: an extended emergence period, rapid growth rates, high fecundity, and required cross breeding for reproduction (Costea et al. 2005). In Ontario, waterhemp emerges from late-May until mid-October; therefore, weed control strategies must provide season-long control (Costea et al. 2005; Soltani et al. 2009; Hedges et al. 2018). As a dioecious weed, waterhemp houses male and female reproductive organs on separate plants, both a male and female plant are required to successfully reproduce (Costea et al. 2005). Obligatory out-crossing influences the rapid remixing and spread of genes from one population to another, increasing the rate at which a population can evolve resistance (Liu et al. 2012). Waterhemp has developed resistance to six herbicide sites-of-action: Group 2 (ALS-inhibitors), Group 4 (synthetic auxins), Group 5 (photosystem II-inhibitors), Group 9 (EPSPS inhibitors), Group 14 (PPO-inhibitors), and Group 27 (HPPD-inhibitors) (Bell et al. 2013; McMullan & Green 2011). Populations in Ontario and Illinois have been confirmed with multiple resistance to four sites-of-action including Group 2, 4, 9 and 14 herbicides (Bell et al. 2013; Schryver et al. 2017). In 2018, the first population resistant to 6 herbicide sites-of-action, Groups 2, 4, 5, 9, 14, and 27, was confirmed in Missouri; 16% of the population had the genes for six-way resistance (Shergill et al. 2018).
Studies completed in Ontario found that a PRE application of S-metolachlor/mesotrione/ atrazine/bicyclopyrone (1259/140/588/35 g a.i. ha\(^{-1}\)) or S-metolachlor/mesotrione/ atrazine (1393/139/524 g ai ha\(^{-1}\)) controlled 95 and 90% of MR waterhemp 8 weeks after application (WAA), respectively (Benoit, unpublished). Mesotrione and bicyclopyrone are also HPPD-inhibitors and Group 27 herbicides. PRE-applied group 27 herbicides, isoxaflutole and tolpyralate, tankmixed with atrazine (1000 g a.i. ha\(^{-1}\)), controlled MR waterhemp 90 and 84%, 12 WAA, respectively (Benoit et al. 2018). Data from five Ontario field trials showed that dicamba/ atrazine (604/1195 g a.i. ha\(^{-1}\)), S-metolachlor/ atrazine (1600/1280 g a.i. ha\(^{-1}\)) and saflufenacil/dimethenamid-P (75/660 g a.i. ha\(^{-1}\)) controlled MR waterhemp 76, 78 and 82% 8 WAA, respectively (Benoit et al. 2017). Given the adaptability of waterhemp to variable environments, its genetic diversity, and prolific seed production; complete control is important to eliminate weed seed return to the soil, reduce the selection intensity for herbicide-resistant biotypes, and decrease the spread of waterhemp.

Group 27 herbicides, applied PRE or POST, are effective herbicides for waterhemp control in corn, but often provide less than 100% control. Two-pass weed control programs need to be evaluated that provide season-long control of waterhemp. To reduce selection pressure for Group 27 resistant biotypes, it is important that Group 27 herbicides are not used both PRE and POST in a two-pass program. The purpose of this research is to identify effective two-pass weed control programs for MR waterhemp control in corn, while upholding proper resistance management practices.
5.3 Materials and Methods

Two studies were conducted at three locations in 2018 for a total of three site-years per study. Locations and soil composition are listed in Table 5.1. Study 1 investigated HPPD-inhibitors applied PRE alone and followed by (fb) glufosinate applied POST; herbicides, application rates, and herbicide application timings presented in Table 5.2. The following HPPD-inhibiting herbicides: isoxaflutole + atrazine, S-metolachlor/mesotrione/bicyclopyrone/atrazine and tolpyralate + atrazine, were selected to be representative of the isoxazole, triketone and pyrazolone herbicide families. The HPPD-inhibitors were tank-mixed with atrazine rates consistent with herbicide labels in Ontario. Study 2 investigated the following three non-HPPD-inhibitor herbicides applied PRE: S-metolachlor/ atrazine, saflufenacil/dimethenamid-P, or dicamba/ atrazine with and without a POST application of mesotrione + atrazine. Herbicides, application rates, and herbicide application timings are presented in Table 5.3. Pre-emergence treatments were selected based on previous studies on waterhemp control in corn in Ontario.

Waterhemp populations at all sites had biotypes that were resistant to Group 2, 5 and 9 herbicides based on greenhouse studies (Schryver et al. 2017). In each study, treatments were arranged in a randomized complete block design with four replications. Each replicate included an untreated weedy control and a weed-free control. The weed-free control was maintained weed-free with S-metolachlor/mesotrione/ atrazine/bicyclopyrone (1259/140/588/35 g ai ha⁻¹) applied PRE fb dicamba/ atrazine (604/1195 g ai ha⁻¹) applied POST and subsequent hand weeding as required. Plots were 8 m long by 2.25 m (3 corn rows space 0.76 m apart) wide with a 2 m ally between replicates. Herbicides were applied using a CO₂-pressurized
backpack sprayer and hand boom equipped with four ULD ultra low drift nozzles. The backpack sprayer was calibrated to deliver a water volume of 200 L ha\(^{-1}\) at 240 kPa.

Seedbed preparation consisted of disk in the fall and two passes with a field cultivator in the spring to prepare the seed bed. The previous crop was soybean at all locations. Corn (DKC 46-82RIB) was planted 4 cm deep at approximately 83 000 seeds ha\(^{-1}\). Preemergence herbicides were applied 1-3 days after planting. Postemergence herbicides were applied when waterhemp escapes were 10 cm tall or when corn reached growth stage V6, whichever occurred first. Crop injury was assessed visually at 2 and 4 weeks after emergence (WAE). Crop injury was evaluated on a scale of 0 to 100; with 0 representing no visual damage and 100 representing complete plant death.

Weed control, a visual estimate of the decrease in waterhemp biomass relative to the untreated control, was evaluated 4, 8 and 12 weeks after the POST application (WAA). In addition, weed control was assessed 1-2 days prior to the POST application. Weed counts and biomass were determined 4 WAA. Counts and dry weights were determined using two 0.25 m\(^2\) quadrats. The quadrats were randomly placed in each plot; waterhemp was counted, cut at the soil surface, placed in a paper bag, and then placed in a kiln and dried to constant moisture and weighed. Yield (t/ha) and moisture (%) were obtained at maturity by harvesting two rows from each plot using a small plot combine. Grain yields were corrected to 15.5% moisture before statistical analysis.

Data were analyzed using the GLIMMIX procedure in SAS v9.4 (Raleigh, NC). Studies were analyzed separately. Environment and replication were random effects. The only fixed effect is herbicide treatment. Normality was tested using the Shapiro-Wilk test conducted using SAS PROC UNIVARIATE. Normality assumptions were confirmed.
by plotting residuals against the predicted estimates, treatments, environment and replication. To compare PRE herbicides to two-pass combinations, PROC COVTEST was used for non-orthogonal contrasts (P=0.05).

5.4 Results

5.4.1 PRE-applied HPPD-inhibiting herbicides with and without glufosinate POST

Tolpyralate + atrazine, isoxaflutole + atrazine, and S-metolachlor/mesotrione/bicyclopyrone/ atrazine, controlled MR waterhemp 80, 84 and 96%, respectively, 4 WAA (Table 5.4). These values remained relatively static for the 8 and 12 WAA ratings. At 12 WAA, tolpyralate + atrazine, isoxaflutole + atrazine, and S-metolachlor/mesotrione/bicyclopyrone/ atrazine provided 77, 84, and 96% waterhemp control. At 4, 8 and 12 WAA, glufosinate applied POST controlled MR waterhemp 81-86%. Weather and field conditions influence the efficacy of glufosinate and control can be variable from year-to-year and location-to-location (Petersen and Hurle 2000).

Waterhemp control with glufosinate in corn and soybean has been variable. Schryver et al. 2017 reported 41% control 12 WAA using 500 g ai. ha$^{-1}$ of glufosinate in soybean. The addition of a late-season glufosinate application can improve season-long control, a two-pass POST program, including an early- and late-season glufosinate application in soybean provided up to 98% control (Schultz 2015). In corn, Benoit et al. 2017 reported 62% control with glufosinate 8 WAA. A disadvantage of glufosinate is the lack of soil residual to control late-emerging flushes of waterhemp. The addition of a residual herbicide to glufosinate will improve full-season control of Amaranth spp. (Tharp and Kells 2002).
At 4 and 8 WAA, there was an 11% improvement in MR waterhemp control when glufosinate was applied POST following isoxaflutole + atrazine applied PRE (Table 4). Similarly, across 4, 8 and 12 WAA assessments, MR WH control was 15-18% better (Table 4; contrast P<0.05) when glufosinate was applied POST following tolpyralate + atrazine applied PRE, respectively. In addition, there was a decrease in waterhemp density and biomass. There was no improvement in MR waterhemp control when glufosinate was applied POST following S-metolachlor/mesotrione/bicyclopyrone/atrazine applied PRE. The application of glufosinate applied POST following any of the PRE herbicides did not result in an increase in corn yield.

5.4.2 PRE-applied non-HPPD-inhibiting herbicides followed by mesotrione + atrazine

Dicamba/atrazine, S-metolachlor/atrazine, and saflufenacil/dimethenamid-P, applied PRE, controlled MR waterhemp 74, 85 and 93%, respectively, 4 WAA. Similar to the results of Study 1, control was similar (+/- 5%) at 8 and 12 WAA. At 4, 8 and 12 WAA, mesotrione + atrazine, applied POST controlled MR waterhemp 93-94%. Reported waterhemp control with mestrione + atrazine is more consistent than with glufosinate. Soltani et al. 2008 reported 86% control of group 2 resistant waterhemp 10 WAA with mesotrione + atrazine (100 + 280 g ai ha\(^{-1}\)). Recent Ontario studies have reported 91, 92 and 95% control of multiple resistant waterhemp 12 WAA using mesotrione + atrazine (100 + 280 g ai. ha\(^{-1}\)) (Benoit unpublished).

In a two-pass program, a PRE application of dicamba/atrazine fb mesotrione + atrazine applied POST improved the control of MR waterhemp 21, 20 and 19%, 4, 8
and 12 WAA, respectively (Table 5.5). There was no difference in waterhemp density and dry weight and corn yield with dicamba/atrazine applied PRE and dicamba/atrazine applied fb mesotrione + atrazine applied POST. In a two-pass program S-metolachlor/atrazine, applied PRE, fb mesotrione + atrazine, applied POST, improved the control of MR waterhemp 10, 13 and 12% at 4, 8 and 12 WAA, respectively. There was no impact on waterhemp density and dry weight and corn yield. When saflufenacil/dimethenamid-P, applied PRE, was compared to the two-pass program there were no differences for any parameter.

5.5 Conclusions and Implications

All two-pass herbicide programs resulted in ≥94% MR waterhemp control 12 WAA. S-metolachlor/mesotrione/bicyclopyrone/atrazine and saflufenacil/dimethenamid-P, applied PRE, controlled MR 96% 12 WAA; there was no benefit from a sequential herbicide applied POST. Saflufenacil/dimethenamid-P and S-metolachlor/mesotrione/bicyclopyrone/atrazine, applied PRE controlled waterhemp resistant to Groups 2, 5, and 9 82 and 91%, respectively 8 WAA (Benoit et al. 2019). In general, there was an improvement in MR waterhemp control with two-pass programs compared to one-pass programs applied PRE or POST. Hedges et al. (2018) and Schryver et al. (2017) reported superior control of MR waterhemp in soybean using two-pass strategies.

In summary, Ontario farmers have several effective herbicide options for MR waterhemp control in corn. A single PRE application of S-metolachlor/mesotrione/bicyclopyrone/atrazine (1259/140/35/288 g a.i. ha⁻¹) or saflufenacil/dimethenamid-P (75/660 g a.i. ha⁻¹) provided MR waterhemp control up to
12 WAA. Single-pass weed control is an attractive option for farmers during the busy growing season as it eliminates the expense of a second application, and POST applications can be delayed due to unsuitable spray conditions. In the event of early season waterhemp escapes, due to herbicide failure, a POST application of either mesotrione + atrazine or glufosinate following a residual PRE herbicide will achieve ≥94% full-season MR waterhemp control while incorporating several distinct modes-of-action. Tank-mixing and rotating multiple effective modes-of-action will reduce the evolution and spread of herbicide-resistant waterhemp.
### 5.6 Figures and Tables

**Table 5.1** Soil classification and composition at each field site with two-pass herbicide program research conducted on multiple-resistant waterhemp in Ontario in 2018.

<table>
<thead>
<tr>
<th>Location</th>
<th>Classification</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>pH</th>
<th>Organic Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottam</td>
<td>Sandy-loam</td>
<td>66</td>
<td>24</td>
<td>10</td>
<td>6.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Walpole 1</td>
<td>Loamy-sand</td>
<td>78</td>
<td>14</td>
<td>8</td>
<td>8.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Walpole 2</td>
<td>Loamy-sand</td>
<td>76</td>
<td>18</td>
<td>6</td>
<td>8.0</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Table 5.2 Application rates of HPPD inhibiting herbicides applied PRE and in two-pass combinations with glufosinate for control of multiple-resistant waterhemp in Ontario in 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (g. a.i ha$^{-1}$)</th>
<th>Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weedy check</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weed-free check</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Isoxaflutole/atrazine</td>
<td>79/ 800</td>
<td>PRE</td>
</tr>
<tr>
<td>$S$-metolachlor/mesotrione/bicyclopyrone/atrazine</td>
<td>1259/140/35/588</td>
<td>PRE</td>
</tr>
<tr>
<td>Tolpyralate/atrazine</td>
<td>30 /560</td>
<td>PRE</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>500</td>
<td>POST</td>
</tr>
<tr>
<td>Isoxaflutole/atrazine fb Glufosinate</td>
<td>79 + 800</td>
<td>PRE</td>
</tr>
<tr>
<td>fb Glufosinate</td>
<td>500</td>
<td>POST</td>
</tr>
<tr>
<td>$S$-metolachlor/mesotrione/bicyclopyrone/atrazine fb Glufosinate</td>
<td>1259/140/35/588</td>
<td>PRE</td>
</tr>
<tr>
<td>fb Glufosinate</td>
<td>500</td>
<td>POST</td>
</tr>
<tr>
<td>Tolpyralate + atrazine</td>
<td>30/560</td>
<td>PRE</td>
</tr>
<tr>
<td>fb Glufosinate</td>
<td>500</td>
<td>POST</td>
</tr>
</tbody>
</table>
Table 5.3 Application rates of preemergence herbicides applied alone and followed by mesotrione+ atrazine in two pass combinations for control of multiple-resistant waterhemp at three locations in Ontario in 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate</th>
<th>Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g. a.i ha(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>Weedy check</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weed-free check</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S-metolachlor/atrazine</td>
<td>1200/960</td>
<td>PRE</td>
</tr>
<tr>
<td>Saflufenacil/dimethenamid-p</td>
<td>735</td>
<td>PRE</td>
</tr>
<tr>
<td>Dicamba/ atrazine</td>
<td>1500</td>
<td>PRE</td>
</tr>
<tr>
<td>Mesotrione/atrazine(^a)</td>
<td>100/280</td>
<td>POST</td>
</tr>
<tr>
<td>S-metolachlor/atrazine</td>
<td>1200/960</td>
<td>PRE</td>
</tr>
<tr>
<td>fb Mesotrione + atrazine(^a)</td>
<td>100/280</td>
<td>POST</td>
</tr>
<tr>
<td>Saflufenacil/dimethenamid-p</td>
<td>735</td>
<td>PRE</td>
</tr>
<tr>
<td>fb Mesotrione + atrazine(^a)</td>
<td>100/280</td>
<td>POST</td>
</tr>
<tr>
<td>Dicamba/ atrazine</td>
<td>1500</td>
<td>PRE</td>
</tr>
<tr>
<td>fb Mesotrione + atrazine(^a)</td>
<td>100/280</td>
<td>POST</td>
</tr>
</tbody>
</table>

\(^a\)applied with 0.2% (v/v) Agral 90
Table 5.4 Means and non-orthogonal contrasts for multiple resistant waterhemp control (%), density (no. m⁻¹), biomass (g m⁻¹) and corn yield (t ha⁻¹) using HPPD-inhibiting herbicides applied PRE and in a two-pass program for control of multiple-resistant waterhemp at three locations in Ontario in 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (g ai ha⁻¹)</th>
<th>App Timing</th>
<th>Control (%)</th>
<th>Density</th>
<th>Biomass</th>
<th>Corn Yield (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weed-Free</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>10.6</td>
</tr>
<tr>
<td>Weedy Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>164</td>
<td>74.3</td>
<td>11.9</td>
</tr>
<tr>
<td>Isoxaflutole/ atrazine</td>
<td>79/800</td>
<td>PRE</td>
<td>84</td>
<td>8</td>
<td>8.2</td>
<td>10.8</td>
</tr>
<tr>
<td>S-metolachlor/ mesotrione/bicyclopyrone/ atrazine</td>
<td>1259/140/35/588</td>
<td>PRE</td>
<td>96</td>
<td>1</td>
<td>0.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Tolpyralate/ atrazine</td>
<td>30/560</td>
<td>PRE</td>
<td>80</td>
<td>26</td>
<td>14.8</td>
<td>12.0</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>500</td>
<td>POST</td>
<td>81</td>
<td>20</td>
<td>2.0</td>
<td>11.7</td>
</tr>
<tr>
<td>Isoxaflutole/ atrazine fb Glufosinate</td>
<td>79/800</td>
<td>PRE</td>
<td>95</td>
<td>4</td>
<td>0.5</td>
<td>12.0</td>
</tr>
<tr>
<td>S-metolachlor/ mesotrione/bicyclopyrone/ atrazine fb Glufosinate</td>
<td>1259/140/35/588</td>
<td>POST</td>
<td>95</td>
<td>5</td>
<td>0.8</td>
<td>11.7</td>
</tr>
<tr>
<td>Tolpyralate/ atrazine fb Glufosinate</td>
<td>30/560</td>
<td>PRE</td>
<td>95</td>
<td>5</td>
<td>0.8</td>
<td>11.7</td>
</tr>
<tr>
<td>Contrasts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoxaflutole/ atrazine vs.</td>
<td>84 vs. 95*</td>
<td>85 vs. 96*</td>
<td>84 vs. 94</td>
<td>8 vs. 1</td>
<td>8.2 vs.</td>
<td>10.8 vs.</td>
</tr>
<tr>
<td>Isoxaflutole atrazine fb Glufosinate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5 vs. 12.0</td>
</tr>
<tr>
<td>S-metolachlor/ mesotrione/bicyclopyrone/ atrazine vs.</td>
<td>96 vs. 97</td>
<td>94 vs. 97</td>
<td>96 vs. 97</td>
<td>1 vs. 2</td>
<td>0.8 vs.</td>
<td>12.6 vs.</td>
</tr>
<tr>
<td>S-metolachlor/ mesotrione/bicyclopyrone/ atrazine fb Glufosinate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3 vs. 12.3</td>
</tr>
<tr>
<td>Tolpyralate/ atrazine vs.</td>
<td>80 vs. 95*</td>
<td>80 vs. 95*</td>
<td>77 vs. 95*</td>
<td>26 vs.</td>
<td>14.8 vs.</td>
<td>12.0 vs.</td>
</tr>
<tr>
<td>Tolpyralate/atrazine fb Glufosinate</td>
<td>5*</td>
<td>0.8*</td>
<td>11.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-----</td>
<td>------</td>
<td>------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>applied with 0.2% v/v Agral 90. * denotes significance (P&lt;0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>
**Table 5.5** Means and non-orthogonal contrasts for multiple resistant waterhemp control (%), density (no. m\(^{-1}\)), biomass (g m\(^{-1}\)) and corn yield (t ha\(^{-1}\)) using single and two-pass herbicide programs at three locations in Ontario in 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (g ai ha(^{-1}))</th>
<th>App Timing</th>
<th>4 WAA</th>
<th>8 WAA</th>
<th>12 WAA</th>
<th>Density</th>
<th>Biomass</th>
<th>Corn Yield (t ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weed-Free Control</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>10.9</td>
</tr>
<tr>
<td>Weedy Control</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>294</td>
<td>99.3</td>
<td>11.9</td>
</tr>
<tr>
<td><em>S</em>-metolachlor/atrazine</td>
<td>1200/960</td>
<td>PRE</td>
<td>85</td>
<td>84</td>
<td>86</td>
<td>10</td>
<td>6.7</td>
<td>11.9</td>
</tr>
<tr>
<td>Saflufenacil/dimethenamid-(P)</td>
<td>75/600</td>
<td>PRE</td>
<td>93</td>
<td>95</td>
<td>96</td>
<td>2</td>
<td>2.1</td>
<td>11.5</td>
</tr>
<tr>
<td>Dicamba/atrazine</td>
<td>604/1195</td>
<td>PRE</td>
<td>74</td>
<td>77</td>
<td>79</td>
<td>21</td>
<td>12.8</td>
<td>12.1</td>
</tr>
<tr>
<td>Mestrionate/atrazine(^a)</td>
<td>100/280</td>
<td>POST</td>
<td>94</td>
<td>93</td>
<td>93</td>
<td>15</td>
<td>1.6</td>
<td>11.3</td>
</tr>
<tr>
<td><em>S</em>-metolachlor/atrazine(^a)</td>
<td>1200/960</td>
<td>PRE</td>
<td>95</td>
<td>97</td>
<td>98</td>
<td>4</td>
<td>0.2</td>
<td>11.6</td>
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<tr>
<td>(fb) Mestrionate/atrazine(^a)</td>
<td>100/280</td>
<td>POST</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>0</td>
<td>0</td>
<td>11.6</td>
</tr>
<tr>
<td>Saflufenacil/dimethenamid-(P)</td>
<td>75/600</td>
<td>PRE</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>0</td>
<td>0</td>
<td>11.6</td>
</tr>
<tr>
<td>(fb) Mestrionate/atrazine(^a)</td>
<td>100/280</td>
<td>POST</td>
<td>98</td>
<td>98</td>
<td>98</td>
<td>0</td>
<td>0</td>
<td>12.1</td>
</tr>
<tr>
<td>Dicamba/atrazine</td>
<td>604/1195</td>
<td>PRE</td>
<td>98</td>
<td>98</td>
<td>98</td>
<td>0</td>
<td>0</td>
<td>12.1</td>
</tr>
<tr>
<td>(fb) Mestrionate/atrazine(^a)</td>
<td>100/280</td>
<td>POST</td>
<td>98</td>
<td>98</td>
<td>98</td>
<td>0</td>
<td>0</td>
<td>12.1</td>
</tr>
</tbody>
</table>

**Contrasts**

<table>
<thead>
<tr>
<th>Contrasts</th>
<th>Rate (g ai ha(^{-1}))</th>
<th>App Timing</th>
<th>4 WAA</th>
<th>8 WAA</th>
<th>12 WAA</th>
<th>Density</th>
<th>Biomass</th>
<th>Corn Yield (t ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S)-metolachlor/atrazine vs.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S)-metolachlor/atrazine (fb) Mestrionate/atrazine</td>
<td>95(^*)</td>
<td>97(^*)</td>
<td>98(^*)</td>
<td>4</td>
<td>0.2</td>
<td></td>
<td></td>
<td>11.6</td>
</tr>
<tr>
<td>Saflufenacil/dimethenamid-(P) vs.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saflufenacil/dimethenamid-(P) (fb) Mestrionate/atrazine</td>
<td>97</td>
<td>97</td>
<td>97</td>
<td>2</td>
<td>0.3</td>
<td></td>
<td></td>
<td>11.6</td>
</tr>
<tr>
<td>Dicamba/atrazine vs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dicamba/atrazine (fb) Mestrionate/atrazine(^a)</td>
<td>98(^*)</td>
<td>98(^*)</td>
<td>98(^*)</td>
<td>0(^*)</td>
<td>0(^*)</td>
<td></td>
<td></td>
<td>12.1</td>
</tr>
</tbody>
</table>
applied with 0.2% v/v Agral 90. * denotes significance (P<0.05)
Chapter 6: Influence of atrazine on the efficacy and consistency of HPP-inhibiting herbicides applied for postemergence control of multiple-resistant Waterhemp (*Amaranthus tuberculatus*).

Lauren Benoit, David C. Hooker, Darren E. Robinson, Peter H. Sikkema.

6.1 Abstract

The application of 4-hydroxyphenylpyruvate dioxygenase-(HPPD) inhibiting herbicides with atrazine provide consistent and effective control of waterhemp resistant to herbicide Groups 2, 5 and 9 in Ontario. Three field trials were conducted in 2018 to determine if the addition of atrazine was needed to maintain the efficacy and consistency of waterhemp control with HPPD-inhibiting herbicides. Five post-emergence HPPD-inhibiting herbicides, including: isoxaflutole (105 g a.i ha\(^{-1}\)), mesotrione (100 g a.i ha\(^{-1}\)), topramezone (12.5 g a.i ha\(^{-1}\)), tembotrione (90 g a.i ha\(^{-1}\)), and tolpyralate (30 g a.i ha\(^{-1}\)) were applied with and without atrazine to 10-cm-tall waterhemp. The addition of atrazine to isoxaflutole improved control of waterhemp 20, 17 and 15% at 4, 8 and 12 WAA, respectively. At 12 WAA, the addition of atrazine to mesotrione and tembotrione improved waterhemp control 2 and 4%, respectively. The addition of atrazine reduced the standard deviation of all treatments for all parameters with the exception of waterhemp control with topramezone at 4 WAA, control with mesotrione at 8 WAA and waterhemp density at 8 WAA with mesotrione. This study found that with the addition of atrazine to HPPD-inhibiting herbicides applied POST tended to increase the consistency of waterhemp control.
6.2 Introduction

Waterhemp (*Amaranthus tuberculatus*) has consistently been ranked as one of the most troublesome weeds in the US Corn Belt. A Missouri waterhemp population has confirmed multiple resistance to six herbicide modes-of-action: Groups 2, 4, 5, 9, 14 and 27 (Shergill et al. 2018). If left uncontrolled for the entire growing season, waterhemp interference has been shown to reduce Ontario corn yields by 48% (Soltani et al. 2009). In Illinois, up to 74% corn yield loss has been recorded (Steckel and Sprague 2004). Ontario waterhemp populations have evolved resistance to four herbicide modes-of-action: Groups 2, 5, 9 and 14 (Benoit et al. 2018). With the evolution of multiple-resistant (MR) waterhemp populations and the concomitant decrease in effective herbicide options, it is crucial that Ontario grain farmers implement waterhemp management practices that provide near-perfect, full season control. This will reduce weed seed return to the soil and reduce the selection intensity for MR biotypes. Using multiple effective herbicide modes-of-action can improve control and reduce the likelihood of selecting for resistant biotypes (Diggle et al. 2003).

Applying two herbicides with distinct modes-of-action together can result in an antagonistic, additive or synergistic effects. Herbicide antagonism results in reduced control, additive control is when the control of the products applied in combination is equal to the expected control, and synergism occurs when the control of the products applied in combination is greater than the expected control based on the products applied individually (Damalas 2004). To determine if two products are synergistic or additive, the Colby’s equation \[ E = X + Y - \frac{XY}{100} \] where ‘E’ represents expected control of the products in combination, and ‘X’ and ‘Y’ represent the control of each
herbicide applied independently. If the control of the combination is greater than 'E' the interaction is synergistic, if the control is equal to 'E' the interaction is additive, and if the control is less then 'E' the interaction is antagonistic (Colby 1967).

One of the most common tank-mixes used by Ontario corn farmers is a 4-hydroxyphenylpyruvate-dioxygenase (HPPD)-inhibiting herbicide with atrazine. Atrazine is a triazine herbicide that was introduced to the market in 1958 as a preemergence (PRE) and postemergence (POST) option for broadleaf weed control in corn (Ulrich et al. 2012). Atrazine occupies the Q_B binding site on the D1 protein of photosystem II, increasing the production of reactive oxygen species (ROS); singlet oxygen and triplet chlorophyll (Shaner et al. 2014). Reactive oxygen species cause lipid peroxidation and cell membrane destruction, resulting in plant death (Bartosz 1997). 4-hydroxyphenylpyruvate-dioxygenase- inhibiting herbicides prevent the conversion of hydroxyphenylpyruvate to homogentisate (Lee et al. 1997). Homogentisate is the precursor to antioxidant compounds: plastiquinone, \( \alpha \)-tocopherols and carotenoids (Lee et al. 1997). Antioxidant compounds quench ROS, reducing or eliminating the impact of oxidative stress on the plant (Trebst et al. 2002). In the absence of plastiquinone, \( \alpha \)-tocopherols and carotenoids to quench ROS, the plant succumbs to oxidative stress resulting in plant death (Trebst et al. 2002). When applied in combination, triazine herbicides, such as atrazine, and HPPD-inhibiting herbicides causes a simultaneous increase in ROS and decrease in antioxidants, resulting in more effective weed control (Abendroth et al. 2006).

Synergistic interaction between atrazine and HPPD-inhibiting herbicides has been reported in waterhemp, common lambsquarters (Chenopodium album L.) and

The objective of this research was to determine if the addition of atrazine as a tankmix partner with HPPD-inhibiting herbicides improves control, and consistency of control, of MR waterhemp in Ontario.

**6.3 Materials and Methods**

Three field trials were conducted in 2018 at three locations in Ontario. Multiple-resistant waterhemp with resistance to Groups 2, 5, and 9 herbicides were present at all sites (Schryver 2017). Soil type and composition of each site is listed in Table 6.1. Trial locations were disked in the fall and cultivated twice in the spring prior to planting. Corn (DKC 46-82RIB) was planted in the spring at approximately 83 000 seed ha\(^{-1}\) to a depth of 4 cm. Trials were arranged in a randomized complete block design, consisting of four replicates. Each replicate included a weedy and a weed-free control. Replicates were separated by a 2 m alley. Plots were 8 m long and 2.25 m (3 corn rows spaced 0.75 m
apart) wide. The weed-free plot was maintained with a preemergence (PRE) application of S-metolachlor/mesotrione bicyclopyrone/ atrazine (2022 g ai ha\(^{-1}\)) followed by dicamba/ atrazine (1800 g ai ha\(^{-1}\)) applied postemergence (POST) at the V3-stage (5-leaf stage) of corn development; hand-weeding was performed throughout the remainder of the growing season as needed. A POST application of glyphosate (450 g ae ha\(^{-1}\)) was applied to the entire trial area, including the weedy control, to eliminate interference of susceptible waterhemp biotypes and other weed species.

Herbicide treatments were applied using a backpack sprayer with CO\(_2\) as the propellant, calibrated to deliver 200 L ha\(^{-1}\) at 250 kPa, and equipped with a 1.5 m boom with four ULD 12002 nozzles (Pentair, New Brighton, MN) spaced 50 cm apart. Herbicide treatments were applied when waterhemp populations reached an average 10 cm in height. Herbicide treatments are presented in Table 6.2. Adjuvants for each treatment are listed in Table 3. Herbicide trade names and manufacturers are listed in Table 4. Atrazine rates and adjuvants were based on label recommendations in Canada.

Corn injury, waterhemp control, waterhemp density and biomass, and corn yield were collected. Corn injury was assessed visually on a percent scale at 1 and 4 weeks after application (WAA); 0 represented no visible injury and 100 represented complete plant death. Waterhemp control was evaluated visually on a 0 to 100 scale, as an estimation of biomass reduction compared to the weedy control at 4, 8, and 12 WAA. Waterhemp density was determined by counting the number of waterhemp within two randomly placed, 0.25m\(^2\) quadrats per plot. Aboveground waterhemp biomass was calculated from the plants within each quadrat; they were then cut at the soil surface, placed inside a paper bag, dried at 49°C for three weeks to consistent moisture, and then weighed.
Two of the corn rows were harvested in the fall using a small-plot combine, yield and moisture were recorded. Grain yields were converted to 15.5% moisture prior to statistical analysis.

PROC GLIMMIX in SAS v. 9.4 (SAS Institute Inc., Cary, NC) was used for data analysis. A covariance analysis determined no treatment by location interaction; therefore data from all locations was combined. Random effects were replicate and trial, and the fixed effect was herbicide treatment. Normality was tested with the Shapiro-Wilk test using SAS PROC UNIVARIATE. Normality assumptions were confirmed by plotting the residuals for predicted, treatment, replicate, and trial. Means were calculated and separated using Tukey’s LSD (p=0.05). Non-orthogonal contrasts were used to determine if there was a benefit to adding atrazine to each HPPD inhibitor individually and as a group. To determine if the addition of atrazine improves the consistency of HPPD-inhibiting herbicides standard deviations of each treatment were calculated using the raw data.

6.4 Results and Discussion

6.4.1 Contrasts

At 1 and 4 WAA, the herbicide treatments evaluated caused ≤3% visible corn injury (data not presented). At 4 WAA, atrazine, applied POST controlled MR waterhemp 66% (Table 6.3); this can be attributed to confirmed triazine resistance at all three locations. At 4 WAA, HPPDs applied alone controlled MR waterhemp 73 to 93%, the addition of atrazine improved waterhemp control to 90 to 99% (Table 5). At 4, 8 and 12 WAA, the addition of atrazine to isoxaflutole improved waterhemp control from 73-83% to 93-98%
(P<0.05). The addition of atrazine to isoxaflutole did not result in a decrease of waterhemp density and biomass. The addition of atrazine to mesotrione resulted in a numeric increase in waterhemp control at 4, 8 and 12 WAA and a greater decrease in density and biomass; however, only waterhemp control at 12 WAA was statistically significant. Hugie et al. (2008) reported a minimum 1:2 ratio of mesotrione to atrazine was required for a synergistic increase in triazine-resistant redroot pigweed control in greenhouse trials, in triazine-susceptible populations the minimum required ratio was 1:2.25. The mesotrione:atrazine ratio used in this study was 1:2.8, chosen to be consistent with the herbicide label. A study conducted in Illinois by Woodyard at al. (2009a) reported a 23-43% increase in control of waterhemp when 280 g a.i. ha\(^{-1}\) of atrazine was added to 105 g a.i. ha\(^{-1}\) of mesotrione. The Woodyard at al. (2009a) study recorded 12-60% control with atrazine (280 g a.i. ha\(^{-1}\)) alone, suggesting the population in question was resistant although it is not explicitly stated in the text. Mesotrione (105 g a.i. ha\(^{-1}\)) provided 48-75% control of waterhemp; reduced control could be attributed to a delayed application due to weather as mentioned by the authors. The addition of atrazine to topramezone resulted in a numeric increase in waterhemp control at 4, 8 and 12 WAA, however only waterhemp control at 4 WAA was statistically significant (p < 0.10). Similarly, The addition of atrazine to tembotrione resulted in a numeric increase in waterhemp control at 4, 8 and 12 WAA, only waterhemp control at 4 and 12 WAA was statistically significant (p <0.10). There was no statistical improvement in waterhemp control when atrazine was added to tolpyralate. Corn grain yield was similar across all treatments.
6.4.2 Standard Deviations

Standard deviations were calculated using raw data. After error due to block and treatment effect is removed, the standard deviation for each treatment is the same, meeting the assumptions of ANOVA. Raw data was used to determine with treatments have the highest variability across different environments. At 4 WAA, the addition of atrazine to a HPPD-inhibiting herbicide reduced the standard deviation of the MR waterhemp control 3.3-9.6% for all treatments with the exception of topramezone (Table 6.4). At 8 and 12 WAA, the addition of atrazine to a HPPD-inhibiting herbicide reduced the standard deviation of waterhemp control an average of 8.0 and 9.7, respectively. Similarly, the addition of atrazine to a HPPD-inhibiting herbicide reduced the standard deviation of waterhemp density and biomass an average of 11 and 0.9, respectively. This data supports that although the addition of atrazine to a HPPD-inhibiting herbicide does not always increase MR waterhemp control, it does improve the consistency of control. This is a clear benefit for Ontario farmers.
6.5 Figures and Tables

Table 6.1 Soil classification and composition at each of the locations used for research on HPPD-inhibiting herbicides with and without the addition of atrazine for control of waterhemp in Ontario in 2018.

<table>
<thead>
<tr>
<th>Location</th>
<th>Classification</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>pH</th>
<th>Organic Matter</th>
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</thead>
<tbody>
<tr>
<td>Cottam</td>
<td>Sandy loam</td>
<td>75</td>
<td>18</td>
<td>7</td>
<td>6.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Walpole Island 1</td>
<td>Sandy loam</td>
<td>75</td>
<td>20</td>
<td>5</td>
<td>7.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Walpole Island 2</td>
<td>Sandy loam</td>
<td>75</td>
<td>20</td>
<td>5</td>
<td>7.5</td>
<td>2.3</td>
</tr>
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Table 6.2 Herbicide active ingredient, adjuvants and manufacturers for research on HPPD-inhibiting herbicides with and without the addition of atrazine for control of waterhemp in Ontario in 2018.

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Adjuvant(s)</th>
<th>Rate (Unit)</th>
<th>Herbicide Manufacturer</th>
<th>Adjuvant Manufacturer</th>
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<td>Atrazine</td>
<td>Assist oil concentrate</td>
<td>1.0 (% v/v)</td>
<td>Syngenta Canada Inc.</td>
<td>BASF Canada Inc.</td>
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<td></td>
<td></td>
<td>140 Research Ln, Guelph, ON.</td>
<td>100 Milverton Dr., Mississauga, ON.</td>
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<tr>
<td>Mesotrione</td>
<td>Agral 90</td>
<td>0.2 (% v/v)</td>
<td>Syngenta Canada Inc.</td>
<td>Syngenta Canada Inc.</td>
</tr>
<tr>
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<td></td>
<td>140 Research Ln, Guelph, ON.</td>
<td>140 Research Ln, Guelph, ON.</td>
</tr>
<tr>
<td>Isoxaflutole</td>
<td>-</td>
<td>-</td>
<td>Bayer CropScience Inc.</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>160 Quarry Park Blvd, Calgary AB.</td>
<td></td>
</tr>
<tr>
<td>Topramezone</td>
<td>Merge</td>
<td>0.5 (% v/v)</td>
<td>BASF Canada Inc.</td>
<td>BASF Canada Inc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100 Milverton Dr., Mississauga, ON.</td>
<td>100 Milverton Dr., Mississauga, ON.</td>
</tr>
<tr>
<td>Tembotrione</td>
<td>A) Hasten</td>
<td>1.75 (l ha⁻¹)</td>
<td>Bayer CropScience LP.</td>
<td>A) Victorian Chemical Company Pty. Ltd.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 TW Alexander Dr. 2 TW Alexander Dr.</td>
<td>83 Maffra St. Coolaroo, Victoria, AUS.</td>
</tr>
<tr>
<td></td>
<td>B) 28% UAN</td>
<td>3.5 (l ha⁻¹)</td>
<td>Research Triangle Park, NC, USA</td>
<td>B) Sylvite</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3221 North Service Rd. Burlington, ON.</td>
</tr>
<tr>
<td>Tolpyralate</td>
<td>A) MSO concentrate</td>
<td>0.5 (% v/v)</td>
<td>Engage Agro Corp.</td>
<td>A) Loveland Products</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1030 Gordon St, Guelph, ON.</td>
<td>3005 Rocky Mountain Ave., Loveland, CO.</td>
</tr>
<tr>
<td></td>
<td>B) 28% UAN</td>
<td>2.5 (% v/v)</td>
<td></td>
<td>B) Sylvite</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3221 North Service Rd. Burlington, ON.</td>
</tr>
</tbody>
</table>
Table 6.3 Non-orthogonal contrasts of visual waterhemp control, density, biomass and grain corn yield using HPPD-inhibiting herbicides with and without the addition of atrazine at three locations in Ontario in 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate</th>
<th>Atr Rate</th>
<th>Visual Weed Control (%)</th>
<th>Density (No. m⁻²)</th>
<th>Biomass (g m⁻²)</th>
<th>Yieldᵃ (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>--- (g ai ha⁻¹) ---</td>
<td>4 WAA</td>
<td>8 WAA</td>
<td>12 WAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weedy</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>190</td>
<td>53.7</td>
</tr>
<tr>
<td>Weed-Free</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Atrazine</td>
<td>1063</td>
<td>66</td>
<td>75</td>
<td>72</td>
<td>8</td>
<td>6.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Alone</th>
<th>Plus atr</th>
<th>Alone</th>
<th>Plus atr</th>
<th>Alone</th>
<th>Plus atr</th>
<th>Alone</th>
<th>Plus atr</th>
<th>Alone</th>
<th>Plus atr</th>
<th>Alone</th>
<th>Plus atr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoxaflutole</td>
<td>105</td>
<td>1063</td>
<td>73</td>
<td>93*</td>
<td>81</td>
<td>98*</td>
<td>83</td>
<td>98*</td>
<td>8</td>
<td>1</td>
<td>2.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Mesotrione</td>
<td>100</td>
<td>280</td>
<td>87</td>
<td>96</td>
<td>97</td>
<td>98</td>
<td>94</td>
<td>96*</td>
<td>3</td>
<td>2</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Topramezone</td>
<td>12.5</td>
<td>500</td>
<td>82</td>
<td>90*</td>
<td>84</td>
<td>94</td>
<td>82</td>
<td>91</td>
<td>24</td>
<td>2</td>
<td>2.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Tembotrione</td>
<td>90</td>
<td>1000</td>
<td>91</td>
<td>99*</td>
<td>95</td>
<td>99</td>
<td>94</td>
<td>98*</td>
<td>1</td>
<td>0</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>Tolpyralate</td>
<td>30</td>
<td>560</td>
<td>93</td>
<td>97</td>
<td>90</td>
<td>98</td>
<td>89</td>
<td>97</td>
<td>3</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
</tr>
</tbody>
</table>

ᵃYield standardized to 15.5% moisture

Abbreviations: Atr=atrazine, WAA=weeks after application.

*(p<0.05), +(p<0.1)
Table 6.4 Standard deviations of visual control, density, and biomass of multiple-resistant waterhemp using HPPD-inhibiting herbicides with and without the addition of atrazine at three locations in Ontario in 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (g ai ha$^{-1}$)</th>
<th>Atr Rate (g ai ha$^{-1}$)</th>
<th>Visual Weed Control (%)</th>
<th>Density (No. m$^{-2}$)</th>
<th>Biomass (g m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 WAA 8 WAA 12 WAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrazine</td>
<td>1063</td>
<td>15.49 13.39 16.2</td>
<td></td>
<td>1.0</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Alone</em></td>
<td><em>Plus</em></td>
<td><em>Alone</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>atr</td>
<td>atr</td>
<td>atr</td>
</tr>
<tr>
<td>Isoxaflutole</td>
<td>105 1063</td>
<td>12.5 9.2 13.7 1.8 14.8</td>
<td>1.6</td>
<td>7 1</td>
<td>1.5 0.5</td>
</tr>
<tr>
<td>Mesotrione</td>
<td>100 280</td>
<td>11.1 6.9 2.9 3.5 7.1</td>
<td>5.7</td>
<td>4 5</td>
<td>1.3 0.8</td>
</tr>
<tr>
<td></td>
<td>12.5 500</td>
<td>11.7 15.3 19.1 11.3</td>
<td>20.6</td>
<td>48 7</td>
<td>4.5 2.9</td>
</tr>
<tr>
<td>Topramzone</td>
<td>90 1000</td>
<td>9.6 0 8.3 0 14.1 1.5</td>
<td>2</td>
<td>0</td>
<td>0.6 0</td>
</tr>
<tr>
<td>Tembotrione</td>
<td>30 560</td>
<td>9.8 2.9 15.1 2.7 18.6</td>
<td>5.5</td>
<td>7 0</td>
<td>0.6 0</td>
</tr>
<tr>
<td>Tolpyralate</td>
<td>30 560</td>
<td>9.8 2.9 15.1 2.7 18.6</td>
<td>5.5</td>
<td>7 0</td>
<td>0.6 0</td>
</tr>
</tbody>
</table>

Abbreviations: Atr=atrazine, WAA=weeks after application.
Chapter 7: General Discussion

7.1 Contributions

This research is of significant value for Ontario grain farmers. The survey confirmed the presence of waterhemp in two additional Ontario counties; Haldimand and Middlesex, and the province of Quebec. Multiple-resistant waterhemp populations with resistance to Herbicide Group 2, 5, 9, and 14 were confirmed at multiple locations in Ontario. This is the first record of a Group 14 resistant weed in the province, and the first record of a four-way multiple-resistant weed in Canada. The most efficacious herbicides applied preemergence, postemergence, and two-pass herbicide programs were identified for control of multiple-resistant waterhemp in corn.

Following the initial survey conducted in 2014 and 2015 that reported GR waterhemp at 48 locations in Ontario, seed was collected from an additional 23 locations in 2016 and 2017, including one seed lot from Middlesex county, one seed lot from Haldimand county, and one seed lot from Quebec. Waterhemp was screened in the greenhouse for resistance to imazethapyr (Group 2), atrazine (Group 5), glyphosate (Group 9), and lactofen (Group 14) with the use of a spray-chamber to apply the active ingredients to individually potted waterhemp plants when they were 10 cm in height. Ten plants from each population were screened for each herbicide. Of the 23 samples tested: 100% had individual plants that were resistant to imazethapyr, 88% had individual plants that were resistant to atrazine, 84% had individual plants that were resistant to glyphosate, and 43% had individual plants that were resistant to lactofen, and 43% of the populations had individual plants that were resistant to all four herbicide
modes-of-action. Leaf samples from 2017 populations that survived an application of lactofen were sent to Dr. Patrick Tranel at the University of Illinois to be tested for the ΔG210 codon deletion that confers resistance to Group 14 herbicides. The codon deletion was confirmed in four of the populations; it is possible that there is an additional mechanism conferring resistance in the other populations.

Herbicides, applied PRE or POST, were evaluated for control of multiple-resistant (Groups 2, 5, and 9) waterhemp in corn. This research found that herbicides, applied PRE, must have extended residual activity to maintain control of waterhemp throughout the entire growing season. At 8 WAA, the most efficacious herbicides applied PRE were S-metolachlor/mesotrione/ atrazine (1393/139/524 g ai ha⁻¹) and S-metolachlor/mesotrione/ atrazine/bicyclopyrone (1259/140/588/35 g ai ha⁻¹) which controlled waterhemp 87 and 91%, respectively. At 8 WAA, the most efficacious herbicides applied POST were dicamba/ atrazine (57/143 g ai ha⁻¹) and mesotrione + atrazine (100 + 280 g ai ha⁻¹) which controlled waterhemp 87 and 92%, respectively.

Relative efficacy of Group 27 herbicides applied PRE and POST was evaluated in field trials completed in 2017 and 2018. Five Group 27 herbicides plus atrazine: isoxaflutole (105 + 1063 g ai ha⁻¹), mesotrione (PRE: 140 + 1490 g ai ha⁻¹, POST: 100 + 288 g ai ha⁻¹), topramezone (12.5 + 500 ai ha⁻¹), tembotrione (90 + 1000 ai ha⁻¹), and tolpyralate (30 + 1000 ai ha⁻¹), were applied PRE and POST in two separate field trials. At 12 WAA, no Group 27 + atrazine treatment, applied PRE, provided control equivalent to the weed-free control. At 12 WAA, topramezone, mesotrione, tolpyralate, and tembotrione, tank mixed with atrazine, applied POST, controlled MR waterhemp 87, 94, 97 and 98%, respectively which was equivalent to the weed-free control. In seasons
where herbicides applied PRE do not receive sufficient rain so they are dissolved in soil water solution and can be taken up by the developing seedlings. A two-pass herbicide program may be necessary to achieve full season control of multiple-resistant waterhemp. For this reason two-pass herbicide programs were evaluated. Field studies were conducted at three locations in 2018. S-metolachlor/mesotrione/ atrazine/bicyclopyrone (1259/140/588/35 ai ha\(^{-1}\)) and saflufenacil/dimethenamid-\(P\) (75/660 ai ha\(^{-1}\)), applied PRE, controlled MR waterhemp 96\% 12 WAA and there was no benefit of a subsequent herbicide applied POST. Group 27 based herbicide tankmixes: isoxaflutole + atrazine (79 + 800 ai ha\(^{-1}\)) and tolpyralate + atrazine (30 + 560 ai ha\(^{-1}\)), applied PRE followed by glufosinate (500 ai ha\(^{-1}\)), applied POST, increased in waterhemp control 10 and 18\%, respectively at 12 WAA. S-metolachlor/ atrazine (1200/960 ai ha\(^{-1}\)) and dicamba/ atrazine (604/1195 ai ha\(^{-1}\)), applied PRE, followed by mesotrione + atrazine (100 + 280 ai ha\(^{-1}\)) applied POST increased waterhemp control 12 and 19\%, respectively at 12 WAA.

All previous research on control of multiple-resistant waterhemp using Group 27 herbicides included the use of atrazine as a tank-mix partner. Field studies to determine if the addition of atrazine to Group 27 herbicides improved weed control were conducted at three locations in 2018. The addition of atrazine (1063 g ai ha\(^{-1}\)) to isoxaflutole (105 g ai ha\(^{-1}\)) increased waterhemp control % from 83 to 98\% 12 WAA. The addition of atrazine to mesotrione, topramezone, tembotrione or tolpyralate did not improve waterhemp control.
7.2 Limitations

Herbicide-resistant weed control is a complex problem. No single study is able to provide a solution on its own; each study provides a small piece of the solution and has shortcomings and limitations. Identifying these limitations allows us to direct future research that will provide improved recommendations for Ontario farmers.

The research completed is both time-sensitive and restricted by human resources. Ideally, every acre in Ontario would be scouted for waterhemp every year. Realistically, this is not an attainable goal. To manage our time as efficiently as possible for the survey on the distribution of waterhemp in Ontario, we chose to identify high priority locations and start there. These locations are often brought to our attention by farmers, area agronomists, and chemical sales reps from fields that have had a herbicide failure. Our survey work does not clearly answer the question “Is waterhemp moving across the province?” Although it is important to know where waterhemp is we cannot truly track the spread unless we also know where it is not. For example, the field where waterhemp was confirmed in Haldimand county in 2017 had not been previously included in our survey. Therefore there is no way to easily identify if it was recently established, or had been there for years.

The field studies conducted were all subject to variability across environments, waterhemp densities and waterhemp biotypes.

One of the largest limitations with this research is the use of chemical herbicides to solve a problem largely created by the use of chemical herbicides. This thesis offers
a short-term solution to a much larger problem. As waterhemp continues to develop resistance to additional modes-of-action, the value of this research will expire.

All herbicides, applied POST, were applied when waterhemp populations reached 10 cm in height, regardless of the corn staging. By the time that waterhemp populations had reached the 10 cm height requirement, the corn was past the stage label restriction for some herbicides. For example, 2,4-D, dicamba and dicamba/atrazine were all applied past the allowable corn stage, a practise not applicable to Ontario farmers.

A similar limitation impacted the two-pass waterhemp control experiments. The POST herbicides were applied when waterhemp reached 10 cm in height. Since the soil applied residual herbicide varied in their efficacy, the POST herbicides were applied on different days with varying environmental conditions. Weather at the time of application has an impact on POST herbicide efficacy, particularly for glufosinate. Either weed-size at time of POST application or POST application dates were going to be inconsistent between treatments. The decision made was to keep weed-size consistent to more accurately reflect what would occur on a commercial farm operation. In the future this could be improved by applying the POST application for all treatments on the same day and applying the POST herbicides when the weed escapes reached 10 cm in height in each individual treatment.

7.3 Future Research

Continuing to monitor the distribution of herbicide-resistant waterhemp in the province is crucial to helping farmers be prepared to address it. Have a predetermined
survey route, in addition to the industry-directed scouting, would allow us to revisit specific locations across the province year-after-year, more accurately determining if newly discovered waterhemp populations are recently established, or just recently found. More research is needed to identify how waterhemp seed is moving across the province, this research would allow farmers to implement practises that reduce waterhemp movement as much as possible.

As previously mentioned, this research is only applicable until waterhemp populations in Ontario develop resistance to additional modes-of-action, particularly Group 27 herbicides. Survey samples should be screened for Group 27 resistance to Group 27 herbicides so that if the resistance is in the province we are able to quickly identify it.

There is a need for research on control strategies that combine cultural, mechanical, biological and chemical weed management strategies to delay the evolution of herbicide resistance.

Competitive indices could be established for the waterhemp biotypes at the different field site locations. Information such as rate-of-growth, crop-competitiveness, and herbicides susceptibility would allow us to understand if some of the treatment differences between locations can be attributed to differences in waterhemp biotypes.
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Appendix- SAS coding

Control using PRE- and POST- herbicides

Data first;
Length trial $ 6;
Input trial block trmt control_28daa control_56daa control_84daa density drywt;
If trmt=1 then delete;
If trmt=2 then delete;
y=control_28daa/100;
if y=0 then y=0.0000000001;
if y=1 then y=0.9999999999;
datalines;

DATA

;
title 'Control 28 DAA';
proc glimmix data=first;
class trial block trmt;
model control_28daa=trmt/
distribution=normal link=identity;
random trial block(trial);
covtest "trial=0"0..;
covtest "block=0"0.;
lsmeans trmt/pdiff adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second;
scatter y=studentresid x=trmt; reline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var control_28daa;
histogram control_28daa/normal kernel;
probplot control_28daa/normal (mu=est sigma=est);
proc sgplot; vbox control_28daa/datalabel;
run;
title 'Control 56 DAA';
proc glimmix data=first;
class trial block trmt;
model control_56daa=trmt/
distribution=normal link=identity;
random trial block(trial);
covtest "trial=0"0..;
covtest "block=0".0.;
lsmeans trmt/pdiff adjust=Tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second;
scatter y=studentresid x=trmt; refline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var control_56daa;
histogram control_56daa/normal kernel;
probplot control_56daa/normal (mu=est sigma=est);
proc sgplot; vbox control_56daa/datalabel;
run;
title 'Control 84 DAA';
proc glimmix data=first;
class trial block trmt;
model control_84daa=trmt/
distribution=normal link=identity;
random trial block(trial);
covtest "trial=0".0.;
covtest "block=0".0.;
lsmeans trmt/pdiff ilink adjust=Tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second;
scatter y=studentresid x=trmt; refline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var control_84daa;
histogram control_84daa/normal kernel;
probplot control_84daa/normal (mu=est sigma=est);
proc sgplot; vbox control_84daa/datalabel;
run;
title 'Density';
proc glimmix data=first;
class trial block trmt;
model density=trmt/
distribution=normal link=identity;
random trial block(trial);
covtest "trial=0".0.;
covtest "block=0".0.;
lsmeans trmt/pdiff adjust=Tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second;
scatter y=studentresid x=trmt; refline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var density;
histogram density/normal kernel;
probplot density/normal (mu=est sigma=est);
proc sgplot; vbox density/datalabel;
run;
title 'Dry Weight';
proc glimmix data=first;
class trial block trmt;
model drywt=trmt/
distribution=normal link=identity;
random trial block(trial);
covtest "trial=0"0 ..;
covtest "block=0"0 .;
lsmeans trmt/pdiff adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second;
scatter y=studentresid x=trmt; refline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var drywt;
histogram drywt/normal kernel;
probplot drywt/normal (mu=est sigma=est);
proc sgplot; vbox drywt/datalabel;
run;

Two-Pass Program

data first;
length trial $ 3;
input trial block trmt beforepost control_28daa control_56daa control_84daa
density drywt;
y=control_28daa/100;
if trmt=1 then delete;
if trmt=2 then delete;
if y=0 then y=0.0000000001;
if y=1 then y=0.9999999999;
datalines;

data
title 'Control 28 DAA';
proc glimmix data=first;
class trial block trmt;
model control_28daa=trmt/
distribution=normal link=identity;
random trial block(trial)trial*trmt;
covtest "trial=0"0...;
covtest "block=0"0..;
covtest "trmt*trial=0"..0.;
lsmeans trmt/pdiff adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second;
scatter y=studentresid x=trmt; refline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var control_28daa;
histogram control_28daa/normal kernel;
probplot control_28daa/normal (mu=est sigma=est);
proc sgplot; vbox control_28daa/datalabel;
run;
Title 'Contrasts';
proc mixed covtest;
class trmt block;
model control_28daa=trmt/outp=second residual;
random block;
contrast 'PRE vs. Two-Pass' trmt 1 1 1 0 -1 -1 -1;
contrast 'POST vs. Two-Pass' trmt 0 0 0 3 -1 -1 -1;
contrast 'PRE vs. POST' trmt 1 1 1 -3 0 0 0;
contrast 'Isox vs. Isox 2-Pass' trmt 1 0 0 0 -1 0 0 0 0 0 0 0 0 0;
contrast 'Acuron vs. Acuron 2-Pass' trmt 0 -1 0 0 1 0 0 0 0 0 0 0 0 0;
contrast 'Tolpyralate vs. Tol 2-Pass' trmt 0 0 -1 0 0 0 1 0 0 0 0 0 0 0;
proc mixed covtest;
class trmt block;
model control_28daa=trmt/outp=second residual;
random block;
estimate 'PRE' intercept 3 trmt 1 1 1 0 0 0 0 /divisor=3;
estimate 'Two-Pass' intercept 3 trmt 0 0 0 1 1 1 1 /divisor=3;
run;
title 'Control 56 DAA';
proc glimmix data=first;
class trial block trmt;
model control_56daa=trmt/
distribution=normal link=identity;
random trial block(trial)trial*trmt block*trmt;
covtest "trial=0"0....;
covtest "block=0"0...;
covtest "trial*trmt=0"..0..;
covtest "block*trmt=0"...0.;
lsmeans trmt/pdiff adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second; scatter y=studentresid x=trmt; reline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second; plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal; var studentresid;
proc univariate data=first; var control_56daa;
histogram control_56daa/normal kernel;
probplot control_56daa/normal (mu=est sigma=est);
proc sgplot; vbox control_56daa/datalabel;
run;
Title 'Contrasts';
proc mixed covtest;
class trmt block;
model control_56daa=trmt/outp=second residual;
random block;
contrast 'PRE vs. Two-Pass' trmt 1 1 1 0 -1 -1 -1;
contrast 'POST vs. Two-Pass' trmt 0 0 0 3 -1 -1 -1;
contrast 'PRE vs. POST' trmt 1 1 1 -3 0 0 0;
contrast 'Isox vs. Isox 2-Pass' trmt 1 0 0 0 -1 0 0 0 0 0 0 0 0 0;
contrast 'Acuron vs. Acuron 2-Pass' trmt 0 -1 0 0 0 1 0 0 0 0 0 0 0 0;
contrast 'Tolpyralate vs. Tol 2-Pass' trmt 0 0 -1 0 0 0 1 0 0 0 0 0 0 0;
proc mixed covtest;
class trmt block;
model control_56daa=trmt/outp=second residual;
random block;
estimate 'PRE' intercept 3 trmt 1 1 1 0 0 0 0 /divisor=3;
estimate 'Two-Pass' intercept 3 trmt 0 0 0 0 1 1 1 /divisor=3;
run;
title 'Control 84 DAA';
proc glimmix data=first;
class trial block trmt;
model control_84daa=trmt/distribution=normal link=identity;
random trial block(trial)trial*trmt;
covtest "trial=0"0...;
covtest "block=0".0..
 covtest "trmt*trial=0"..0.;
lsmeans trmt/pdiff ilink adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second; scatter y=studentresid x=trmt; reline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second; plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal; var studentresid;
proc univariate data=first; var control_84daa;
histogram control_84daa/normal kernel;
Probplot control_84daa/normal (mu=est sigma=est);
proc sgplot; vbox control_84daa/datalabel;
run;
Title 'Contrasts';
proc mixed covtest;
class trmt block;
model control_84daa=trmt/outp=second residual;
random block;
contrast 'PRE vs. Two-Pass' trmt 1 1 1 0 -1 -1 -1;
contrast 'POST vs. Two-Pass' trmt 0 0 0 3 -1 -1 -1;
contrast 'PRE vs. POST' trmt 1 1 1 -3 0 0 0;
contrast 'Isox vs. Isox 2-Pass' trmt 1 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0;
contrast 'Acuron vs. Acuron 2-Pass' trmt 0 -1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0;
contrast 'Tolpyralate vs. Tol 2-Pass' trmt 0 0 -1 0 0 0 1 0 0 0 0 0 0 0 0 0 0;
proc mixed covtest;
class trmt block;
model control_84daa=trmt/outp=second residual;
random block;
estimate 'PRE' intercept 3 trmt 1 1 1 0 0 0 0 /divisor=3;
estimate 'Two-Pass' intercept 3 trmt 0 0 0 0 1 1 1 /divisor=3;
run;
title 'Density';
proc glimmix data=first;
class trial block trmt;
model density=trmt/distribution=normal link=identity;
random trial block(trial)trial*trmt;
covtest "trial=0"0 ...;
covtest "block=0" .0 ...;
covtest "trmt*trial=0" .0 ..;
lsmeans trmt pdiff adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred
residual(noblp)=mresid;
run;
proc sgplot data=second;
scatter y=studentresid x=trmt; reline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var density;
histogram density/normal kernel;
Probplot density/normal (mu=est sigma=est);
proc sgplot; vbox density/datalabel;
run;
Title 'Contrasts';
proc mixed covtest;
class trmt block;
model density=trmt/outp=second residual;
random block;
contrast 'PRE vs. Two-Pass' trmt 1 1 1 0 -1 -1 -1;
contrast 'POST vs. Two-Pass' trmt 0 0 0 3 -1 -1 -1;
contrast 'PRE vs. POST' trmt 1 1 1 -3 0 0 0;
contrast 'Isox vs. Isox 2-Pass' trmt 1 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0;
contrast 'Acuron vs. Acuron 2-Pass' trmt 0 -1 0 0 0 1 0 0 0 0 0 0 0 0 0 0;
contrast 'Tolpyralate vs. Tol 2-Pass' trmt 0 0 -1 0 0 0 1 0 0 0 0 0 0 0 0 0;
proc mixed covtest;
class trmt block;
model density=trmt/outp=second residual;
random block;
estimate 'PRE' intercept 3 trmt 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 /divisor=3;
estimate 'Two-Pass' intercept 3 trmt 0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 /divisor=3;
run;
title 'Dry Weight';
proc glimmix data=first;
class trial block trmt;
model drywt=trmt/
distribution=normal link=identity;
random trial block(trial)trial*trmt;
covtest "trial=0"...;
covtest "block=0"...;
covtest "trmt*trial=0"...
lsmeans trmt/pdiff adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred
residual(noblup)=mresid;
run;
proc sglplot data=second;
scatter y=studentresid x=trmt; reline 0;
proc sglplot data=second; vbox studentresid/group=trmt datalabel;
proc sgsclatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var drywt;
histogram drywt/normal kernel;
probplot drywt/normal (mu=est sigma=est);
proc sglplot; vbox drywt/datalabel;
run;
Title 'Contrasts';
proc mixed covtest;
class trmt block;
model drywt=trmt/outp=second residual;
random block;
estimate 'PRE vs. Two-Pass' intercept 3 trmt 1 1 1 0 -1 -1 -1;
estimate 'POST vs. Two-Pass' intercept 0 0 0 3 -1 -1 -1;
estimate 'PRE vs. POST' intercept 1 1 1 -3 0 0 0;
estimate 'Isox vs. Isox 2-Pass' intercept 1 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0;
estimate 'Acuron vs. Acuron 2-Pass' intercept 0 -1 0 0 0 1 0 0 0 0 0 0 0 0 0 0;
estimate 'Tolpyralate vs. Tol 2-Pass' intercept 0 0 -1 0 0 0 1 0 0 0 0 0 0 0 0 0;
proc mixed covtest;
class trmt block;
model drywt=trmt/outp=second residual;
random block;
estimate 'PRE' intercept 3 trmt 1 1 1 0 0 0 0 /divisor=3;
estimate 'Two-Pass' intercept 3 trmt 0 0 0 0 1 1 1 /divisor=3;
run;
**Group 27 Herbicides +/- Atrazine**

```plaintext
Data first;
Length trial $ 3;
Input trial block trmt injury_7daa injury_28daa control_28daa control_56daa
control_84daa;
y=control_28daa/100;
if trmt=1 then delete;
if trmt=2 then delete;
if y=0 then y=0.0000000001;
if y=1 then y=0.9999999999;
datalines;

DATA;

title 'Injury 7 daa';

proc glmmix data=first;
class trial block trmt;
model injury_7daa=trmt/
distribution=normal link=identity;
random trial block(trial);
covtest "trial=0" 0..;
covtest "block=0" .0.;
lsmeans trmt/pdiff adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred
residual(noblup)=mresid;
run;

proc sgplot data=second;
scatter y=studentresid x=trmt; refline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;

proc univariate data=first;
var injury_7daa;
histogram injury_7daa/normal kernel;
probplot injury_7daa/normal (mu=est sigma=est);
proc sgplot; vbox injury_7daa/datalabel;
run;

title 'Injury 28 daa';

proc glmmix data=first;
class trial block trmt;
model injury_28daa=trmt/
distribution=normal link=identity;
random trial block(trial);
covtest "trial=0" 0..;
covtest "block=0" .0.;
lsmeans trmt/pdiff adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred
residual(noblup)=mresid;
run;

proc sgplot data=second;
scatter y=studentresid x=trmt; refline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
```

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proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var injury_28daa;
histogram Injury_28daa/normal kernel;
probplot injury_28daa/normal (mu=est sigma=est); proc sgplot; vbox injury_28daa/datalabel;
run;
title 'Control 28 DAA';
proc glimmix data=first;
class trial block trmt;
model control_28daa=trmt/
distribution=normal link=identity;
random trial block(trial);
covtest "trial=0"0..;
covtest "block=0".0.;
lsmeans trmt/pdiff adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second;
scatter y=studentresid x=trmt; reline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var control_28daa;
histogram control_28daa/normal kernel;
probplot control_28daa/normal (mu=est sigma=est); proc sgplot; vbox control_28daa/datalabel;
run;
title 'Control 56 DAA';
proc glimmix data=first;
class trial block trmt;
model control_56daa=trmt/
distribution=normal link=identity;
random trial block(trial);
covtest "trial=0"0..;
covtest "block=0".0.;
lsmeans trmt/pdiff adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second;
scatter y=studentresid x=trmt; reline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var control_56daa;
histogram control_56daa/normal kernel;
probplot control_56daa/normal (mu=est sigma=est);
proc sgplot; vbox control_56daa/datalabel;
run;
title 'Control 84 DAA';
proc glmmix data=first;
class trial block trmt;
model control_84daa=trmt/
distribution=normal link=identity;
random trial block(trial);
covtest "trial=0",0.;
covtest "block=0",0.;
lsmeans trmt/pdiff ilink adjust=tukey lines;
output out=second student=studentresid residual=resid predicted=pred residual(noblup)=mresid;
run;
proc sgplot data=second;
scatter y=studentresid x=trmt; reline 0;
proc sgplot data=second; vbox studentresid/group=trmt datalabel;
proc sgsscatter data=second;
plot studentresid*(pred trmt trial block);
proc univariate data=second plot normal;
var studentresid;
proc univariate data=first;
var control_84daa;
histogram control_84daa/normal kernel;
probplot control_84daa/normal (mu=est sigma=est);
proc sgplot; vbox control_84daa/datalabel;
run;
Title 'Contrasts';
proc mixed covtest;
class trmt block trial;
model control_28daa=trmt/outp=second residual;
random block(trial) trial trial*trmt;
contrast 'Isoxaflutole' trmt 0 1 -1 0 0 0 0 0 0 0 0 0;
contrast 'Mesotrione' trmt 0 0 0 1 -1 0 0 0 0 0 0 0;
contrast 'Topramezone' trmt 0 0 0 0 1 -1 0 0 0 0 0;
contrast 'Tembotrione' trmt 0 0 0 0 0 0 1 -1 0 0 0;
contrast 'Tolpyralate' trmt 0 0 0 0 0 0 0 0 1 -1;
run;
proc mixed covtest;
class trmt block trial;
model control_56daa=trmt/outp=second residual;
random block(trial) trial trial*trmt;
contrast 'Isoxaflutole' trmt 0 1 -1 0 0 0 0 0 0 0 0 0;
contrast 'Mesotrione' trmt 0 0 0 1 -1 0 0 0 0 0 0 0;
contrast 'Topramezone' trmt 0 0 0 0 1 -1 0 0 0 0 0;
contrast 'Tembotrione' trmt 0 0 0 0 0 0 1 -1 0 0 0;
contrast 'Tolpyralate' trmt 0 0 0 0 0 0 0 0 1 -1;
run;
proc mixed covtest;
class trmt block trial;
model control_84daa=trmt/outp=second residual;
random block(trial) trial trial*trmt;
contrast 'Isoxaflutole' trmt 0 1 -1 0 0 0 0 0 0 0 0 0;
contrast 'Mesotrione' trmt 0 0 0 1 -1 0 0 0 0 0 0 0;
contrast 'Topramezone' trmt 0 0 0 0 0 1 -1 0 0 0 0;
contrast 'Tembotrione' trmt 0 0 0 0 0 0 0 1 -1 0 0;
contrast 'Tolpyralate' trmt 0 0 0 0 0 0 0 0 0 1 -1;
run;