

**Glyphosate-Resistant Giant Ragweed (*Ambrosia trifida* L.) in Ontario: Survey
and Control in Soybean (*Glycine max* L.)**

**by
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ABSTRACT

Glyphosate-Resistant Giant Ragweed (*Ambrosia trifida* L.) in Ontario: Survey and Control in Soybean (*Glycine max* L.)

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Giant ragweed is an extremely competitive weed and poor control in soybean could lead to significant yield losses for Ontario producers. In 2008, a giant ragweed biotype near Windsor, ON was not controlled with glyphosate and further testing confirmed it as the first glyphosate-resistant (GR) weed in Canada. Giant ragweed seed was collected from 102 locations in Essex (70), Kent (21), Lambton (10) and Waterloo (1) counties to document the occurrence and distribution of GR giant ragweed in Ontario. Giant ragweed seedlings were sprayed with glyphosate at 1800 g a.e. ha⁻¹, and evaluated 1, 7, 14 and 28 days after application (DAA). Results from the survey concluded that there are 47 additional locations in southwestern Ontario with GR giant ragweed. The majority of the sites were found in Essex county, but there was one location in both Chatham-Kent and Lambton counties. Field trials were established at six sites with GR giant ragweed during the 2010 and 2011 growing seasons. The objectives were to determine the level of giant ragweed control with increasing doses of glyphosate, and glyphosate tank mixes applied either preplant or postemergence. Control of giant ragweed increased with higher doses of glyphosate, but only at doses that are not economical for producers. The most effective glyphosate tank mixes were 2, 4-D ester, saflufenacil, linuron, and cloransulam-methyl providing up to 98, 94, 99 and 97% control 4 weeks after application (WAA), respectively. Glyphosate plus dicamba in dicamba-tolerant soybean provided up to 100% giant ragweed control, 4 WAA at the three confined field trial locations.

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1.1 Introduction

Glyphosate-resistant (GR) crops have been rapidly adopted by Ontario growers for reasons such as excellent weed control, excellent crop safety, simplicity of application, relatively low cost of weed control, reduced fuel costs and improved soil conservation (Nandula 2010). The wide spread adoption and repeated use of glyphosate has led to selection of weeds species that are naturally tolerant to glyphosate, late emerging weed species that emerge after the last application of glyphosate, and weed biotypes that are resistant to glyphosate. In response to the increased incidence of GR weeds, seed companies are now developing crop hybrids and cultivars with resistance to multiple herbicides including glyphosate plus dicamba and glyphosate plus 2, 4-D. Crops with new herbicide resistance traits may be an important tool for the management of GR weeds (Loux 2008; Nandula 2010).

Giant ragweed (*Ambrosia trifida* L.) has become a challenge for farmers, input suppliers and local weed scientists because it is an extremely competitive weed that can reduce yields in both soybean (*Glycine max* L.) and corn (*Zea mays* L.) if proper control strategies are not implemented. Traditionally, control with glyphosate or effective acetolactate-synthase (ALS) inhibiting herbicides was adequate. More recently, control options have failed and fields are left with giant ragweed escapes. In the United States, some giant ragweed biotypes are resistant to both the ALS-inhibiting and glycine herbicides in several states (Heap 2012). In Canada, giant ragweed in southwestern Ontario was the first weed identified with resistance to glyphosate and

preliminary research suggests that some biotypes are also resistant to the ALS-inhibiting herbicide, cloransulam-methyl.

GR giant ragweed may have arrived in Ontario for several reasons. The conditions for the selection of GR biotypes exist, such as the repeated use of glyphosate in an intensive GR cropping system. Giant ragweed is a genetically diverse species which is known to favour the selection of resistant biotypes. GR giant ragweed was first reported in Ohio approximately 240 km from the first confirmed site in Ontario. Research has shown that the gene which confers resistance to glyphosate in giant ragweed is a paternally inherited trait (François Tardif, unpublished research). Therefore, it is possible that GR giant ragweed pollen may have arrived in Ontario from Ohio. Due to high selection intensity in many fields, it is expected that GR giant ragweed will be identified in additional fields in southwestern Ontario. Identifying the distribution of GR giant ragweed in Ontario and developing strategies for its control in soybean under Ontario crop production practices are important steps in addressing this new weed management challenge in the province.

1.2 Biology of Giant Ragweed

1.2.1 Origin and distribution

Giant ragweed, also known as great ragweed, kinghead and tall ragweed (Alex 2001) is a native plant to North America. It is found in various regions of southern Canada and the midwestern and eastern portions of the United States of America (Abul-Fatih and Bazzaz 1980; Bassett and Crompton 1982; Hunt and Bazzaz 1980). Bassett and Crompton (1982) stated that giant ragweed is particularly abundant in southern parts of Manitoba, Ontario, Quebec and a few areas of New Brunswick, Prince Edward Island and Nova Scotia. Harrison et al. (2001) reported that giant ragweed is widely abundant in South America and Asia.

Giant ragweed originally moved into Canada from the south by the retreat of the last glacial ice (Bassett and Crompton 1982). However, it is only within the last two centuries that giant ragweed has become abundant in agricultural landscapes (Bassett and Crompton 1982). This species was previously known to occur in river valleys, meadows, roadsides, fencerows and drainage ditches and occasionally in low, cultivated flood plain fields (Alex 2001; Bassett and Crompton 1982; Johnson et al. 2007). Presently, giant ragweed is most problematic in agronomic crops in the southern most portion of southwestern Ontario; Essex county. According to Johnson et al. (2007), giant ragweed populations have moved from their primary habitats into many fertile fields across the Corn Belt. Hartzler et al. (2002) reported that giant ragweed has adapted to survive in agronomic fields in both the eastern and western part of the Corn Belt.

Giant ragweed has evolved and been able to proliferate under current agronomic practices, including earlier planting and less tillage (Johnson et al. 2007). Similarly, farmers in southwestern Ontario are seeding soybean cultivars and corn hybrids earlier in the spring which coincides with the emergence of giant ragweed seedlings. However, the infestation of giant ragweed in southwestern Ontario fields is variable. Some fields contain high densities of giant ragweed, while others have only a few small patches (personal observation). Bassett and Terasmae (1962) stated that the increase in populations of giant ragweed coincided with the settlement of early pioneers, the breakup of land for agricultural purposes, and the disruption of previously undisturbed habitats.

1.2.2 Description

Giant ragweed is an erect, herbaceous, annual dicot weed that is a member of the Asteraceae family (Bassett and Crompton 1982). Mature plants can be very large and may reach heights of up to six m depending on their environment (Abul-Fatih and Bazzaz 1979a; Bassett

and Crompton 1982; Johnson et al. 2007). The underground portion of the plant consists of a fibrous rooted, relatively short taproot. Seedlings are easily identified by their large, spoon shaped cotyledons that are 9 to 16 mm wide, 25 to 45 mm long, and up to 2 mm thick (Johnson et al. 2007). The stems are usually much-branched and moderately hairy (Alex 2001).

Giant ragweed leaves are highly variable in shape and size. Leaves of giant ragweed are frequently arranged opposite along the stem with two per node. However, the ends of the smaller branches at the time of reproduction may bare an alternate leaf arrangement consisting of one leaf per node (Alex 2001; Bassett and Crompton 1982). The leaves are large at the end of long petioles, palmate or simple in shape, rounded in outline and mainly three to five lobed with serrate margins. The lobes are either smooth or coarsely toothed, and the uppermost small leaves are usually not lobed at all. Occasionally, biotypes have been found with most or all leaves not lobed, but ovate in shape and only shallowly toothed (Alex 2001). Leaf surfaces are usually rough because of stiff hairs that point toward the leaf apex.

Giant ragweed is a monoecious species; both male and female flower heads are present on the same plant (Alex 2001; Bassett and Crompton 1982). The flowers are dull green and inconspicuous, but very numerous and form distinctive inflorescences (Alex 2001). The male, pollen producing flowers are in elongated clusters at the ends of branches forming spikes. Each individual flower within the cluster hangs downward on a short stalk like a “tiny inverted umbrella” (Alex 2001). Seed producing female flower heads are large and bunched in groups of two to four near the base of each male spike.

In Ontario, flowering occurs from mid-July to October and is an important cause of hay fever during the months of August and September in southwestern Ontario. The tiny male flowers are able to generate vast quantities of pollen. Johnson et al. (2007) reported that a single

plant can produce 10 million pollen grains in one day, or the equivalent of one billion pollen grains during its life cycle.

Giant ragweed “seed” is much larger than its close relative common ragweed (*Ambrosia artemisiifolia* L.). The seed can reach up to 10 mm long with numerous, distinguishable, lengthwise spikes or ridges ending in sharp spines around the upper most portion. Frequently, this characteristic is composed of one central spike surrounded by a circle of five or more marginal points (Bassett and Crompton 1982) and resembles a crown (Johnson et al. 2007). The true seed is located within the hard outer covering and enclosed in a smooth, black testa.

1.2.3 Growth and development

Giant ragweed requires a period of seed dormancy for at least eight weeks after dispersal (Jeff Stachler, personal communication). In general, seed dormancy can be attributed to an inhibitory mechanism within the embryo or by a physical constraint imposed by the embryo covering structures (Schutte et al. 2004; Stoller and Wax 1974). For giant ragweed, dormancy can be broken by subjecting seed to an environment that involves a sequential reduction of embryo and coat-imposed dormancy (Schutte et al. 2004). Research by Schutte et al. (2004) noted that giant ragweed seed dormancy was reduced most rapidly by moist, cold (4°C) conditions. The authors concluded that to overcome giant ragweed dormancy, seed must be subjected to cold conditions. In Ontario giant ragweed seed dormancy was broken by exposure to cool (3.5-5.5°C), moist soil for three months (personal observation). Fu et al. (2008) observed a high rate of germination two to four months after giant ragweed seed burial.

Giant ragweed is one of the earliest annual weeds to germinate and emerge in the spring. Harrison et al. (2001) observed emergence in Ohio beginning in early March and continued through mid-July. Similarly, Schutte et al. (2008) observed giant ragweed emergence in Ohio

from April 5 to July 7. This early and prolonged emergence pattern has been observed in Ontario where giant ragweed will emerge as early as late March and continue through July (personal observation). Hartzler et al. (2002) found giant ragweed emergence characteristics to be variable. In Ohio, populations exhibited a later initial emergence date but occurred over a prolonged period, whereas in Iowa 95% of total emergence occurred within 29 days. Illinois biotypes were found to exhibit emergence characteristics similar to biotypes in both Ohio and Iowa. In contrast, Stoller and Wax (1973), in an earlier study reported that giant ragweed finished emerging before May 15. Abul-Fatih and Bazzaz (1979b) reported that giant ragweed seedlings emerged first in early March before other prominent annuals such as lamb's-quarters (*Chenopodium album* L), common ragweed, velvetleaf (*Abutilon theophrasti* Medic.) and giant foxtail (*Setaria faberii* Herrm.).

Giant ragweed seed can germinate under various temperatures. Abul-Fatih and Bazzaz (1979b) reported that seeds of giant ragweed were able to germinate in a range of temperatures from 8 to 41°C with an optimum of 10 to 24°C. Stachler (2008) observed giant ragweed germination at temperatures as low as 2°C.

Giant ragweed seeds can germinate and emerge under varying soil moisture content and depths. Abul-Fatih and Bazzaz (1979b) found the highest germination rate to occur between 20 and 33% soil moisture but can occur anywhere between 17 and 55%. In the same study, germination occurred at depths between 0.5 cm and 16 cm, while optimal emergence occurred from a depth of 2 cm. Harrison et al. (2007) observed optimal giant ragweed emergence from a depth of 5 cm, but observed emergence from as deep as 20 cm. Giant ragweed has become more successful at germinating on the soil surface. Previous research reported only 1% germination at

the soil surface (Abul-Fatih and Bazzaz 1979b). More recent research reported up to 19% germination when placed on top of the soil (Harrison et al. 2007).

Early germination and emergence contributes to the survival and fitness of giant ragweed plants. Abul-Fatih and Bazzaz (1979b) indicated that early emerging plants made better use of resources while late emerging plants exhibited reduced fitness. This suggests selection among giant ragweed biotypes favours those with early germination (Abul-Fatih and Bazzaz 1979b).

Previous studies reported on the growth and biomass allocation of giant ragweed. Growth of giant ragweed was variable, and the weight of individual plants depended on the density of the population (Abul-Fatih et al. 1979). Mean dry weight per plant was 11 g and 320 g in the highest and lowest density, respectively. In contrast, above-ground biomass of 1590 g m⁻² was reported in the lowest density and 3058 g m⁻² in the highest density. Abul-Fatih et al. (1979) observed that plants growing in highly dense populations were always single stemmed, while those growing in the lowest density produced many branches. Baysinger and Sims (1991) reported mature plant biomass production to be 1138 g m⁻² and 2065 g m⁻² at the lowest and highest densities, respectively.

1.2.4 Reproduction

Giant ragweed is wind pollinated. The stigmas of female flowers become receptive prior to the release of pollen by the separate male flowers. This suggests cross pollination is favoured in giant ragweed. Bassett and Crompton (1982) found that plants bagged before flowering were able to produce viable seeds. However, progeny from the self-pollinated plants experienced reduced growth, loss of vigour, and a reduction in overall fitness.

Distance of pollen travel has been documented but the results are variable. Giant ragweed pollen may travel greater than 200 km (Alex 2001). Volenberg (2005) reported that viable giant

ragweed pollen could travel at least 60 m from its source plant. Giant ragweed pollen was deposited 100 m from the source but may remain airborne at one km (Raynor et al. 1970). Pollen from common ragweed was able to travel up to 198 m from the source plants (Dierking and Smeda 2007).

Hybridization between giant and common ragweed may occur under field conditions or through embryo culture techniques. Basset and Crompton (1982) noted the discoveries of hybrid populations (common ragweed♀ X giant ragweed♂) in Nova Scotia and Quebec. This was consistent with Vincent and Cappadocia (1987) who obtained hybrid plants by crosses of common ragweed♀ X giant ragweed♂ from seed collected in Montreal, Canada. Plants of the reciprocal crosses (giant ragweed♀ X common ragweed♂) have been obtained, but only through embryo culture techniques (Vincent et al. 1988; Vincent and Cappadocia 1987). Seeds from the cross giant ragweed♀ X common ragweed♂ did not produce viable plants because of underdeveloped embryos (Vincent and Cappadocia 1987). Hybrid ragweed plants crossed to either giant or common ragweed may produce viable seed, but in most cases embryos will abort (Vollenberg 2005). Interspecific hybridization between ragweed species suggests that herbicide resistance traits could transfer to at least the first generation (Stachler 2008).

Compared to other annual weed species, giant ragweed produces a relatively low amount of seed. Seed number produced per plant is variable and depends on the density and plasticity of the population. Abul-Fatih and Bazzaz (1979b) found that plants produced 5000 +/- 1500 seeds per m² or 275 seeds per plant. Baysinger and Sims (1991) reported mean seed production per plant was 1399 and 16 at the lowest and highest density, respectively. Johnson et al. (2007) noted the seed production from a single giant ragweed plant in a soybean and corn field could reach

5100 seeds, or 4320 seeds per m², respectively. In contrast, Fu et al. (2008) reported a much lower giant ragweed seed density of 732 seeds per m² in the top three cm of soil.

1.2.5 Competition

As one of the earliest emerging annual weeds, giant ragweed establishes dominance very early in the growing season. It is fast growing and dominates other weed species by reducing their numbers and growth (Abul-Fatih et al. 1979; Bassett and Crompton 1982). When present, giant ragweed has the ability to negatively affect all other plants because of its highly flexible germination, emergence and growth characteristics (Abul-Fatih et al. 1979; Abul-Fatih and Bazzaz 1980). The provinces of Ontario, Quebec, Manitoba, Saskatchewan and British Columbia have classified either ragweed or giant ragweed as a primary noxious weed (Bassett and Crompton 1982; Rice 1997). According to the United States Department of Agriculture (USDA 2009), states such as California, Delaware, Illinois, Minnesota and New Jersey have labeled it as a noxious weed.

Seedlings of giant ragweed have large cotyledons with a high photosynthetic rate (Abul-Fatih and Bazzaz 1979b). This characteristic allows for an early competitive advantage because the young plants are able to grow rapidly, and as a result, grow above the other species present (Abul-Fatih and Bazzaz 1979b). High photosynthetic rates in mature plants promote large quantities of carbohydrates to reproductive efforts (Bazzaz and Carlson 1979). Giant ragweed may eliminate other species from a community by depleting water, light, nutrients and other resources (Abul-Fatih and Bazzaz 1979b).

Giant ragweed can be very competitive in soybean resulting in large yield losses. Webster et al. (1994) reported a soybean yield reduction of up to 77% at a giant ragweed density of one plant per m². Giant ragweed leaves can respond to shading in a soybean canopy by

developing shade tolerant leaves (Webster et al. 1994). Therefore, giant ragweed can outgrow soybeans early in the season, and maintain vigorous growth within the soybean canopy. Baysinger and Sims (1991, 1992) reported soybean yield loss of up to 92% at the highest giant ragweed density. Full season interference in the untreated plots resulted in complete yield loss. In their studies, the giant ragweed emerged simultaneously with the soybeans. To prevent yield loss from giant ragweed in soybean, fields should be kept weed-free for 8 to 10 weeks after soybean emergence. The critical weed free period for soybeans is typically four to six weeks after emergence (Barrentine 1974; Bloomberg et al. 1982; Coble et al. 1981; Coble and Ritter 1978; Williams and Hayes 1984). This suggests that giant ragweed must be controlled for an extended period of time in soybean compared to other annual weed species. When soybeans were kept weed free for the entire season, soybean plants weighed 85% more compared to those from the weedy checks (Baysinger and Sims 1991). Giant ragweed has been shown to be one of the most competitive weed species in soybean (Webster et al. 1994). Additionally, giant ragweed causes problems with mechanical harvesting and may reduce the quality of seed (Baysinger and Sims 1992; personal observation).

Giant ragweed interference results in large yield losses in corn. Harrison et al. (2001) reported a 13% loss in corn yield with a giant ragweed density of one plant per 10 m², when both the crop and the weeds emerged at the same time. At the highest giant ragweed density, yield loss of up to 60% occurred. Previous research on the competitiveness of various weed species suggests that giant ragweed is the most competitive annual weed of corn. Harrison et al. (2001) predicted if giant ragweed density was 14 plants per 10 m² and emerged simultaneously with the corn, yield loss would be as high as 90%.

1.2.6 Seed mortality

Giant ragweed seeds are susceptible to pre- and post-dispersal predation (Abul-Fatih 1979b; Harrison et al. 2001; Harrison et al. 2003; Harrison et al. 2007). Abul-Fatih et al. (1979b) noted insect feeding of involucre began in August before seed shed, and was greatest in exposed plants in a low density population. They reported that out of 5000 seeds per m², only 340 seeds per m² were still present in the soil the following spring, and only 90 seedlings emerged. Predation was caused by earthworms, rodents, birds, insects and decay by fungi and bacteria. Pre-dispersal seed predation is a major contributor to the overall decline of viable seeds. Harrison et al. (2001) reported that up to 62% of seed rain collected was nonviable and up to 19% of the involucre were infested with insect larvae that had consumed the embryo. Vitolo and Stiles (1987) determined that 35% of seeds collected from a giant ragweed population occurring in soybeans were nonviable. Amatangelo (1974) documented that 86% of the seeds collected from a non cropped area in northeast Ohio were nonviable, of which 11% were infested with insect larvae.

Post-dispersal predation of giant ragweed seed contributes to the seed decline from the seedbank. Harrison et al. (2003) reported that rodents and invertebrates contributed the most to giant ragweed seed mortality. Rodents were the greatest predators for involucre during the fall and winter, whereas invertebrates became more active during the spring and summer. Giant ragweed seed losses due to predation may be as high as 80% between seed rain in the autumn and seedling emergence the following spring. Seed loss from the seedbank reached 88% over a 12 month period. Harrison et al. (2003) concluded that 90% of giant ragweed seed deposited and left on the surface of a no-till corn field for 12 months was predated by insects and mice. Giant

ragweed seed is an attractive food source for rodents and invertebrates because of its high nutritional value (Harrison et al. 2003).

Seed mortality is related to burial depth and seed size. Stoller and Wax (1973) determined seed bank losses were inversely proportional to burial depth, and after a four year period, less than five percent of seeds in the top layer of soil remained viable. Schmoll et al. (2004) reduced giant ragweed emergence by 90% after four years in a conventional tillage system. More recently, Harrison et al. (2007) was able to predict high levels of seed mortality. Their model predicted >90% depletion of the active seed bank after four growing seasons when all seed size-burial depth combinations were taken into consideration.

According to Regnier et al. (2004, 2008), earthworm species may provide safe sites for giant ragweed seed and increase establishment in an environment normally conducive to seed mortality. The authors reported that *Lumbricus terrestris* collected over 60% of the giant ragweed seed rain and in the subsequent spring, over 60% of the giant ragweed seedlings emerged from *L. terrestris* burrows. This indicates that giant ragweed seeds remain viable and protected from vertebrate predation in earthworm burrows. Methods to promote seed mortality must be used in conjunction with other control measures. Minimizing seed burial, implementing good practices that prevent new additions to the soil seed bank, and maintaining cropping practices favourable to rodent and invertebrate habitats could dramatically reduce giant ragweed populations (Harrison et al. 2007).

1.3 Glyphosate

Glyphosate [N-(phosphonomethyl)glycine] is often regarded as the most important herbicide ever discovered. It is the best selling and fastest growing agrochemical in the world (Baylis 2000). Currently, glyphosate is used globally because of its many favourable

characteristics and its very broad spectrum of weed control (Baylis 2000). However, glyphosate must be carefully managed to secure its use for future generations (Duke and Powles 2008a).

Glyphosate was first discovered and synthesized by Dr. Henri Martin of Cilag; a small pharmaceutical company in Switzerland (Franz et al. 1997). It was reported that Dr. Martin synthesized over 7 g of glyphosate in 1950, but Cilag showed no interest in the chemical and Dr. Martin's synthesis was never reported in the literature (Franz et al. 1997). Two decades later in 1970, J. E. Franz of Monsanto Co. synthesized glyphosate while searching for a systemic perennial herbicide. By 1974, glyphosate was formulated as a monoisopropylamine salt and introduced in several world markets under the trade name Roundup® as a post emergence, nonselective herbicide (Franz et al. 1997). Presently, glyphosate is used extensively in both North and South America especially in Canada, USA, Argentina and Brazil (Duke and Powles 2008b). The rapid adoption of GR crops of soybean, corn, cotton (*Gossypium hirsutum* L.) and canola (*Brassica campestris* L.) can be attributed to its success with over 80% of the 100 million hectares of biotech crops being grown in these four countries (Duke and Powles 2008b; Owen 2008).

1.3.1 Strengths and weaknesses

Several factors contribute to the success of glyphosate as an herbicide. Environmentally, several reports concluded that glyphosate has very little, negative effects on the environment (Dyer 1994; Franz et al. 1997; Williams et al. 1999). When applied at normal use rates, glyphosate that reaches the soil is tightly bound, and little movement in soil occurs (Duke and Powles 2008a). Lack of activity in the soil prevents glyphosate from being used as a preemergence herbicide. Contradictory to most reports, glyphosate may not always bind tightly to soil (Sprankle et al. 1975a). Glyphosate undergoes rapid microbial degradation in the soil, and

is non volatile, thus it does not contribute to atmospheric contamination (Carlisle and Trevors 1988; Dill et al. 2010; Duke and Powles 2008a; Franz et al. 1997).

Glyphosate was found to be one of the least toxic pesticides to animals (Duke and Powles 2008a; Franz et al. 1997). An extensive review by Williams et al. (2000) concluded that when glyphosate is used according to label directions there are no deleterious effects to humans or animals. These studies were in agreement with major health organizations such as Health Canada, the Pest Management Regulatory Agency (PMRA) and the United States Environmental Protection Agency (USEPA). However, Franz et al. (1997) reported on a study (Chakravarty and Chatarpaul 1990) that showed an inhibitory effect on the growth of some fungi when exposed to low levels of glyphosate. In contrast, Duke and Powles (2008a) noted that normal exposure of glyphosate to fungi in the environment had no adverse effects on growth.

Most plants rapidly absorb glyphosate and distribute phytotoxic levels throughout the plant (Duke and Powles 2008a). Under optimal conditions, glyphosate is rapidly absorbed by plant foliage and translocated to the rhizomes of perennial plants (Franz et al. 1997; Gottrup et al. 1976; Sprankle et al. 1975b). However, absorption can vary significantly between weed species leading to some differences in susceptibility to glyphosate (Baylis 2000; Duke and Powles 2008a). Once absorbed through the plant leaf tissue, glyphosate is able to move systemically via the xylem and phloem and enter the metabolic sinks throughout the plant following a source to sink relationship (Duke and Powles 2008a; Franz et al. 1997; Sprankle et al. 1975b). Typically, meristematic regions are the “sinks” within a plant, thus, glyphosate accumulates in young, actively growing leaves, roots and storage organs (Duke and Powles 2008a; Franz et al. 1997; Gottrup et al. 1976).

1.3.2 Mode of action

The mode of action of glyphosate has been thoroughly studied. An extensive list of studies and reviews have been published, many were reported in Franz et al. (1997). Presently, it is generally accepted that glyphosate is the only herbicide known to primarily target and inhibit the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) (Amrhein et al. 1980a; Amrhein et al. 1980b; Duke and Powles 2008a; Franz et al. 1997; Sikorski and Gruys 1997; Steinrucken and Amrhein 1980). Inhibition of EPSPS leads to high levels of shikimate-3-phosphate in glyphosate treated plants (Amrhein et al. 1980a). Plants treated with glyphosate lack key aromatic amino acids such as tryptophan, phenylalanine and tyrosine which act in the conversion of shikimate-3-phosphate via EPSPS into chorismate. Aromatic amino acids are involved in the role of protein synthesis, natural plant defenses and feedback inhibition within the shikimate pathway. Plant growth stops four to seven days after the application of glyphosate, and is followed by chlorosis and necrosis of the growing point. Plant death occurs 7 to 21 days after exposure to glyphosate.

1.4 Herbicide Resistance

Weed resistance has been documented in most herbicide families. Repeated applications of the same herbicide on the same field have contributed to the widespread occurrence of herbicide resistant weeds (Nandula 2010). The first herbicide resistant weed was 2, 4-D resistant wild carrot (*Daucus carota* L.) found in Ontario, Canada in 1957 (Sikkema, personal communication). This was followed by resistance to the triazine herbicides first reported in 1968 (Booker 2009; Ryan 1972). Herbicide resistance in giant ragweed has been reported in several American states, and was first documented in 1998 with resistance to the ALS-inhibiting herbicides (Heap 2012; Taylor et al. 2002). Herbicide resistance in a weed can be 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). Factors affecting the evolution of herbicide resistance in weeds include gene mutation, fitness, seed longevity, mating behaviour, gene flow, herbicide effectiveness, persistence, and cultural practices (Booker 2009; Nandula 2010).

1.4.1 Multiple resistance

Multiple resistance occurs when weeds evolve resistance to more than one herbicide mode of action. A redroot pigweed (*Amaranthus retroflexus* L.) biotype in Pennsylvania was found to be resistant to ALS and photosystem II-inhibitors (Heap 2012). Common ragweed biotypes in Ohio were found with multiple herbicide resistance to PPO and ALS-inhibitors (Heap 2012; Stachler et al. 2007). Tall waterhemp biotypes (*Amaranthus tuberculatus* Moq.) in Illinois, Missouri and Iowa are resistant to three different modes of action (Heap 2012). A rigid ryegrass (*Lolium rigidum* Gaud.) biotype evolved resistance to three different herbicide modes of action in western Australia (Neve 2004).

Reported cases of herbicide resistance continue to grow annually. As of February 8, 2012, the International Survey of Herbicide Resistant Weeds lists 372 herbicide resistant biotypes, 200 different species (116 dicots and 84 monocots) in over 570,000 fields (Heap 2012).

1.4.2 Mechanisms of resistance

There are numerous mechanisms of herbicide resistance. The mechanisms include: modified target site, enhanced detoxification or metabolism, reduced absorption/translocation, sequestration, and gene amplification / over expression of the target site (Nandula 2010). In most cases, the mechanism of resistance is due to an altered target site where binding affinity of the herbicide is negatively affected (Powles and Preston 2006). A tryptophan to leucine substitution at the ALS enzyme resulted in poor giant ragweed control with the herbicide cloransulam

(Patzoldt and Tranel 2002). Triazine resistant weeds have exhibited resistance due to a modification at the target site, as well as enhanced detoxification (Gronwald 1994). Reduced absorption and translocation (Fuerst and Vaughn 1990; Tanaka et al. 1986) and sequestration (Lasat et al. 1997) resulted in resistance to the herbicide paraquat. In a recent study, gene amplification conferred herbicide resistance in Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Gaines et al. 2010). The agronomic, biochemical and genetic basis of resistance have been reviewed previously (Holt et al. 1993; Powles and Preston 2006).

1.5 Glyphosate-Resistant Weeds

Glyphosate's unique mode of action, limited metabolism in plants, and lack of residual activity in soil led some to believe that glyphosate resistance in weeds was unlikely (Bradshaw et al. 1997). Glyphosate was used for over 20 years, yet resistance to glyphosate in weeds was unheard of until a decade ago (Nandula 2010; Powles et al. 1998; Pratley et al. 1999). As a non-selective herbicide with no soil activity, glyphosate used in burndown applications could only exert a short, powerful selection event on those weeds that were present at the time of application (Powles 2008). However, in 1996 following the introduction of GR crops, glyphosate was suddenly used as a selective herbicide and resistant weed populations began emerging as a significant problem (Duke and Powles 2008a; Powles 2008). The wide adoption of GR crops has increased the reliance on glyphosate for weed management. Consequently, experts now realize that GR weeds are increasing rapidly (Green et al. 2008; Owen 2008; Powles 2008).

In 1996, a population of rigid ryegrass was the first example that glyphosate resistance could occur following repeated use (Powles et al. 1998). In their study, the resistant biotype was found in an orchard where glyphosate had been applied two or three times a year for 15 years. Soon after, in 1997, a GR goosegrass [*Eleusine indica* (L.) Gaertn] biotype was found in an

orchard in Malaysia (Heap 2012; Lee and Ngim 2000; Ng et al. 2004a). Horseweed (*Conyza Canadensis* L.) in Delaware, USA was the first dicot weed species, and the first weed species selected in a GR crop to evolve resistance to glyphosate (Heap 2012; VanGessel 2001).

Weeds with multiple resistance that include glyphosate have been discovered. The GR goosegrass biotype found in Malaysia is also resistant to the ACCase-inhibiting herbicides. This was the first multiple resistant weed biotype that included resistance to glyphosate. Several rigid ryegrass biotypes in Australia have multiple resistance that includes glyphosate. Some biotypes have resistance to four modes of action. In total, there are 10 different weed species with multiple resistance that include glyphosate (Heap 2012).

As of February 8, 2012 the International Survey of Herbicide Resistant Weeds, lists 21 different weed species (11 dicots, 10 monocots) that are resistant to glyphosate. GR weed biotypes have been identified in North America, South America, Europe, Asia, and Australia (Heap 2012).

1.6 Mechanisms of Resistance to Glyphosate

Mechanisms that confer glyphosate resistance can be placed into two main categories that include target-site-based resistance of the EPSPS enzyme, and nontarget-site-based resistance such as reduced translocation (Perez-Jones et al. 2007; Perez-Jones and Mallory-Smith 2010; Powles and Preston 2006). More recently, reports by Gaines et al. (2010) and Ge et al. (2010) identified novel mechanisms of glyphosate resistance by gene amplification and sequestration, respectively. However, both mechanisms are still considered either target-site-based or nontarget-site-based. Within GR weed biotypes, individuals can have single or multiple mechanisms causing the resistance (Dinelli et al. 2006; Michitte et al. 2007; Powles and Preston 2006).

1.6.1 Target-Site Glyphosate Resistance

The first case of target-site-based glyphosate resistance occurred in a Malaysian population of goosegrass. Research conducted by Baerson et al. (2002) demonstrated that an amino acid substitution from proline to serine at position 106 (Pro106-Ser) in the EPSPS enzyme was responsible for glyphosate resistance in a goosegrass population. The different EPSPS gene resulted in a goosegrass biotype that was able to withstand five times the concentration of glyphosate that was required to inhibit EPSPS in susceptible populations. Other studies on GR goosegrass found similar results. Ng et al. (2004a) found the same proline to serine substitution at position 106 as Baerson et al. (2002). In addition, they found a proline to threonine substitution at position 106 (Pro106-Thr) that also resulted in glyphosate resistance. These studies reveal that at least two different mutations within the EPSPS enzyme of goosegrass biotypes lead to amino acid changes at Pro106, conferring glyphosate resistance (Powles and Preston 2006).

Weed scientists have found altered EPSPS in other weed species. Wakelin and Preston (2006) identified an amino acid substitution of proline to threonine at position 106 in an Australian population of rigid ryegrass. Similarly, decreased sensitivity of EPSPS to glyphosate was observed in a Californian population of rigid ryegrass. Simarmata and Penner (2008) reported a proline to serine substitution in the amino acid code of this rigid ryegrass biotype. An altered target site in populations of glyphosate resistant Italian ryegrass (*Lolium multiflorum* Lam.) from California was identified as the mechanism of resistance (Jasieniuk et al. 2008). Perez et al. (2004) attributed glyphosate resistance in a population of Italian ryegrass to either an altered target site on the EPSPS enzyme, or enhanced metabolism. Culpepper et al. (2006) reported on a palmer amaranth biotype, and suggested an altered target site conferred resistance.

Glyphosate resistance can be due to overproduction of the target enzyme by gene amplification and over-expression (Perez-Jones and Mallory-Smith 2010). Feng et al. (1999) conducted research on GR rigid ryegrass from Australia. The authors suggested that over-expression of EPSPS conferred resistance to glyphosate in that particular biotype. Brewer and Oliver (2009) confirmed two populations of common ragweed with a 21-fold resistance to glyphosate compared to the susceptible biotypes. The authors suggested that glyphosate resistance in common ragweed could be explained by increased shikimate pathway activity by the overproduction of EPSPS. More recently, Gaines et al. (2010) conducted research on a population of palmer amaranth from Georgia. They demonstrated that the basis of resistance in palmer amaranth is due to EPSPS gene amplification that results in high levels of EPSPS expression.

1.6.2 Nontarget-Site Glyphosate Resistance

Glyphosate is a symplastically translocated herbicide. In treated plants, glyphosate enters the symplast and is translocated via the phloem, following a source to sink relationship (Bromilow and Chamberlain 2000; Bromilow et al. 1993; Perez-Jones and Mallory-Smith 2010). Therefore, reduced translocation of the herbicide glyphosate could be an important mechanism of resistance in weed species.

Reduced translocation was the primary mechanism of resistance to glyphosate in some rigid ryegrass biotypes. Lorraine-Colwill et al. (2003) documented an accumulation of glyphosate in the tip of treated leaves from a population of rigid ryegrass in Australia. Resistant biotypes showed a strong tendency to move glyphosate upwards in the xylem. Similar results were found by Wakelin et al. (2004) in four different populations of rigid ryegrass in Australia. The altered translocation of glyphosate moved more herbicide to the tip of treated leaves

compared to the susceptible plants. Additionally, susceptible plants allocated twice as much glyphosate in the shoot meristematic zone compared to the resistant biotypes. Other rigid ryegrass populations have exhibited glyphosate resistance due to reduced rates of translocation (Yu et al. 2007; Yu et al. 2009).

Horseweed has evolved resistance to glyphosate and was first reported from a population in Delaware (VanGessel 2001). Glyphosate resistance in horseweed from several populations in the U.S. was attributed to reduced translocation (Feng et al. 2004). Phloem loading was disrupted due to an alteration in cellular distribution and consequently, glyphosate translocation was reduced (Feng et al. 2004). Koger and Reddy (2005) documented similar results. They found a decrease in translocation of glyphosate in resistant biotypes of horseweed from Mississippi, Arkansas, Delaware and Tennessee. Dinelli et al. (2006) also collected horseweed seed from the U.S. (Delaware, Virginia, Arkansas, Washington, and Ohio). They reported that resistant biotypes impaired the movement of glyphosate within the plant as well as other mechanisms that contributed to resistance.

Reduced glyphosate translocation has endowed resistance in other weed species. Perez-Jones et al. (2007) and Nandula et al. (2008) identified populations of Italian ryegrass with reduced rates of translocation as a mechanism of resistance. An Italian ryegrass biotype from Chile had lower spray retention, lower absorption, and an altered translocation pattern of glyphosate when compared to a known susceptible (Michitte et al. 2007). Palmer amaranth in Tennessee accumulated similar levels of shikimate in both susceptible and resistant biotypes indicating glyphosate resistance might be due to reduced translocation (Steckel et al. 2008). Dinelli et al. (2008) reported on the mechanism of glyphosate resistance in hairy fleabane

(*Conyza bonariensis* L.), and concluded that impaired translocation and high EPSPS transcript levels were the reason for resistance.

1.7 Inheritance of Glyphosate Resistance

The inheritance of glyphosate resistance has been studied in several weed species. In general, resistance has been attributed to an incompletely dominant single nuclear gene (Christoffers and Varanasi 2010; Preston and Wakelin 2008). This was observed in a biotype of Italian ryegrass which inherited resistance by a single, nuclear, and incompletely dominant gene (Ng et al. 2004b). This was consistent with horseweed found in Delaware, USA (Zelya et al. 2004, 2007) and rigid ryegrass from New South Wales, Australia (Lorraine-Colwill et al. 2001). However, glyphosate resistance is not always a single-gene trait. Yu et al. (2007) reported on a rigid ryegrass biotype with two mechanisms of resistance indicating the presence of at least two genes. This was consistent with Simarmata et al. (2005) who reported on a rigid grass population from California. The simple mode of inheritance in some GR weeds indicates the potential for rapid spread under intense glyphosate selection pressure (Lorraine-Colwill et al. 2001).

1.8 Glyphosate-Resistant Giant Ragweed

GR giant ragweed was first identified in Ohio, USA in 2004 (Stachler 2008; Stachler et al. 2006). Currently, GR giant ragweed has been documented in ten states in the U.S. Some populations of GR giant ragweed contain multiple resistance (Heap 2012; Stachler 2008). Giant ragweed was the first weed species in Canada to evolve resistance to glyphosate (Sikkema et al. 2009).

The level of resistance to glyphosate among giant ragweed populations varies substantially, and is likely due to its high genetic variability (Jeff Stachler, personal communication). Initial dose response studies conducted by Stachler (2008) reported that the

GR₅₀ values in resistant populations from Ohio and Indiana ranged from 8.3 to 23.9 kg a.e. ha⁻¹. In Minnesota, giant ragweed was able to survive three glyphosate applications in one season while greenhouse tests reported a four-fold level of resistance (Gunsolus 2008). In Arkansas, a giant ragweed biotype was 7.2 times more tolerant to glyphosate than the susceptible check (Still et al. 2008). More recently, a giant ragweed biotype from Tennessee was reported to exhibit a 5.3-fold level of resistance to glyphosate compared to the susceptible biotype (Norsworthy et al. 2010).

In Ontario, greenhouse dose response experiments on a GR population demonstrated a 24-fold resistance level (unpublished data). Plants from this population were able to survive rates of glyphosate up to two times the field rate, while susceptible plants were killed by rates as low as a quarter of the field rate (Sikkema et al. 2009). Field experiments conducted in 2009 and 2010 in Ontario have shown that giant ragweed biotypes could survive glyphosate at rates as high as 12 times the labeled rate or 10 800 g a.e. ha⁻¹ (unpublished data).

1.8.1 Mechanism of resistance

Research conducted thus far has not determined the mechanism of resistance in the GR giant ragweed biotype in Ontario. However, reduced translocation is the suspected mechanism in most GR biotypes (Norsworthy et al. 2010; personal observation; Stachler 2008). Mueller et al. (2003) reported on a GR horseweed biotype from Tennessee with elevated levels of shikimate similar to that of giant ragweed found in Tennessee (Norsworthy et al. 2010). Elevated levels of shikimate indicated that resistance was not due to an altered target site. This was consistent with other reports on horseweed from Tennessee in which reduced translocation was the mechanism of resistance to glyphosate (Feng et al. 2004; Koger and Reddy 2005).

Many GR giant ragweed biotypes exhibit an injurious response similar to that of a contact herbicide. Rapid necrosis and curling of the leaf margins has been observed in many resistant biotypes (personal observation). The rapid response to glyphosate is unique, and not expected for a systemic herbicide such as glyphosate. This response is consistent with other giant ragweed biotypes found in Indiana and Ohio. The injury observed on the mature leaves of giant ragweed after treatment with glyphosate has led researchers to speculate that reduced translocation is the mechanism of resistance. In contrast, some GR giant ragweed biotypes from Ontario do not exhibit the rapid necrosis response and it is speculated that a second mechanism is conferring resistance to glyphosate (personal observation).

The actual mechanisms of resistance due to reduced translocation are not known (Preston and Wakelin 2008; Shaner 2009). However, research on several hypotheses in the literature is ongoing. In general, results have indicated an unidentified barrier preventing glyphosate from being loaded into the phloem (Shaner 2009). Potential barriers that would contribute to reduced translocation of glyphosate include: first, alterations in a phosphate transporter responsible for active cellular uptake of glyphosate (Feng et al 2004; Shaner 2009); second, the evolution of a new transporter that pumps glyphosate into the vacuoles thus sequestering glyphosate and preventing transport into the phloem (Ge et al. 2010; Shaner 2009); and third, the evolution of a new transporter that actively pumps glyphosate into the apoplast, and away from the target site (Lorraine-Colwill et al. 2003; Shaner 2009).

1.9 Hypotheses and Objectives

Giant ragweed is the first weed in Canada to evolve resistance to glyphosate. There is limited information available on this new problem facing Ontario growers. Research is needed to determine if glyphosate-resistant (GR) giant ragweed is wide spread in southwestern Ontario. Research is also needed to evaluate options for control of GR giant ragweed with herbicides registered for use in Ontario. The hypotheses of this research are:

1. GR giant ragweed is confined to one location at the Windsor airport.
2. Alternative herbicides will not control GR giant ragweed in soybean.
3. GR giant ragweed will not reduce yield in soybean.

The research objectives are to:

- i. Conduct a survey and document the occurrence and distribution of GR giant ragweed in southwestern Ontario.
- ii. Conduct field studies to identify alternative options for control in GR soybean.
- iii. Evaluate the efficacy of dicamba plus glyphosate for the control of GR giant ragweed in dicamba-tolerant (DT) soybean.

2.0 Occurrence and distribution of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in southwestern Ontario

2.1 Abstract

Giant ragweed is the first confirmed glyphosate-resistant (GR) weed in Canada. A survey was conducted to document the distribution of GR giant ragweed in southwestern Ontario. Giant ragweed seed was collected from 102 sites in Essex (70), Chatham-Kent (21), Lambton (10) and Waterloo counties (1) during the autumn of 2009 and 2010 prior to soybean harvest. Plants were grown in a growth room, sprayed with glyphosate at 1800 g a.e. ha⁻¹ and classified as resistant or susceptible. GR giant ragweed has been confirmed at 47 additional locations in three counties (Essex, Chatham-Kent and Lambton). The results from this survey indicate that GR giant ragweed biotypes occur across a greater area in southwestern Ontario than originally thought. This survey provides an important baseline for future surveys in the province.

2.2 Introduction

Giant ragweed (*Ambrosia trifida* L.) is a troublesome weed found in southern Canada as well as in midwestern and eastern portions of the United States of America (Abul-Fatih and Bazzaz 1980; Bassett and Crompton 1982; Hunt and Bazzaz 1980). It is an annual dicot that is a member of the Asteraceae family (Bassett and Crompton 1982). This species was previously known to occur in river valleys, meadows, roadsides, fencerows, drainage ditches and occasionally in low, cultivated flood plain fields (Alex 2001; Bassett and Crompton 1982; Johnson et al. 2007). Over the past two decades giant ragweed has moved into fertile fields as it has adapted to current agronomic practices in southwestern Ontario, across the USA Corn Belt

(Hartzler et al. 2002; Johnson et al. 2007) and increasingly in the mid-south of the USA (Norsworthy et al. 2010; Steckel 2007).

Several factors have contributed to the increased success of giant ragweed in row crop production such as earlier planting and less tillage (Johnson et al. 2007). In southwestern Ontario, farmers seed soybean and corn early enough that giant ragweed emerges after spring tillage or application of burndown herbicides. Giant ragweed seedling emergence begins in March and continues until late July which complicates control strategies.

Giant ragweed is extremely competitive and is considered to be one of the most problematic summer annual weeds (Gibson et al. 2005). Giant ragweed may reduce growth of all neighbouring annual plants because of its highly flexible germination, rapid growth and variable growth characteristics (Abul-Fatih et al. 1979; Abul-Fatih and Bazzaz 1980). Nearby species are often eliminated from a community because water, light, nutrients and other resources are quickly depleted (Abul-Fatih and Bazzaz 1979).

Giant ragweed can be very competitive in soybean resulting in large yield losses. Webster et al. (1994) reported 77% yield loss in soybean with a giant ragweed density of one plant m⁻². Baysinger and Sims (1991) reported soybean yield losses of up to 92% with a giant ragweed density of two plants m⁻² when giant ragweed and soybeans emerged at the same time. Giant ragweed can outgrow soybeans early in the season, and maintain vigorous growth within the soybean canopy (Webster et al. 1994). To prevent yield loss from giant ragweed in soybean, fields should be kept weed-free for 8 to 10 weeks after soybean emergence (Baysinger and Sims 1991). This is a longer weed-free period requirement than most other annual weed species (Barrentine 1974; Bloomberg et al. 1982; Coble and Ritter 1978; Coble et al. 1981; Williams and Hayes 1984).

Historically, giant ragweed was controlled effectively in soybean with glyphosate or acetolactate synthase (ALS) inhibiting herbicides (Franey and Hart 1999; Taylor et al. 2002). Consistent control of giant ragweed has become difficult as many previously effective herbicides have failed resulting in escapes (Johnson et al. 2007). Glyphosate-resistant (GR) giant ragweed was first identified in Ohio, USA in 2004 (Heap 2012; Stachler 2008; Stachler et al. 2006). Since its first documentation in Ohio, GR giant ragweed has been confirmed in nine additional states in the U.S.

In 2008, a giant ragweed biotype near Windsor, Ontario, Canada was not controlled with glyphosate at the manufacturer's recommended dose. Greenhouse dose response experiments were conducted, and the resistant plants had a 24-fold resistance level relative to the susceptible control (data unpublished). Plants from the Windsor biotype were able to survive rates of glyphosate up to two times the field rate, while susceptible plants were killed by rates as low as a quarter of the field rate (Sikkema et al. 2009). Based on this research, giant ragweed was documented as the first weed species in Canada with resistance to glyphosate (Sikkema et al. 2009). Preliminary research suggests that some of these giant ragweed biotypes are resistant to both glyphosate and ALS inhibiting herbicides (data unpublished).

Giant ragweed populations are genetically diverse, in part, because of its high rate of cross-pollination, a trait often found in resistance prone species (Boerboom and Owen 2006; Johnson et al. 2007). Other weed species such as common ragweed (*Ambrosia artemisiifolia* L.), palmer amaranth (*Amaranthus palmeri* S. Wats.), common waterhemp (*Amaranthus tuberculatus* [Moq.] Sauer var. *rudis* [Sauer] Costea and Tardif), Italian ryegrass (*Lolium multiflorum* Lam.) and rigid ryegrass (*Lolium rigidum* Gaud.) are known for their genetic diversity and biotypes with resistance to several different herbicide modes of action have been reported (Busi and

Powles 2011; Falk et al. 2005; Heap 2012; Nordby et al. 2007; Norsworthy et al. 2008; Stachler and Loux 2007). The repeated use of glyphosate in an intensive GR cropping system has led to the selection of several GR weed biotypes worldwide (Heap 2012).

Most farmers in Ontario have adopted GR field crops. In the fall of 2009, giant ragweed escapes were visible in several fields in southwestern Ontario. Surveys have been reported in the literature to document various weed characteristics such as emergence and distribution patterns (Ramirez 2010), farmers' perceptions of problem weed species (Gibson et al. 2005), and most commonly to document cases of herbicide resistance (Baumgartner et al. 1999; Bourgeois and Morrison 1997; Davis et al. 2008; Falk et al. 2005). Surveys documenting herbicide resistance can serve as an important baseline to create awareness of the importance of herbicide resistance management. Therefore, the objective of this study was to determine the distribution of GR giant ragweed in southwestern Ontario.

2.3 Material and Methods

2.3.1 Seed Collection

Giant ragweed seed was collected from a total of 102 sites over a two year period (2009, 2010) in southwestern Ontario. Samples were collected in September and October of each survey year prior to soybean harvest when giant ragweed seed reached maturity. Seeds were identified as mature when they were visibly brown to black in colour. Seed was collected from fields in Essex (70), Chatham-Kent (21), Lambton (10) and Waterloo (1) counties. Two populations from the county of Chatham-Kent were used as susceptible controls: one collected from a wood lot near Ridgetown and another collected from a ditch bank near Chatham, Ontario. An initial single

dose screen (450 g a.e. ha⁻¹) with the Ridgetown and Dover populations confirmed susceptibility to glyphosate.

Site selection was non-random and consistent with other surveys for weed resistance reported in the literature (Davis et al. 2008; Ellis et al. 2010; Falk et al. 2005). Most of the giant ragweed seed was collected from Essex county where soybean is the predominant field crop, and giant ragweed escapes in soybean are easily visible from the roadside. Therefore, of the 102 seed samples collected, 101 were from soybean fields and one was from a corn field. For the majority of the sites, seed was collected from soybean fields where giant ragweed was the only weed present. The assumption was that glyphosate controlled all of the other weed species in the field, therefore increasing the likelihood of finding GR giant ragweed biotypes. Sites included in the survey were located by three different methods: first, growers who observed poor control of giant ragweed with glyphosate contacted us; second, personnel from agricultural retailers identified fields with poor giant ragweed control with glyphosate; and third, while travelling to known problem fields, if giant ragweed was observed from the road, seed was collected from those fields (Beckie et al. 2000). At the time of seed collection, herbicide-use history was not collected because the focus was placed on collecting seed from as many sites as possible before soybean harvest. Herbicide-use history is confidential, but some growers did agree to provide spray records after screening with glyphosate confirmed resistance.

At every sample location the following data were recorded: grower name (if known), road name, nearest intersection, date sampled, approximate field area infested, giant ragweed phenotype, distribution pattern of giant ragweed, and absence or presence of other weed species. A handheld global positioning unit (Garmin GPSMAP 76CSx) was used to record the field coordinates.

Plant sampling procedures were consistent with methods reported by Beckie et al. (2000), Davis et al. (2008) and Stachler (2008). Giant ragweed seed heads were collected by clipping the stems below the flower heads. Mature seed was collected from at least 20 different plants, or alternatively, at least two paper bags (7 by 30 cm across and 43 cm high) were filled with giant ragweed seed heads. A similar amount of seed was collected from each plant and bulked into one composite sample for each location. Seeds and plant tissue were stored in paper bags and dried under greenhouse conditions at a daytime and nighttime temperature of 23 and 18°C, respectively, with a photoperiod of 16 hours for at least two weeks. Seed heads were threshed using a bench top soybean thresher. Seed was cleaned and insect predated seeds were removed from the sample. Cleaned seed was cataloged and temporarily stored in a freezer at -12°C.

2.3.2 Resistance Testing

Giant ragweed seed has a dormancy requirement for at least eight weeks following seed dispersal (Jeff Stachler, personal communication). To break dormancy, methods consistent with Stachler (2008) were used. Greenhouse transplant trays (18 cells) were filled halfway using a potting media (PRO-MIX PGX). Seeds from each population were placed in an individual cell, labeled using plastic stakes, and then covered with approximately two cm of potting media. The trays containing seed and potting media were watered, and the excess water was allowed to drain. The trays were placed in a refrigerator under constant darkness for at least eight weeks at a temperature between 3 and 6°C. After six to eight weeks, the seed from a few cells were unearthed and checked for germination. If germinated seeds were observed, all cells were checked and seedlings were transplanted.

Germinated seeds (radical/root visible and/or shoot elongation) were transplanted into the same potting mix in 400 mL square pots. Seedlings were watered and fertilized immediately

after planting and placed in a growth room. Growth conditions were set for a daytime and nighttime temperature of 25 and 20°C, respectively, with a photoperiod of 16 hours. Plants were lightly watered and fertilized with a 20-20-20 solution daily. Seeds that did not germinate were left in the moist potting media and placed in the refrigerator until germination occurred. For most populations, at least 20 plants were screened with glyphosate.

Plants had two- to four-nodes (four to eight leaves) at the time of glyphosate application. The application of glyphosate was made as plants were established from germinated seed. Up to four weed germination flushes were required to assess the population response to the application of glyphosate. Glyphosate (Roundup[®] Weathermax) was applied at 1800 g a.e. ha⁻¹ or two times the recommended field rate. Culpepper et al. (2006) demonstrated that GR palmer amaranth were approximately two-fold more tolerant to glyphosate in the field compared to plants grown in the greenhouse. Beckie et al. (2000) noted that herbicide efficacy is often higher in a controlled environment than in field conditions because uptake and translocation is optimal. However, others have noted a decrease in glyphosate efficacy under growth room conditions (Doug Sammons, Monsanto, St. Louis, personal communication) and that sensitive populations of giant ragweed routinely survive lower doses (450 g a.e. ha⁻¹) of glyphosate. Therefore, all populations were screened at a conservative 2X rate to avoid any false positives. Glyphosate was applied with a laboratory chamber sprayer using a single 8002 even flat fan nozzle (TeeJet, Wheaton, IL) positioned 46 cm above the leaves calibrated to deliver a spray volume of 210 L ha⁻¹ at 276 kPa. After glyphosate application, plants were immediately transferred to the growth room.

Visual assessments were made one day after application (DAA) and 1, 2, and 4 weeks after application (WAA). Plants were identified as either susceptible (S) or resistant (R) at each rating date (Beckie et al. 2000). Plants were considered S if they responded similarly to the

susceptible check and if the apical growing point was visibly dead (necrotic). In addition, previous observations have shown that in many R populations, leaf necrosis may occur within 24 h of treatment application. Therefore, plants at the 1 d evaluation were classified as R if necrosis of the oldest leaves was evident. At 1, 2, and 4 WAA, plants were classified as R if the growing point remained alive. At the 4 WAA assessment, giant ragweed populations were designated either S or R and the percentage of plants tested that were R was calculated (Beckie et al. 2000; Owen et al. 2007).

2.4 Results and Discussion

Giant ragweed seed was collected in 2009 from 53 sites in Essex (32), Chatham-Kent (18), and Lambton (3) counties. In addition to the initial Windsor site documented in 2008 (Fig. 2-1), there are several other sites with GR giant ragweed in southwestern Ontario. Of the 53 populations collected in 2009, 18 (34%) had at least one resistant plant when assessed at 4 WAA (Table 2-1). All resistant sites were found in Essex County. The percentage of GR giant ragweed plants at each site ranged from less than 10% to greater than 90% (Table 2-1). Plants from the susceptible populations were completely killed at 4 WAA and never responded to glyphosate with rapid necrosis. Plants from the other 34 sites (42%) were either completely controlled or were inconclusive as to their susceptibility to glyphosate (24%). Inconclusive plants were severely stunted and distorted at the apical growing point, and did not grow after glyphosate application. For the purpose of this survey, inconclusive plants were considered as susceptible.

The survey was expanded in 2010 to include 49 additional sites. Giant ragweed seed was collected from sites in Essex (38), Chatham-Kent (3), Lambton (7) and Waterloo (1) counties. Results from the 2010 survey concluded that an additional 29 sites (59%) had at least one giant

ragweed survive 4 WAA (Table 2-1). Resistant sites were found in Essex (27), Chatham-Kent (1) and Lambton (1) counties. The percentage of GR giant ragweed at different sites ranged from less than 10% to greater than 70% (Table 2-1). Similar results were observed in the 2009 survey and are consistent with Beckie et al. (2000) who noted that field-collected samples of populations suspected to be resistant almost always contain seeds from both R and S biotypes. Plants from the susceptible populations were completely controlled at 4 WAA. Plants from the other 20 sites were completely controlled (12%), or were inconclusive (29%) and considered susceptible.

GR giant ragweed has been confirmed at 47 new locations in southwestern Ontario (Fig. 2-1). Resistance was not identified at the other 55 sample sites. These sites were therefore classified as susceptible. This reinforces the fact that poor control in the field is not always due to herbicide resistance.

The geographic distribution ranges from the south on Pelee Island, to the western and southwestern portion of Essex County, to the western edge of Chatham-Kent, and north to the southwest border of Lambton County. In general, several sites were found in concentrated groups along the periphery of Essex county near the southern shore of Lake St. Clair, near the Detroit river in the far west of Essex county, and near the north shore of Lake Erie in the extreme southwest of Essex county.

When the survey began in 2009, there was only one site documented in the NW corner of Essex county with GR giant ragweed (Fig. 2-1). GR giant ragweed has now been identified at many other sites, as far as 63 km from the initial site. This widespread distribution of GR giant ragweed across three counties can be due to a combination of gene flow from pollen dispersal, seed movement and local selection pressure. Gene flow via pollen from resistant plants and

physical seed movement from harvesters can be an important contributor to the spread of resistant biotypes across a wide geographic area. Pollen can travel up to 200 km but viable pollen travels over a distance of less than 100 m (Alex 1992; Volenberg et al. 2005). Pollen flow from site to site could explain why so many resistant fields are located within relatively close proximity to one another. In other studies, the spread of GR giant ragweed and other herbicide resistant weed species has been attributed to pollen movement (Baumgartner 1999; Brabham and Johnson 2010; Falk 2005). A second possible explanation for the movement of GR giant ragweed is seed on machinery. Several growers in southwestern Ontario, particularly in Essex county, manage farms over 1000 hectares. In general they operate large harvesters that move from site to site during the harvest season increasing the risk of weed seed movement. Additionally, a few growers with GR giant ragweed have indicated that their fields are harvested by custom operators who harvest crops for several different growers. In addition to pollen flow and seed movement, multiple founder events may have taken place simultaneously at a number of independent sites. It is common, particularly in Essex county for growers to plant GR soybeans in successive years with no rotation to other crops, and they utilize glyphosate for giant ragweed control year after year. Due to selection pressure at those sites, GR giant ragweed biotypes may have originated from within a population as opposed to arriving by pollen flow or seed movement.

Results from this survey may over estimate the GR frequency at each site because locations were selected non-randomly where giant ragweed was clearly visible from the road, or based on farmer/retail personnel complaints. In many instances, seed was collected from distinct patches of survivors – presumably plants that escaped previous glyphosate applications. Because seed collection occurred just prior to soybean harvest, susceptible biotypes were likely controlled

by the previous glyphosate applications. On the other hand, the glyphosate dose of 1800 g a.e. ha⁻¹ may have killed plants that had a lower level of resistance. This was observed in populations that consistently responded to glyphosate with rapid necrosis of the oldest leaves 1 d after treatment, but did not survive 4 WAA (data not shown).

These results indicate that sites in southwestern Ontario differ in the percentage of GR giant ragweed in a surviving population. This was consistent with field observations recorded at the time of seed collection. Visual estimation of the field area infested with giant ragweed ranged from less than 1% to greater than 80% across all frequency classes. Sites with the most surviving giant ragweed plants at the time of seed collection (i.e. >30% infested) generally resulted in <50% of the plants resistant (Table 2-1). The distribution of giant ragweed plants at these sites was often scattered or in strips throughout the field and not confined to distinct patches. Conversely, sites that had few surviving plants at the time of seed collection (i.e. <5% infested) were often found in patches and generally resulted in greater than 50% of the samples containing GR giant ragweed (Table 2-1). Beckie et al. (2000) noted that resistant weeds often have a patchy distribution. Therefore, it is likely that the proportion of resistant seeds collected from a patch would be higher than seeds collected from a larger population that infests a greater area. This is consistent with Bourgeois and Morrison (1997) who reported an increase in the number of samples classified as R when wild oat seed was collected from patches.

This is the first survey to document a glyphosate-resistant weed in Canada. Through field scouting, growers may be able to manage GR giant ragweed more easily if populations are identified and destroyed when they are still confined to small patches. At sites where giant ragweed was observed across the entire field, the plants were often distributed in strips. This likely indicates that harvesting equipment is spreading seeds from patches at the early stages of

resistance. Most resistant sites surveyed in this study can be managed if corrective measures are implemented immediately. Such measures would include a diverse crop rotation, timely tillage, the use of herbicides with different modes of action, and cleaning machinery before moving to other sites (Brown and Whitwell 1988; Falk et al. 2005; Shaner et al. 2011; Stachler, 2008). This survey has established baseline information on the occurrence of GR giant ragweed in Ontario and is an important step in addressing this new weed management challenge in the province.

Table 2-1. The number of populations of giant ragweed in each glyphosate resistance frequency class, the percent of field infested with giant ragweed at seed collection, and the number of plants screened in the two years of the survey

Frequency class (% R)	Number of populations			Field infested (%)	Plants screened (#)
	2009	2010	Total		
0	35	20	55	<1-80	16-30
1-10	2	2	4	<1-15	20-28
11-20	1	5	6	<1-25	14-42
21-30	2	3	5	<1-75	20-24
31-40	4	3	7	<1-40	18-39
41-50	2	8	10	<1-80	13-47
51-60	2	3	5	<1-25	17-29
61-70	1	3	4	<1-5	21-24
71-80	2	2	4	<1-20	16-23
81-90	1	0	1	5	41
91-100	1	0	1	5	20
All classes	53	49	102	-	2152

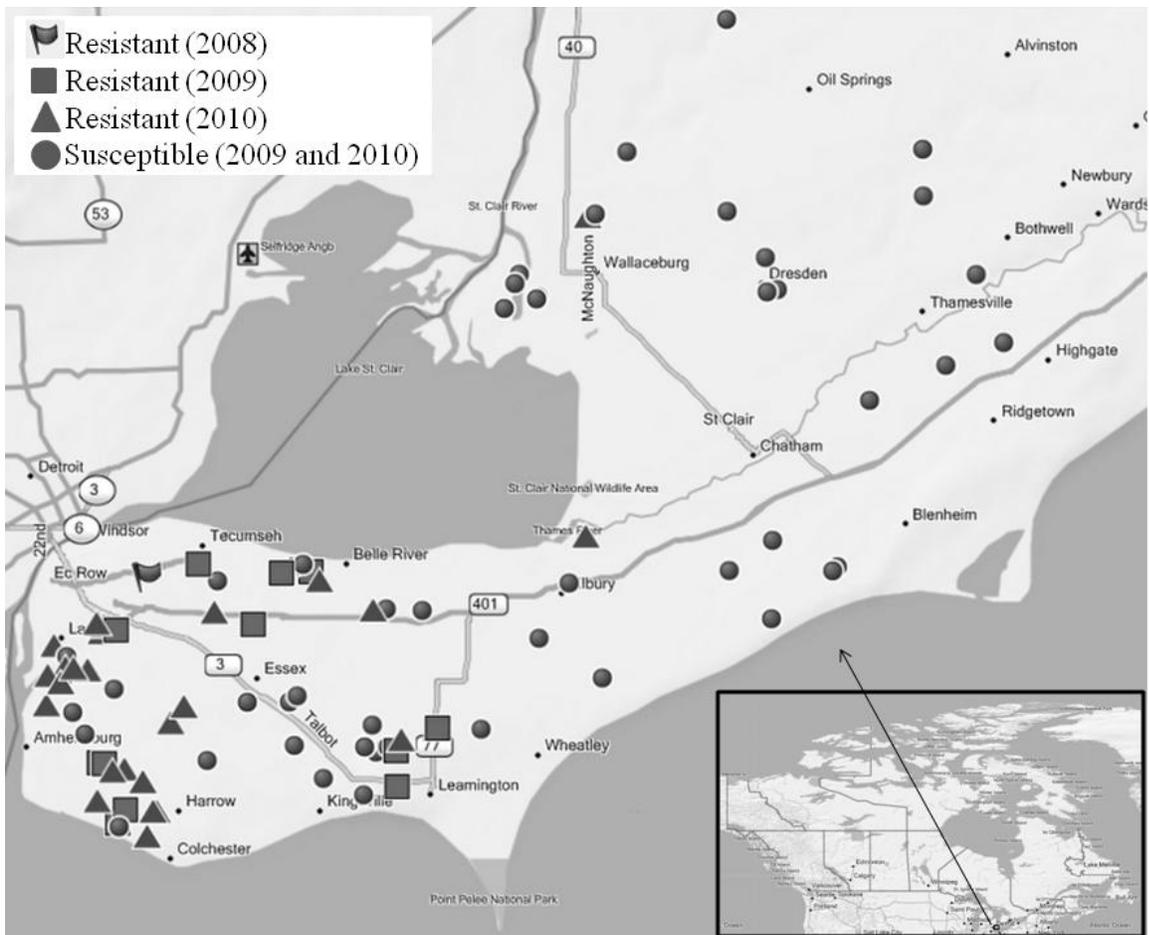


Figure 2-1. Survey locations of glyphosate-resistant and -susceptible giant ragweed from seed collected in the fall of 2009 and 2010 in southwestern Ontario (six resistant and one susceptible location on Pelee Island not shown).

3.0 Glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) control with preplant herbicides in soybean (*Glycine max* L.)

3.1 Abstract

Giant ragweed populations in southwestern Ontario have evolved resistance to the herbicide glyphosate. Glyphosate-resistant (GR) giant ragweed interference in field crops can lead to significant yield losses. Eleven field trials (five with preplant (PP) burndown only and six with PP burndown plus residual herbicides) were conducted over a 2-yr period (2010-2011) on Ontario farms with GR giant ragweed to evaluate the efficacy of various PP herbicides applied prior to soybean planting. Glyphosate applied at the recommended field dose failed to adequately control GR giant ragweed. The PP only herbicides: 2, 4-D ester, cloransulam-methyl and saflufenacil applied alone and with glyphosate provided 97-99, 68-100 and 71-94% control, respectively and resulted in soybean yields equivalent to the weed-free check. Combinations of glyphosate plus cloransulam-methyl or linuron controlled GR giant ragweed 8 weeks after application (WAA), 75-95 and 95-98%, respectively. Residual control with glyphosate plus linuron resulted in soybean yield equivalent to the weed-free check. Based on these results, GR giant ragweed can be controlled prior to soybean planting in southwestern Ontario.

3.2 Introduction

Soybean (*Glycine max* L.) is an important agronomic crop in southwestern Ontario. The total area planted to soybean in Canada has increased from 1,180,00 ha in 2007 to 1,550,000 ha in 2011 (Statistics Canada 2011). In 2011, Ontario accounted for the majority of the soybean production with 64% of the total area planted (McGee 2011). Weed control in soybean is important to maximize yield and profitability. Growers in southwestern Ontario will often plant

soybean in early May to increase the vegetative growth period and the number of pods per plant in hope of increasing yield (OMAFRA 2002). Unfortunately, earlier soybean planting often exposes the germinating seedlings to cooler soil temperatures which may delay emergence. Delayed emergence may allow more time for early emerging broad-leaved weeds such as giant ragweed (*Ambrosia trifida* L.) to establish and compete with young soybean seedlings (Soltani et al. 2008). In no-tillage production systems that are commonly implemented in southwestern Ontario, growers often apply a PP burndown nonselective herbicide to control emerged weeds prior to soybean planting (Soltani et al. 2008; Thompson et al. 2007). This is important because it allows growers to plant into a weed free field and provides the crop with a competitive advantage over weeds (Thompson et al. 2007).

Giant ragweed is a native, annual, broadleaf weed found in southwestern Ontario as well as in midwestern and eastern portions of the United States (Abul-Fatih and Bazzaz 1980; Bassett and Crompton 1982; Hunt and Bazzaz 1980; Johnson et al. 2007). Historically, giant ragweed was predominantly found in undisturbed areas such as river valleys, meadows, roadsides, fencerows and drainage ditches (Bassett and Crompton 1982; Johnson et al. 2007), but in the past few decades this species has adapted to current agronomic production systems and is now a common weed of agronomic crops in southwestern Ontario. Today, giant ragweed is considered a common weed in agronomic crops of the eastern Corn Belt, the midwestern United States and increasingly in the southern United States (Gibson et al. 2005; Hartzler et al. 2002; Johnson et al. 2007; Norsworthy et al. 2010; Steckel 2007).

Giant ragweed interference can result in large yield losses in soybean. After emergence, its rapid growth rate and large leaf area gives it an early competitive advantage over agronomic crops (Abul-Fatih and Bazzaz 1979). As little as one plant m^{-2} can reduce soybean yield up to

77% (Webster et al. 1994). Baysinger and Sims (1991) reported soybean yield loss of 52% with as few as two giant ragweed plants m⁻². Harrison et al. (2001) reported 13% yield loss in corn with a giant ragweed density of only one plant per 10 m², when the corn and giant ragweed emerged simultaneously.

Management of giant ragweed in row crops has become difficult (Johnson et al. 2007). In previous reports, acetolactate synthase (ALS) inhibitors such as cloransulam-methyl, chlorimuron-ethyl and imazethapyr provided adequate control of giant ragweed (Baysinger and Sims 1992; Franey and Hart 1999; Taylor et al. 2002). However, over reliance on ALS-inhibitors has led to the selection of ALS resistant giant ragweed populations (Johnson et al. 2007; Taylor et al. 2002; Zelaya and Owen 2004). After the introduction of GR soybeans in 1996, ALS-resistant giant ragweed could be managed with postemergence (POST) applications of glyphosate (Johnson et al. 2007; Taylor et al. 2002; Stachler 2008). The repeated use of glyphosate has led to the selection of resistant giant ragweed populations in the United States. GR giant ragweed was first identified in Ohio, USA in 2004 and has since been confirmed in nine additional states (Heap 2012; Stachler 2008; Stachler et al. 2006).

In 2008, a giant ragweed biotype near Windsor, Ontario, Canada was not controlled in soybean after two applications of glyphosate at the recommended field rate. Seed was collected and greenhouse testing confirmed that the Windsor biotype was resistant to glyphosate (Sikkema et al. 2009). The giant ragweed biotype from Windsor was the first weed species in Canada to evolve resistance to glyphosate.

Many soybean growers in southwestern Ontario rely on glyphosate for weed management. This was evident in 2011, as the total area planted to GR soybean cultivars in eastern Canada reached 72% (Stratus, personal communication). GR giant ragweed will continue

to be selected for with the repeated use of glyphosate under a mainly GR cropping system (Dill et al. 2008). More recent research evaluated GR giant ragweed control under greenhouse conditions in the United States (Norsworthy et al. 2010, 2011). Previous research suggests that the critical weed-free period for most annual weed species in soybean is four to six weeks after seeding (Barrentine 1974; Bloomberg et al. 1982; Coble et al. 1981; Coble and Ritter 1978; Williams and Hayes 1984). In southwestern Ontario, the majority of giant ragweed seedlings emerge prior to soybean planting. Soybean growers may take advantage of this application timing and control GR giant ragweed prior to planting. However, there is currently limited information on the effect of PP herbicide applications for the control of GR giant ragweed under Ontario's environmental conditions. Therefore, the objective of this research was to evaluate the efficacy of various PP herbicides for the control of GR giant ragweed under field conditions in Ontario.

3.3 Materials and Methods

A total of 11 field experiments were established on Ontario farms with GR giant ragweed over a two-year period (2010 and 2011) to evaluate the efficacy of PP herbicides. One set of experiments evaluated herbicides with limited residual activity (enhanced burndown), and a second set of experiments evaluated glyphosate tank mixes with residual herbicides for full season control (burndown plus residual). In 2010, there was one enhanced burndown and one burndown plus residual trial at a location near Windsor (L1). In 2011, there were four enhanced burndown and five burndown plus residual trials at locations near Windsor (L2 and L3), Belle River (L4), LaSalle (L5) and Amherstburg (L6). Glyphosate resistance was confirmed at each

location prior to the establishment of field trials (unpublished data). Soil characteristics and agronomic information for each location are presented in Table 3-1.

The experiments were arranged in a randomized complete block design with three replications at L1, L2, and L4 and four replications at L3, L5, and L6. At L1, L4 and L5, the first, third and first replications respectively, were removed prior to analysis due to low giant ragweed density. Herbicides in the enhanced burndown study included glyphosate (900 g a.e. ha⁻¹) applied alone, and carfentrazone (17.5 g a.i. ha⁻¹ + COC at 1.0% vol/vol), saflufenacil (25 g a.i. ha⁻¹ + COC at 1.0% v/v), 2, 4-D ester (500 g a.i. ha⁻¹), glufosinate (500 g a.i. ha⁻¹), cloransulam-methyl (17.5 g a.i. ha⁻¹ + NIS at 0.250% v/v + 28% UAN 2.50% vol/vol), or chlorimuron-ethyl (9 g a.i. ha⁻¹ + NIS at 0.2% v/v) applied alone and with glyphosate (900 g a.e. ha⁻¹). At 4 weeks after application (WAA) an over spray of glyphosate (900 g a.e. ha⁻¹) plus fomesafen (240 g a.i. ha⁻¹) was applied POST after completion of visual estimates of giant ragweed control, giant ragweed biomass and giant ragweed density to control any late emerging giant ragweed that could interfere with soybean yield. Herbicides in the burndown plus residual study included glyphosate (900 g a.e. ha⁻¹) applied alone or with chlorimuron-ethyl (9 g a.i. ha⁻¹), cloransulam-methyl (35 g a.i. ha⁻¹), linuron (2250 g a.i. ha⁻¹), metribuzin (1120 g a.i. ha⁻¹), flumetsulam (70 g a.i. ha⁻¹), imazethapyr (100 g a.i. ha⁻¹), or clomazone (846 g a.i. ha⁻¹). The herbicide rates used are the highest labeled rate registered for use in Ontario. Each experiment included a weedy and weed-free check. In 2010, the weed free check plots were maintained with either an application of glufosinate (1000 g a.i. ha⁻¹) or glyphosate (1800 g a.e. ha⁻¹) plus saflufenacil (25 g a.i. ha⁻¹) applied PP followed by hand hoeing as required. In 2011, all weed-free check plots were maintained with glyphosate (900 g a.e. ha⁻¹) plus 2, 4-D ester (500 g a.i. ha⁻¹) applied PP followed by hand hoeing as required.

Herbicides were applied with a CO₂-pressurized backpack sprayer equipped with ULD 120-02 flat fan nozzles (Hypro, New Brighton, MN) calibrated to deliver 200 L ha⁻¹ of water at 210 kPa. Herbicide applications were made with a 1.5 m boom with four nozzles spaced 50 cm apart over the center of the plot. Plots were six to eight m long depending on the location. Size of giant ragweed and date of applications are presented in Table 3-1.

Visual estimates of soybean injury were made during giant ragweed control assessments if soybean emergence had occurred. For L1 and L4, and L2, L3, and L6 soybean injury was rated 2 and 4, and 4 WAA, respectively. At L5, soybean emergence occurred after the last control assessment and therefore injury ratings were not recorded. Injury ratings were on a scale of 0 to 100%, where a rating of 0 was defined as no plant injury and a rating of 100 was defined as plant death. Giant ragweed control was rated 1, 2, and 4 WAA for the enhanced burndown experiment and 1, 2, 4, and 8 WAA for the burndown plus residual experiment. Control was rated on a scale of 0 to 100%, where 0 was defined as no control and 100 was defined as complete giant ragweed control. At 4 WAA, giant ragweed density and biomass (shoot dry weight) in each plot was determined by counting giant ragweed plants and cutting the plants at the soil surface from two 0.25 m² quadrats. Plants were bagged by plot, and dried at 60°C to constant moisture content, and the dry weights were recorded. At crop maturity in 2010, soybeans were harvested by collecting 10 m of row from each plot at L1. In 2011, soybean from two m of row from each plot at all trial locations was harvested by hand. Soybeans were threshed in a plot combine or a stationary thresher, and the grain weight and moisture was recorded. Yields were adjusted to 13.0% moisture.

All data were subjected to ANOVA using the MIXED procedure in SAS (Ver. 9.1, SAS Institute Inc., Cary, NC). Variances were partitioned into the random effects of location (year

and location), replication (within location), and location by treatment interaction, and the fixed effect of herbicide treatment. Significance of random effects and their interaction with fixed effects was tested using the Z-test of the variance estimate, while the significance of fixed effects was tested using the F-test. For all variables there was a significant location by treatment interaction. Therefore, locations were analyzed separately or combined into groups that resulted in a non-significant interaction. Error assumptions of the variance analysis (random, independent and homogenous) were confirmed by examining residual plots. Data were tested for normality using the Shapiro-Wilk statistic as generated by the UNIVARIATE procedure in SAS. When necessary, a transformation (natural log, square root, arcsine square root) of the data was applied and the transformation which generated the highest Shapiro-Wilk statistic was chosen. For the enhanced burndown trial, giant ragweed shoot dry weight data at L4 and L5 were log transformed, soybean injury data at L2 and L4, and giant ragweed shoot dry weight at L3 and L6 were square-root transformed, and giant ragweed control data 1 WAA at L4 and L5, 2 WAA at L1 and L3, L4, L5, 4 WAA at L1 and L3, L4, L5 and L6 were arcsine square-root transformed. For the burndown plus residual trial, giant ragweed shoot dry weight and soybean yield data were log transformed, giant ragweed control data 8 WAA at L1 and L2 were square-root transformed, and giant ragweed control data 4 WAA at L4 and L5 were arcsine square-root transformed. After interpretation, treatment means were transformed back to the original scale for presentation. Means were separated using Fisher's protected LSD at $P < 0.05$.

3.4 Results and Discussion

3.4.1 Enhanced burndown

At 4 WAA, soybean injury up to 7% (delayed emergence and growth) was observed at L3 and L4 from application of 2, 4-D ester alone and with glyphosate (data not shown). However, injury was transient and there was no effect on soybean yield. This is consistent with the findings of Soltani et al. (2008) who reported 0.5% injury and no effect on yield when 2, 4-D ester was applied at a higher rate of 705 g a.e. ha⁻¹ seven days before planting.

All of the herbicide treatments evaluated improved the control of GR giant ragweed compared to the weedy check. At 1 WAA, control data from L1 and L3 and L4 and L5 could be combined, whereas L6 was analyzed separately. Glyphosate provided 25 to 51% control (Table 3-2). Giant ragweed injury symptoms due to glyphosate application was rapid necrosis of the mature leaf tissue; a unique response associated with the resistant biotypes that is consistent with previous research (Brabham and Johnson 2010; Norsworthy et al. 2010; personal observation). The resistant biotypes resume growth from new axillary buds at the base of the plant or from the primary growing point. In contrast, saflufenacil applied alone or in a tank mix with glyphosate provided the most consistent control and was equivalent to the weed-free check for groups L1 and L3, and L6 (Table 3-2). For group L4 and L5, saflufenacil treatments provided up to 98% control (Table 3-2). Control with 2, 4-D ester, carfentrazone, chlorimuron-ethyl, cloransulam-methyl and glufosinate applied alone or with glyphosate was up to 73, 89, 62, 78 and 87%, respectively (Table 3-2). For most of the herbicides evaluated, there was no difference in control when they were applied alone or in a tank mix with glyphosate. The exceptions were carfentrazone (all groups) and chlorimuron-ethyl (L6); where the control was improved when applied in a tank mix with glyphosate (Table 3-2).

At 2 WAA, data from L1 and L3 could be combined, whereas L4, L5, and L6 were analyzed separately. Control with all treatments evaluated was generally lower for group L1 and L3 compared to L4, L5, and L6, and may be attributed to larger giant ragweed (2 to 22 cm) at the time of application (Table 3-3). Glyphosate provided 24 to 63% control across all groups. In previous reports, glyphosate applied at 840 g a.e. ha⁻¹ provided 95 to 98% control of a susceptible giant ragweed biotype in soybean (Maertens et al. 2002; Wiesbrook et al. 2001). In another study, glyphosate applied at 870 g a.e. ha⁻¹ provided only 11% control of a GR biotype 3 WAA (Still et al. 2008). Control with 2, 4-D ester, cloransulam-methyl, glufosinate and saflufenacil applied alone or with glyphosate ranged from 74 to 98%. These treatments were the most consistent across groups (Table 3-3). GR giant ragweed control with carfentrazone and chlorimuron-ethyl applied alone or with glyphosate was variable across groups and ranged from 15 to 85% (Table 3-3). Control with carfentrazone (L1 and L3, L4, L6) and chlorimuron-ethyl (L1 and L3, L5, L6) improved when tank mixed with glyphosate, and was consistent with the earliest assessment (Table 3-3). In contrast, at L6 only, glufosinate applied alone improved control compared to glyphosate plus glufosinate tank mix (Table 3-3). Previous research has also reported antagonism with combinations of glyphosate and glufosinate (Dotray et al. 2009; Whitaker et al. 2011).

At 4 WAA, control data from L1 and L3, and L5 and L6 could be combined, and L4 was analyzed separately. At L4, glyphosate provided 70% control of GR giant ragweed compared to only 21 and 40% control at L1 and L3, and L1 and L6, respectively (Table 3-4). This is consistent with the findings of Stachler (2008) who reported 45 to 77% control of GR giant ragweed in a field dose response study. The difference in control with glyphosate may be due to differences in the proportion of glyphosate susceptible and resistant biotypes among the

experimental sites. Previous greenhouse survey results from these locations suggest that the proportion of GR giant ragweed biotypes at L1 and L3, L4, and L5 and L6 was 75 to 100, 14, and 43 to 50%, respectively (Vink et al. 2011). Across all groups, 2, 4-D ester, and glyphosate plus 2, 4-D ester provided control equivalent to the weed-free check (Table 3-4). This is consistent with the findings of Robinson and Johnson (2010), but in contrast to other reports in the literature (Johnson et al. 2007; Loux et al. 2006). Cloransulam-methyl applied alone or with glyphosate (L4, L5 and L6), and saflufenacil alone (L5 and L6) provided control equivalent to that observed in the weed-free check (Table 3-4). Franey and Hart (1999) reported up to 97% giant ragweed control with cloransulam-methyl applied alone to 10 to 15 cm tall plants. In contrast, Still et al. (2008) applied cloransulam-methyl at a higher rate of 24 g a.i. ha⁻¹ and reported only 64% GR giant ragweed control. For the other groups, saflufenacil provided 71 to 88% control and there was no difference between applications alone or with glyphosate (Table 3-4). This is in contrast to results reported by Waite et al. (2008) who observed 100% GR giant ragweed control with a PP application of glyphosate plus saflufenacil. Glufosinate alone or with glyphosate provided 71 to 93% control across all groups (Table 3-4). In a greenhouse study, glufosinate applied at 590 g a.i. ha⁻¹ provided at least 90% control of GR giant ragweed 4 WAA (Norsworthy et al. 2010). In a field study, glufosinate applied at a lower rate of 410 g a.i. ha⁻¹ provided 50 to 86% control (Hoss et al. 2003). For group L1 and L3, cloransulam-methyl applied alone provided 91% control of GR giant ragweed compared to only 68% when tank mixed with glyphosate. This is consistent with the findings of Stachler (2008) who reported only 69% control of GR giant ragweed with glyphosate plus cloransulam-methyl. In contrast, control with carfentrazone at L1 and L3, and L4 and chlorimuron-ethyl at L1 and L3, and L5 and L6 improved when tank mixed with glyphosate (Table 3-4).

For GR giant ragweed shoot dry weight, data from L1 was analyzed separately and data from L3 and L6, and L4 and L5 could be combined (Table 3-5). Reduction in GR giant ragweed shoot dry weight correlated with the level of control. Glyphosate reduced shoot dry weight 26 to 74%, and was equivalent to the weedy check at L3 and L6, and L4 and L5 (Table 3-5). Conversely, 2, 4-D ester, cloransulam-methyl and glyphosate plus 2, 4-D ester reduced shoot dry weight equivalent to the weed-free check across all groups (Table 3-5). Glufosinate at L1, saflufenacil at L1, and L4 and L5, glyphosate plus cloransulam-methyl at L1, and L4 and L5, glyphosate plus glufosinate at L1, and L4 and L5, and glyphosate plus saflufenacil at L1 reduced giant ragweed shoot dry weight equivalent to the weed-free check (Table 3-5). In contrast, carfentrazone applied alone reduced giant ragweed shoot dry weight only 20 to 44% and was equivalent to the weedy check across all groups (Table 3-5). In recent greenhouse experiments, control of GR giant ragweed with carfentrazone was found to be dependent on the size of plants at application and provided 89, 62 and 46% control of two-, four-, and six-node giant ragweed, respectively (Norsworthy et al. 2010). In contrast, carfentrazone applied alone to two-node GR giant ragweed provided 96 to 100% control (Norsworthy et al. 2011). At L1, glyphosate plus carfentrazone reduced shoot dry weight 58% and was an improvement over carfentrazone applied alone. Chlorimuron-ethyl applied alone reduced shoot dry weight 4 to 71%, but was equivalent to that observed in the weedy check at groups L3 and L6, and L4 and L5. Chlorimuron-ethyl has provided variable control of giant ragweed in other reports (Baysinger and Sims 1992; Still et al. 2008; Taylor et al. 2002). Glyphosate plus chlorimuron-ethyl reduced shoot dry weight 47 to 86% (Table 3-5).

GR giant ragweed interference reduced soybean yield 49% in the untreated check (Table 3-5). Glyphosate resulted in soybean yield equivalent to that observed in the weedy check. In a

previous report, glyphosate applied at a lower rate of 420 g a.e. ha⁻¹ failed to control giant ragweed and soybean yield was reduced 31% (Maertens et al 2002). Conversely, 2, 4-D ester, cloransulam-methyl, saflufenacil, glyphosate plus 2, 4-D ester, glyphosate plus cloransulam-methyl and glyphosate plus saflufenacil resulted in yields equivalent to the weed-free check (Table 3-5). Soybean yields with glufosinate and glyphosate plus glufosinate were reduced 18 to 24% (Table 3-5). Carfentrazone and chlorimuron-ethyl applied alone or with glyphosate resulted in soybean yields equivalent to the weedy check (Table 3-5).

3.4.2 Burndown plus residual

There was no soybean injury from the herbicides evaluated (data not shown). Data sets were analyzed in groups L1 and L2, L3 and L6, and L4 and L5 for control 1 WAA and L1, L2 and L5, and L3, L4 and L6 for control 2 WAA. All treatments evaluated improved control of GR giant ragweed compared to the weedy check. However, glyphosate did not provide adequate control of GR giant ragweed and control ranged from only 25 to 45% (Table 3-6). In contrast, glyphosate plus linuron provided 69 to 82 and 88 to 97% control 1 and 2 WAA, respectively. At 2 WAA, control with glyphosate plus linuron was equivalent to the weed-free check (Table 3-6). Glyphosate plus metribuzin provided 55 to 70 and 33 to 93% control 1 and 2 WAA, respectively. For group L2 and L5, control was equivalent to that observed in the weed-free check at 2 WAA (Table 3-6). Glyphosate plus cloransulam-methyl provided 56 to 88% control at the earlier assessments and was equivalent to that observed in the weed-free check for group L2 and L5 at 2 WAA (Table 3-6). Glyphosate plus chlorimuron-ethyl, clomazone, flumetsulam or imazethapyr provided up to 58, 52, 69 and 71% control, respectively (Table 3-6).

For control assessments at 4 and 8 WAA, data were analyzed in groups L1 and L2, L3 and L6, and L4 and L5. Glyphosate provided only 32 to 54 and 20 to 45% control 4 and 8 WAA,

respectively (Table 3-7). This is consistent with the findings of Norsworthy et al. (2010) who reported less than 50% GR giant ragweed control in a greenhouse study with glyphosate applied at 870 g a.e. ha⁻¹ to four- to six-node plants. For group L1 and L2, 4 WAA, control with glyphosate was equivalent to that observed in the weedy check (Table 3-7). Conversely, glyphosate plus linuron provided the most consistent control at 4 and 8 WAA, and was equivalent to the weed-free check across all groups (Table 3-7). Control with glyphosate plus metribuzin was variable at the later assessments and was consistent with earlier control ratings. Control ranged from 69 to 96 and 60 to 71%, 4 and 8 WAA respectively, and was equivalent to the weed-free check for groups L1 and L2, and L3 and L6 at 4 WAA, and L1 and L2, at 8 WAA (Table 3-7). Hasty et al. (2004) reported only 42% giant ragweed control with metribuzin applied 30 d before planting at 527 g a.i. ha⁻¹. Glyphosate plus cloransulam-methyl provided 77 to 96 and 75 to 95% control 4 and 8 WAA, respectively, and was more consistent at the later assessments compared to control 1 and 2 WAA. In general, control was equivalent to the weed-free check at the later assessments (Table 3-7). Glyphosate plus chlorimuron-ethyl, clomazone, flumetsulam or imazethapyr did not provide adequate control of GR giant ragweed and was often equivalent to glyphosate applied alone (Table 3-7). This is consistent with other reports in the literature where flumetsulam provided 2 to 24% control of giant ragweed when applied preemergence (PRE) at 75 g a.i. ha⁻¹ (Taylor et al. 2002) and imazethapyr applied PRE at a lower rate of 70 g a.i. ha⁻¹ provided 0 to 12% control of giant ragweed (Taylor et al. 2002). In contrast, clomazone applied PRE at 840 g a.i. ha⁻¹ followed by glyphosate applied POST provided up to 98% giant ragweed control in soybean (Wiesbrook et al. 2001).

For giant ragweed shoot dry weight, there was a nonsignificant trial by treatment interaction and data could be combined. Reduction in giant ragweed shoot dry weight was

consistent with the visual estimates of control. Glyphosate reduced giant ragweed shoot dry weight 37% and was equivalent to the weedy check (Table 3-8). In contrast, glyphosate plus linuron reduced giant ragweed shoot dry weight 99% and was equivalent to the weed-free check (Table 3-8). Glyphosate plus metribuzin or cloransulam-methyl effectively reduced giant ragweed shoot dry weight 95 to 97%, respectively. Glyphosate plus chlorimuron-ethyl, clomazone, flumetsulam or imazethapyr reduced shoot dry weight 67 to 77% (Table 3-8).

For soybean yield, there was a nonsignificant trial by treatment interaction and data could be combined. GR giant ragweed interference reduced soybean yield 72% and all treatments evaluated increased soybean yield compared to the weedy check (Table 3-8). However, giant ragweed interference still reduced soybean yield 69% when glyphosate was applied alone. In contrast, glyphosate plus linuron resulted in soybean yield equivalent to the weed-free check which is consistent with control assessments. Giant ragweed interference reduced soybean yield 43 and 30%, with glyphosate plus metribuzin or glyphosate plus cloransulam-methyl, respectively but were the next best options after linuron (Table 3-8). Giant ragweed interference reduced soybean yield 56 to 65% with glyphosate plus chlorimuron-ethyl, clomazone, flumetsulam or imazethapyr (Table 3-8).

In summary, this research shows that GR giant ragweed in soybean can be managed with effective PP herbicides applied alone or when tank mixed with glyphosate. In this study, the best control of GR giant ragweed in the enhanced burndown experiment was achieved with 2, 4-D ester, cloransulam-methyl and saflufenacil applied alone or as a tank mix with glyphosate. Glyphosate plus cloransulam-methyl or linuron applied PP were the most effective treatments in the burndown plus residual experiments. Growers are advised to implement diverse weed management practices that achieve high levels of control. Growers should control weeds early,

incorporate as many other herbicides with different modes of action as possible and use the full labeled rate that controls the most problematic weed in the field (Sammons et al. 2007). Future research is needed to evaluate glyphosate tank mixes applied PP followed by POST in crop applications. Research is also needed to evaluate the effect of size of giant ragweed at the time of application on herbicide efficacy under Ontario's environmental conditions.

Table 3-1. Location characteristics, agronomic information, and giant ragweed height at the time of treatment application for preplant burndown experiments conducted during 2010 and 2011

Location	Year	Nearest town	Soil Texture	Soil OM %	Soil pH	Soybean Cultivar	Planting Date	Planting Population (seeds ha ⁻¹)	Row Spacing (cm)	Treatment Application Date	Giant Ragweed Height (cm)
1	2010	Windsor	Sandy clay	3.8	6.9	Dekalb 31-10	27-May	513,980	38	25-May	12-22
2	2011	Windsor	Sandy clay	4.0	7.3	Dekalb 31-10	7-June	444,789	38	21-May	2-7
3	2011	Windsor	Loam	2.8	6.9	Pioneer 92Y80	15-June	420,079	38	2-June	2-17
4	2011	Belle River	Clay	3.3	6.8	Dekalb 31-10	7-June	444,789	38	3-June	2-12
5	2011	LaSalle	Loam	2.6	7.5	Dekalb 31-10	13-June	467,029	38	21-May	1-9
6	2011	Amherstburg	Clay loam	3.7	7.9	Pioneer 92Y80	8-June	568,342	19	20-May	1-7

Table 3-2. Percent control of glyphosate-resistant giant ragweed at 1 WAA for various preplant burndown herbicides^z

Treatment	Rate (g ae/ai ha ⁻¹)	Control 1 WAA		
		L1 and L3	L4 & L5	L6
			(%)	
Weedy check		0 h	0 j	0 h
Weed-free check		100 a	100 a	100 a
2,4-D ester	500	68 bcd	79 de	69 cd
Carfentrazone ^y	17.5	24 g	50 hi	74 c
Chlorimuron-ethyl ^v	9	37 fg	54 gh	37 g
Cloransulam-methyl ^{xw}	17.5	59 cde	78 de	64 de
Glufosinate	500	76 b	85 cd	85 b
Glyphosate	900	25 g	41 i	51 f
Saflufenacil ^y	25	91 a	97 b	98 a
Glyphosate + 2,4-D ester	900 + 500	73 bc	82 cd	74 c
Glyphosate + carfentrazone ^y	900 + 17.5	56 de	63 fg	89 b
Glyphosate + chlorimuron-ethyl ^v	900 + 9	52 ef	62 fg	58 ef
Glyphosate + cloransulam-methyl ^{xw}	900 + 17.5	55 de	71 ef	63 de
Glyphosate + glufosinate	900 + 500	76 b	87 c	86 b
Glyphosate + saflufenacil ^y	900 + 25	95 a	98 b	98 a

^zAbbreviations: L1, Windsor; L3, Windsor; L4, Belle River; L5, LaSalle; L6, Amherstburg; WAA, weeks after treatment application.

^yIncluded COC (1.0% vol/vol).

^xIncluded non-ionic surfactant (0.25% vol/vol).

^wIncluded 28% UAN (2.5% vol/vol).

^vIncluded non-ionic surfactant (0.2% vol/vol).

^{a-j}Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P < 0.05.

Table 3-3. Percent control of glyphosate-resistant giant ragweed at 2 WAA for various preplant burndown herbicides^z

Treatment	Rate (g ae/ai ha ⁻¹)	Control 2 WAA			
		L1 and L3	L4	L5	L6
Weedy check		0 g	0 h	0 i	0 h
Weed-free check		100 a	100 a	100 a	100 a
2,4-D ester	500	80 bc	95 abc	93 bc	76 e
Carfentrazone ^y	17.5	15 f	50 g	25 gh	63 f
Chlorimuron-ethyl ^v	9	30 ef	83 de	40 f	46 g
Cloransulam-methyl ^{xw}	17.5	86 bc	97 ab	93 bc	88 bcd
Glufosinate	500	84 bc	98 ab	85 d	88 bc
Glyphosate	900	24 f	63 fg	37 fg	51 g
Saflufenacil ^y	25	84 bc	94 bcd	97 b	97 a
Glyphosate + 2,4-D ester	900 + 500	85 bc	95 abc	94 bc	80 de
Glyphosate + carfentrazone ^y	900 + 17.5	47 de	75 ef	23 h	78 e
Glyphosate + chlorimuron-ethyl ^v	900 + 9	52 d	85 cde	55 e	63 f
Glyphosate + cloransulam-methyl ^{xw}	900 + 17.5	74 c	94 bcd	94 bc	81 cde
Glyphosate + glufosinate	900 + 500	84 bc	98 ab	88 cd	79 e
Glyphosate + saflufenacil ^y	900 + 25	91 b	96 abc	95 bc	94 ab

^zAbbreviations: L1, Windsor; L3, Windsor; L4, Belle River; L5, LaSalle; L6, Amherstburg; WAA, weeks after treatment application

^yIncluded COC (1.0% vol/vol).

^xIncluded non-ionic surfactant (0.25% vol/vol).

^wIncluded 28% UAN (2.5% vol/vol).

^vIncluded non-ionic surfactant (0.2% vol/vol).

^{a-i}Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P < 0.05.

Table 3-4. Percent control of glyphosate-resistant giant ragweed at 4 WAA for various preplant burndown herbicides^z

Treatment	Rate (g ae/ai ha ⁻¹)	Control 4 WAA		
		L1 and L3	L4	L5 and L6
			(%)	
Weedy check		0 i	0 i	0 i
Weed-free check		100 a	100 a	100 a
2,4-D ester	500	99 ab	99 ab	97 ab
Carfentrazone ^y	17.5	11 h	34 h	29 h
Chlorimuron-ethyl ^v	9	18 gh	72 fg	34 h
Cloransulam-methyl ^{xw}	17.5	91 bc	100 a	99 ab
Glufosinate	500	71 de	93 bcde	81 cde
Glyphosate	900	21 gh	70 fg	40 gh
Saflufenacil ^y	25	71 de	80 efg	94 abc
Glyphosate + 2,4-D ester	900 + 500	97 ab	98 abc	98 ab
Glyphosate + carfentrazone ^y	900 + 17.5	37 fg	65 g	51 fgh
Glyphosate + chlorimuron-ethyl ^v	900 + 9	48 ef	85 defg	60 efg
Glyphosate + cloransulam-methyl ^{xw}	900 + 17.5	68 de	97 abcd	93 abc
Glyphosate + glufosinate	900 + 500	70 de	93 bcde	74 def
Glyphosate + saflufenacil ^y	900 + 25	82 cd	88 cdef	92 bcd

^zAbbreviations: L1, Windsor; L3, Windsor; L4, Belle River; L5, LaSalle; L6, Amherstburg; WAA, weeks after treatment application.

^yIncluded COC (1.0% vol/vol).

^xIncluded non-ionic surfactant (0.25% vol/vol).

^wIncluded 28% UAN (2.5% vol/vol).

^vIncluded non-ionic surfactant (0.2% vol/vol).

^{a-i}Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P < 0.05.

Table 3-5. Glyphosate-resistant giant ragweed shoot dry weight and soybean yield for various preplant burndown herbicides^z

Treatment	Rate (g ae/ai ha ⁻¹)	Giant ragweed shoot dry weight			Soybean Yield
		L1	L3 and L6	L4 and L5	Combined
			(g m ⁻²)		(t ha ⁻¹)
Weedy check		337.5 d	55.7 g	33.9 f	1.44 f
Weed-free check		0.0 a	0.0 a	0.0 a	2.82 a
2,4-D ester	500	0.0 ab	0.1 ab	0.0 ab	2.59 abc
Carfentrazone ^y	17.5	268.8 d	41.5 fg	18.9 f	1.57 f
Chlorimuron-ethyl ^v	9	133.5 c	53.6 g	10.0 def	1.83 ef
Cloransulam-methyl ^{xw}	17.5	0.0 ab	1.5 abc	0.1 ab	2.60 ab
Glufosinate	500	4.9 ab	4.9 bc	2.0 bc	2.30 bcd
Glyphosate	900	86.7 bc	41.0 fg	12.5 ef	1.89 def
Saflufenacil ^y	25	2.7 ab	10.8 cde	1.2 abc	2.38 abc
Glyphosate + 2,4-D ester	900 + 500	0.0 ab	0.2 ab	0.0 ab	2.83 a
Glyphosate + carfentrazone ^y	900 + 17.5	141.7 c	23.1 def	11.3 ef	1.72 ef
Glyphosate + chlorimuron-ethyl ^v	900 + 9	80.6 bc	29.4 efg	4.7 cde	1.81 ef
Glyphosate + cloransulam-methyl ^{xw}	900 + 17.5	15.2 ab	8.2 bcd	0.4 ab	2.45 abc
Glyphosate + glufosinate	900 + 500	38.4 ab	11.0 cde	1.3 abc	2.13 cde
Glyphosate + saflufenacil ^y	900 + 25	0.0 ab	6.5 bcd	2.3 bcd	2.65 ab

^zAbbreviations: L1, Windsor; L3, Windsor; L4, Belle River; L5, LaSalle; L6, Amherstburg; WAA, weeks after treatment application

^yIncluded COC (1.0% vol/vol).

^xIncluded non-ionic surfactant (0.25% vol/vol).

^wIncluded 28% UAN (2.5% vol/vol).

^vIncluded non-ionic surfactant (0.2% vol/vol).

^{a-g}Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at $P < 0.05$.

Table 3-6. Percent control of glyphosate-resistant giant ragweed at 1 and 2 WAA for various preplant burndown plus residual herbicides^z

Treatment	Rate (g ae/ai ha ⁻¹)	Control 1 WAA			Control 2 WAA		
		L1 and L2	L3 and L6	L4 and L5	L1	L2 and 5	L3, L4 and L6
		(%)					
Weedy check		0 d	0 f	0 f	0 e	0 d	0 f
Weed-free check		100 a	100 a	100 a	100 a	100 a	100 a
Glyphosate	900	36 bc	41 e	41 de	25 d	38 c	45 e
Glyphosate + chlorimuron-ethyl	900 + 9	45 bc	56 d	57 bc	28 cd	58 b	58 d
Glyphosate + cloransulam-methyl	900 + 35	56 b	64 cd	60 bc	75 b	88 a	79 b
Glyphosate + clomazone	900 + 846	27 c	40 e	38 e	38 cd	51 bc	52 de
Glyphosate + flumetsulam	900 + 70	37 bc	53 d	53 cd	38 cd	58 b	69 c
Glyphosate + imazethapyr	900 + 100	52 bc	55 d	56 bcd	45 c	65 b	71 c
Glyphosate + linuron	900 + 2250	82 a	77 b	69 b	88 ab	97 a	94 a
Glyphosate + metribuzin	900 + 1120	55 b	70 bc	56 bcd	33 cd	93 a	83 b

^zAbbreviations: L1, Windsor; L2, Windsor; L3, Windsor; L4, Belle River; L5, LaSalle; L6, Amherstburg; WAA, weeks after treatment application

^{a-f}Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P < 0.05.

Table 3-7. Percent control of glyphosate-resistant giant ragweed at 4 and 8 WAA for various preplant burndown plus residual herbicides^z

Treatment	Rate (g ae/ai ha ⁻¹)	Control 4 WAA			Control 8 WAA		
		L1 and L2	L3 and L6	L4 and L5	L1 and L2	L3 and L6	L4 and L5
Weedy check		0 e	0 e	0 e	0 d	0 d	0 d
Weed-free check		100 a	100 a	100 a	100 a	100 a	100 a
Glyphosate	900	32 de	37 d	54 d	20 c	21 c	45 c
Glyphosate + chlorimuron-ethyl	900 + 9	40 cde	49 c	72 cd	24 c	31 c	63 c
Glyphosate + cloransulam-methyl	900 + 35	77 ab	91 a	96 ab	75 ab	81 b	95 ab
Glyphosate + clomazone	900 + 846	48 bcd	57 bc	66 cd	34 bc	30 c	48 c
Glyphosate + flumetsulam	900 + 70	41 cd	53 bc	75 cd	43 bc	26 c	61 c
Glyphosate + imazethapyr	900 + 100	57 bcd	63 b	78 cd	39 bc	31 c	55 c
Glyphosate + linuron	900 + 2250	95 a	99 a	97 ab	96 a	98 a	95 ab
Glyphosate + metribuzin	900 + 1120	69 abc	88 a	96 bc	60 ab	71 b	67 bc

^zAbbreviations: L1, Windsor; L2, Windsor; L3, Windsor; L4, Belle River; L5, LaSalle; L6, Amherstburg; WAA, weeks after treatment application

^{a-e}Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P < 0.05.

Table 3-8. Glyphosate-resistant giant ragweed shoot dry weight and soybean yield for various preplant burndown plus residual herbicides

Treatment	Rate (g ae/ai ha ⁻¹)	Giant ragweed shoot dry weight	Soybean yield
		Combined (g m ⁻²)	Combined (t ha ⁻¹)
Weedy check		78.4 e	0.80 f
Weed-free check		0.0 a	2.90 a
Glyphosate	900	49.6 de	0.91 e
Glyphosate + chlorimuron-ethyl	900 + 9	25.9 cd	1.09 de
Glyphosate + cloransulam-methyl	900 + 35	2.6 b	2.04 b
Glyphosate + clomazone	900 + 846	17.9 c	1.01 de
Glyphosate + flumetsulam	900 + 70	20.6 c	1.29 cd
Glyphosate + imazethapyr	900 + 100	18.6 c	1.02 de
Glyphosate + linuron	900 + 2250	0.2 a	2.61 a
Glyphosate + metribuzin	900 + 1120	3.7 b	1.64 bc

^{a-f}Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P < 0.05.

4.0 Glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in Ontario: Dose response and control with postemergence herbicides

4.1 Abstract

Giant ragweed is competitive with agronomic crops such as corn and soybean, and can cause significant yield losses. Rapid adoption of glyphosate-resistant (GR) crops and a concomitant increase in the reliance on glyphosate for weed management has led to the evolution of GR giant ragweed in Ontario, Canada. Field studies were conducted to evaluate the level of resistance in giant ragweed biotypes from Ontario, and to evaluate the effectiveness of various postemergence (POST) herbicides in soybean. The effective dose (ED) to provide 50, 80 and 95% giant ragweed control was up to 1658, 9991 and >43200 g a.e. ha⁻¹ 4 weeks after application (WAA), respectively. For effective control, growers would need to apply glyphosate 18 times greater than the recommended field application dose. Glyphosate applied at the recommended field dose of 900 g a.e. ha⁻¹ provided up to 57% control and resulted in soybean yield equivalent to the weedy check. Cloransulam-methyl applied POST provided up to 99% control, reduced giant ragweed density 98%, reduced giant ragweed shoot dry weight 99% and resulted in soybean yield equivalent to the weed-free check. Chlorimuron-ethyl, fomesafen, imazethapyr and imazethapyr plus bentazon applied alone or with glyphosate did not provide adequate control of GR giant ragweed. Based on these results, some GR giant ragweed biotypes from Ontario have evolved a high level of resistance to glyphosate. Cloransulam-methyl applied POST was the only herbicide that provided adequate control and suggests that additional weed management tactics will need to be implemented in order to effectively manage GR giant ragweed.

4.2 Introduction

Glyphosate [N-(phosphonomethyl)glycine] was developed by John E. Franz of Monsanto Co., and was first tested as a herbicide in 1970 (Franz et al. 1997). By 1974, glyphosate was commercially introduced in several markets as a postemergence (POST), non-selective herbicide for the control of weeds prior to crop planting (Duke and Powles 2008; Franz et al. 1997; Powles 2008). Glyphosate inhibits the enzyme EPSPS (5-enolpyruvylshikimate 3-phosphate synthase) in the shikimic acid pathway and leads to the depletion of the aromatic amino acids tryptophan, tyrosine, and phenylalanine which are important for protein synthesis and secondary metabolism (Amrhein 1980; WSSA 2011). As a systemic herbicide, glyphosate is translocated from foliage to the roots, rhizomes, and apical tissues and controls hard-to-kill perennials such as Canada thistle (*Cirsium arvense* L.), johnsongrass (*Sorghum halepense* L.), nutsedge (*Cyperus esculentus* L.) and quackgrass (*Elymus repens* L.) (Franz et al. 1997).

Prior to the mid 1990's, the use of glyphosate was limited in field crop production because it also killed treated crops (Dill et al. 2010). The introduction of glyphosate-resistant (GR) crops, mainly soybean (*Glycine max* L.), canola (*Brassica campestris* L.), cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.) between 1996 and 1998 allowed growers to apply POST applications of glyphosate to the crop for the control of emerged weeds without crop damage (Powles 2008; Reddy and Norsworthy 2010). Since then, GR crops have been rapidly adopted for reasons including excellent weed control, wide margin of crop safety, simplicity of application, lower cost of weed control, reduced fuel costs and improved soil conservation through no-tillage management (Feng et al. 2010; Nandula 2010). In 2008, GR corn, cotton and soybean were grown on 77% of the total corn, cotton and soybean planted in the United States (Reddy and Norsworthy 2010). In 2011, the area planted with GR corn and

soybean in eastern Canada reached 90 and 72%, respectively (Stratus Agri-Marketing Inc., Guelph ON, personal communication).

GR crops have led to changes in herbicide use patterns because glyphosate is often the only herbicide used for weed control (Reddy and Norsworthy 2010). In GR cropping systems, glyphosate is often applied preplant (PP) or preemergence (PRE), POST in crop, and post harvest as a stand-alone selective herbicide (Shaner et al. 2011). From 1997 to 2003, the total active ingredient of glyphosate used in the United States in corn increased from 0.6 million kg year⁻¹ to 5.6 million kg year⁻¹. In soybean, glyphosate use has increased dramatically from 2.9 million kg year⁻¹ in 1995 to 41.7 million kg year⁻¹ in 2006 (Reddy and Norsworthy 2010; USDA 2012).

The repeated use of glyphosate has increased selection pressure for weeds that are naturally difficult to control as well as the evolution of GR weed biotypes (Duke and Powles 2009). Glyphosate-resistance was first reported in a rigid ryegrass (*Lolium rigidum* Gaud.) population from an orchard in Australia, and soon after in goosegrass (*Eleusine indica* L.) in Malaysia (Lee and Ngim 2000; Powles et al. 1998). The first report of evolved glyphosate-resistance in a GR cropping system was in Canada fleabane (*Conyza canadensis* L.) in the state of Delaware (VanGessel 2001). Glyphosate-resistance has now been reported in 21 different weeds and is especially prevalent in the *Amaranthus*, *Ambrosia*, *Conyza*, and *Lolium* species (Heap 2012).

Glyphosate-resistance in giant ragweed (*Ambrosia trifida* L.) was first reported in Ohio, in 2004 (Stachler 2008) but has since been reported in nine additional states (Heap, 2012). Westhoven et al. (2008) suggested that GR giant ragweed could be found throughout the state of Indiana. GR giant ragweed biotypes from two populations in Arkansas have a 2.3- to 7.2-fold resistance level compared to a susceptible biotype (Norsworthy et al. 2011). In Tennessee, a GR

giant ragweed biotype had a 5.3-fold resistance level relative to a susceptible biotype (Norsworthy et al. 2010). In 2008, a giant ragweed biotype from a field near Windsor, Ontario, Canada was not controlled after two applications of glyphosate at the manufacturer's recommended dose. Seeds were collected and greenhouse experiments confirmed resistance to glyphosate. Plants from the Windsor population survived glyphosate up to two times the field dose (1800 g ae ha⁻¹) while the susceptible biotype was controlled at doses as low as a quarter of the field dose (Sikkema et al. 2009). The giant ragweed biotype from Windsor was the first weed in Canada to evolve resistance to glyphosate and populations have since been confirmed at 47 additional locations (Vink et al. 2011). Some biotypes are also resistant to the acetolactate synthase (ALS) inhibiting herbicide, cloransulam-methyl (unpublished data).

Giant ragweed is an erect, herbaceous, annual dicot weed that is a member of the Asteraceae family. Seedlings are easily identified by their large, spoon shaped cotyledons that are 9 to 16 mm wide, 25 to 45 mm long, and up to 2 mm thick (Johnson et al. 2007). Mature plants can grow up to six meters in height, and are often at least one meter taller than the crop with which it is competing (Johnson et al. 2007). Flowering occurs from mid-July to October, and a single plant can produce more than a billion pollen grains during its life cycle (Johnson et al. 2007). Pollen from ragweed is an important cause of hay fever; an allergenic reaction that affects an estimated 30 million people in the United States (Knowlton 2011).

Poor control of giant ragweed in agronomic crops can result in large yield losses. In a study conducted by Webster et al. (1994), giant ragweed outgrew soybean early in the season and maintained growth within the soybean canopy throughout the growing season. In the same study, giant ragweed interference from as little as one plant per m² reduced soybean yield up to

77%. In corn, yield losses as high as 90% were predicted if giant ragweed density was 14 plants per 10 m², when it emerged simultaneously with the corn (Harrison et al. 2001).

Unpredictable germination and emergence of giant ragweed has contributed to management challenges for growers. In previous reports, giant ragweed seedlings emerged in March before other annual weed species (Abul-Fatih and Bazzaz 1979). In an earlier report, giant ragweed finished emerging before May 15 (Stoller and Wax 1973). More recent research suggests an early and prolonged emergence pattern. Schutte et al. (2008) observed giant ragweed emergence in Ohio from April 5 to July 7. This early and prolonged emergence pattern has been observed in Ontario where giant ragweed will emerge as early as late March and continue through July (personal observation).

Glyphosate applied POST will no longer control GR giant ragweed biotypes in Ontario. Furthermore, prolonged emergence of giant ragweed complicates control strategies and results in late emerging plants that are not controlled with alternative PP or PRE herbicides. Giant ragweed can be controlled in corn with dicamba based herbicides, but options in soybean are limited (Sikkema et al. 2009). The objective of this research was to determine the level of resistance to glyphosate in different GR giant ragweed populations, and evaluate the efficacy of various POST herbicides for the control of GR giant ragweed in soybean under field conditions in Ontario. This research will contribute towards the development of recommendations for the control of GR giant ragweed in Ontario.

4.3 Materials and Methods

A total of ten field experiments were established on Ontario farms with GR giant ragweed in 2011. One set of experiments evaluated the response of giant ragweed to varying

doses of glyphosate (field dose response), and another set evaluated various herbicides registered for POST application in soybean (POST herbicides). The experiments were conducted at locations near Windsor (L1 and L2), Belle River (L3), LaSalle (L4) and Amherstburg (L5). Glyphosate resistance was confirmed at each location prior to the establishment of field trials (Vink et al. 2011). Field preparation included chisel plow, disking or no-tillage in the autumn followed by no-tillage management in the spring. Soil characteristics and agronomic information for each location are presented in Table 4-1.

The experiments were arranged in a randomized complete block design with three to four replications. Dose response treatments included glyphosate applied at 112.5, 225, 450, 900, 1800, 2700, 5400, 10800, 21600, or 43200 g a.e. ha⁻¹. Herbicides included in the POST herbicides experiment were glyphosate (900 g a.e. ha⁻¹) applied alone, and chlorimuron-ethyl (9 g a.i. ha⁻¹ + non-ionic surfactant at 0.2% vol/vol + 28% UAN at 2 L ha⁻¹), cloransulam-methyl (17.5 g a.i. ha⁻¹ + non-ionic surfactant at 0.25% vol/vol + 28% UAN at 2.5% vol/vol), fomesafen (240 g a.i. ha⁻¹ + crop oil concentrate at 0.5% vol/vol), imazethapyr (100 g a.i. ha⁻¹ + non-ionic surfactant at 0.25% vol/vol + 28% UAN at 2 L ha⁻¹), or imazethapyr plus bentazon (75 and 840 g a.i. ha⁻¹ + 28% UAN at 2 L ha⁻¹) applied alone and in a tank mix with glyphosate (900 g a.e. ha⁻¹). The herbicide rates used in the POST experiment are the highest rate registered for use in Ontario. Each experiment included a weedy and weed-free check. All weed-free check plots were maintained with glyphosate (900 g a.e. ha⁻¹) plus 2, 4-D ester (500 g a.e. ha⁻¹) applied PP followed by hand hoeing as required.

Herbicides were applied with a CO₂-pressurized backpack sprayer equipped with ULD 120-02 flat fan nozzles (Hypro, New Brighton, MN) calibrated to deliver 200 L ha⁻¹ of water at 210 kPa. Herbicide applications were made with a 1.5 meter boom with four nozzles spaced 50

cm apart over the center of the plot. Plots were six to eight m long depending on location. Size of giant ragweed and date of application varied according to location (Table 4-1).

Visual estimate of soybean injury was evaluated up to 4 weeks after application (WAA) when soybean emergence corresponded with control assessment dates. Injury ratings were on a scale of 0 to 100%, where a rating of 0 was defined as no plant injury and a rating of 100 was defined as plant death. Giant ragweed control was rated 1, 2, 4, and 8 WAA. Control was rated on a scale of 0 to 100%, where 0 was defined as no control and 100 was defined as complete giant ragweed control. At 4 WAA, giant ragweed density and biomass (shoot dry weight) in each plot was determined by counting giant ragweed plants and cutting the plants at the soil surface from two 0.25 m² quadrats. Plants were bagged by plot, dried at 60°C to constant weight, and the dry weights were recorded. At crop maturity, soybean from two m of row from each plot was harvested by hand. Soybeans were threshed in a stationary thresher, and the grain weight and moisture was recorded. Yields were adjusted to 13.0% moisture.

4.3.1 Statistical analysis

4.3.1.1 Field dose response

Data were subjected to ANOVA using the MIXED procedure in SAS (Ver. 9.1, SAS Institute Inc., Cary, NC). Variances were partitioned into the random effects of location, replication within location, and the location by dose interaction and the fixed effect of glyphosate dose. Significance of random effects and their interaction with fixed effects were tested using the Z-test of the variance estimate, while the significance of fixed effects were tested using the F-test. For giant ragweed control 1 and 2 WAA, there was a non-significant ($P>0.05$) location by dose interaction and data could be combined. For giant ragweed control 4 and 8 WAA, density, and shoot dry weight, locations were analyzed separately or combined in groups that resulted in a

non-significant interaction. Giant ragweed shoot dry weight and density were expressed as a percent of the weedy check. Soybean yields were expressed as a percent of the weed-free check. At L3, the third replication was removed prior to analysis due to low giant ragweed density. At L5, plots 308 to 312 and 408 to 412 were excluded from the analysis after low giant ragweed densities were observed as a result of pooling water after rainfall. Soybean yield at L1 was excluded from the analysis due to giant ragweed shading of adjacent plots.

Non-linear regressions were performed using the PROC NLIN procedure in SAS. The regression models were chosen by examining scatter plots of the observed responses (Bowley 2008) or from previous reports in the literature (Seefeldt 1995). A sigmoidal log-logistic curve:

$$Y = C + (D-C) / \{1 + \exp [B (\ln (\text{dose}) - \ln (I_{50}))]\}$$

was used to regress giant ragweed control and soybean yield with glyphosate dose where Y is percent giant ragweed control or soybean yield, C is the lower limit, D is the upper limit, B is the slope of the line (negative for control), and I_{50} is the dose giving 50 percent of the response between the upper and lower limits. For soybean yield at L2, the log-logistic curve failed to fit the data and a segmented linear regression was used (Bowley 2008; Christy Shropshire, personal communication). The equation(s) were of the form:

left segment:

and right segment:

where a_0 is the intercept of the left segment, b_1 is the slope of the left segment, br_1 is the slope of the right segment, and j is the junction point at which the two equations join. Giant ragweed density and shoot dry weight were regressed using an inverse exponential equation of the form:

where a is the lower asymptote, b is the reduction in Y from intercept to a and c is the slope.

The effective dose (ED) of glyphosate was calculated using the appropriate regression equation. For giant ragweed control and soybean yield, ED50, 80, and 95 values represented the glyphosate dose that was required to provide 50, 80, and 95% control or 50, 80, and 95% soybean yield relative to the weed-free check, respectively. For density and shoot dry weight, ED50, 20, and 5 values were calculated to correspond with the dose of glyphosate required to reduce density and shoot dry weight by 50, 80, and 95%, respectively.

4.3.1.2 Postemergence herbicides

Data were subjected to ANOVA using the MIXED procedure in SAS (Ver. 9.1, SAS Institute Inc., Cary, NC). Variances were partitioned into the random effects of location, replication (within location), and location by treatment interaction, and the fixed effect of herbicide treatment. Significance of random effects and their interaction with fixed effects was tested using the Z-test of the variance estimate, while the significance of fixed effects was tested using the F-test. For giant ragweed control and soybean yield there was a significant location by treatment interaction and locations were analyzed separately or combined into groups that resulted in a non-significant interaction. Giant ragweed density and shoot dry weight data could be combined. Residual plots were examined to confirm the assumptions of variance analysis (random, independent and homogeneous) and the Shapiro-Wilk test was used to confirm normality. When necessary, a transformation (natural log, square root, arcsine square root) of the data was applied and the transformation which generated the highest Shapiro-Wilk statistic was chosen. Giant ragweed density data were log transformed, shoot dry weight data were square-root transformed, and giant ragweed control data 4 WAA at L1, L4 and L5, and 8 WAA at L2

and L3 were arcsine square-root transformed. After interpretation, treatment means were transformed back to the original scale for presentation of the results. Means were separated using Fisher's protected LSD at $P < 0.05$.

4.4 Results and Discussion

4.4.1 Dose response

The recommended glyphosate dose of 900 g a.e. ha⁻¹ did not provide acceptable control of GR giant ragweed. At 1 WAA, the dose required to provide 80% giant ragweed control was 6718 g a.e. ha⁻¹ or eight times the recommended dose. Glyphosate applied at 43200 g a.e. ha⁻¹ provided 93% control and therefore the ED₉₅ was predicted to be greater than the highest dose evaluated in this study (Table 4-2). At the earliest assessment, giant ragweed injury due to glyphosate treatment was rapid necrosis of the mature leaf tissue, as well as slight chlorosis of the newest leaves. This unique phenotypic response associated with the mechanism of resistance is consistent with a GR giant ragweed biotype from Indiana (Brabham et al. 2011). By 2 WAA, the ED₅₀, ED₈₀ and ED₉₅ for GR giant ragweed was 1212, 4332 and 37764 g a.e. ha⁻¹, or 1, 5 and 42 times the normal use dose, respectively.

At the later control assessments (4 and 8 WAA), the GR giant ragweed population at L1 was more robust and resumed growth more rapidly than the giant ragweed at L2, L3, L4 and L5. Giant ragweed height in the weedy check was approximately 20 cm taller than the giant ragweed at the other trial locations. In previous research, the lethal dose (LD) required to kill 50% of susceptible giant ragweed accessions from Arkansas ranged from 164 to 335 g a.e. ha⁻¹ (Norsworthy et al. 2011). In contrast, ED₅₀, ED₈₀ and ED₉₅ at L1 were 1658, 9991 and >43200 g a.e. ha⁻¹, respectively. For the same level of control at the locations L2, L3, L4 and L5,

glyphosate would need to be applied at doses of 1106, 3890 and 15957 g a.e. ha⁻¹, respectively (Table 4-2). Based on the GR₅₀'s in this study, GR giant ragweed in Ontario is up to 10-fold more resistant than a susceptible biotype from Arkansas. Furthermore, 95% GR giant ragweed control may be achieved at doses 18 to >48 times the normal dose. These doses are neither economical nor legal for growers in Ontario. By 8 WAA, the level of resistance at L1 further separated from the other locations. The GR₅₀ was 18982 g a.e. ha⁻¹ compared to 2430 g a.e. ha⁻¹ at L2, L3, L4, and L5 combined; an 8-fold difference in the level of control (Table 4-2).

Giant ragweed density and shoot dry weight generally correlated with the level of control. At L1, the doses required to reduce density by 50, 80 and 95% were 6734, 18179 and >43200 g a.e. ha⁻¹, respectively. At the other locations combined, the doses required to reduce density by the same amounts were 6077, 13908 and 25757 g a.e. ha⁻¹, respectively (Table 4-2). The L1 biotype had a 1.0-, 1.3- and at least 1.7-fold greater resistance to glyphosate for reduction in density (Table 4-2). Reduction in giant ragweed shoot dry weight was similar to density, except for ED₉₅ values. The doses required to reduce shoot dry weight by 50, 80 and 95% were 3813, 11143 and 22234 g a.e. ha⁻¹ for the L1 location and 953, 2665 and >43200 g a.e. ha⁻¹, respectively for the other locations combined. Based on the results from the combined locations, growers that would normally apply glyphosate at 900 g a.e. ha⁻¹ would only achieve approximately 50% reduction in giant ragweed shoot dry weight (Table 4-2). Stachler (2008) reported even higher levels of resistance in GR biotypes collected from Ohio and Indiana which required 8270 to 23940 g a.e. ha⁻¹ glyphosate to reduce shoot fresh weight by only 50%.

Higher doses of glyphosate were required to achieve soybean yields comparable to the weed-free check. For locations combined (L3, L4, L5), the doses required to achieve 80 and 95% of the weed-free check were 6931 and 13785g a.e.ha⁻¹, respectively. These doses are 7.7- to

15.3-fold greater than the recommended dose. At L2, poor soybean emergence due to heavy rain after planting resulted in the formation of a “crust” at the soil surface. As a result, soybean yield did not respond similarly to the other locations. The doses required to achieve soybean yield equivalent to 80 and 95% of the weed-free check were greater than the highest dose evaluated in this study (Table 4-2).

GR giant ragweed populations vary in the level of resistance. Based on the results of this research the ED₅₀ and ED₉₅ for GR giant ragweed 4 WAA ranged from 1106 to 1658 and 15957 to >43200 g a.e. ha⁻¹, respectively. This corresponds to 1.2 to 1.8 and 17.7 to greater than 48 times the recommended dose in Ontario. Norsworthy et al. (2010) reported on GR giant ragweed from Tennessee. In their greenhouse study, the LD required to kill 50 and 90% of the resistant accession was 2176 and 12400 g a.e. ha⁻¹, respectively. In another greenhouse study, GR giant ragweed biotypes from Arkansas were not as resistant as the Tennessee biotypes with a LD₅₀ and LD₉₀ of 765 to 1181 and 2278 to 2753 g a.e. ha⁻¹, respectively (Norsworthy et al. 2011). In their study, the LD₉₀ values were 2.7 to 3.3 times the normal dose of glyphosate in Arkansas.

The results of this research demonstrate that some giant ragweed biotypes can survive very high doses of glyphosate. Growers will need to alter their weed management practices and no longer rely on glyphosate for the control of GR biotypes. Alternative management strategies may include effective residual herbicides, diverse crop rotation, and the use of effective postemergence herbicides.

4.4.2 Postemergence herbicides

There was no soybean injury from the herbicides evaluated (data not shown). GR giant ragweed control data at 1 WAA were combined into groups L1 and L4, and L2 and L3, whereas L5 was analyzed separately. For the remaining control assessments, data were combined into

groups L1, L4 and L5 and L2 and L3. At the earliest assessments, all herbicides evaluated increased GR giant ragweed control compared to the weedy check (Table 4-3). However, glyphosate alone provided up to only 44 and 50% control 1 and 2 WAA, respectively. In contrast, glyphosate plus fomesafen provided control equivalent to the weed-free check at L1, L4 and L5 but only 61% control at L2 and L3, 1 WAA. The difference may be due to larger giant ragweed at the time of application (Table 4-1). Norsworthy et al. (2010) also reported variable control with fomesafen depending on the size of GR giant ragweed at application. Control with fomesafen applied alone ranged from 27 to 86%. At 2 WAA, cloransulam-methyl alone and with glyphosate also provided control equivalent to the weed-free check (Table 4-3). Chlorimuron-ethyl, imazethapyr, and imazethapyr plus bentazon applied alone and with glyphosate generally provided similar levels of control which ranged from 41 to 73% (Table 4-3). Taylor et al. (2002) applied chlorimuron-ethyl and imazethapyr at higher rates of 13 and 140 g a.i. ha⁻¹, respectively and reported control within the range observed in this study 2 WAA. At the earliest assessments, adding glyphosate to some herbicides improved control compared to single applications. Control with imazethapyr at L2 and L3, and L5, as well as chlorimuron-ethyl at L5 improved when tank mixed with glyphosate 1 WAA. At 2 WAA, control with fomesafen increased when tank mixed with glyphosate.

At 4 and 8 WAA, GR giant ragweed control was improved with all herbicide treatments compared to the weedy check (Table 4-4). However, glyphosate applied alone provided only 43 to 57 and 29 to 41% control. This is consistent with Stachler (2008) who reported 32% GR giant ragweed control with glyphosate applied at 840 g a.e. ha⁻¹. Similarly, Johnson et al. (2007) reported 39% control with glyphosate applied at a higher rate of 1680 g a.e. ha⁻¹. In contrast, cloransulam-methyl applied alone provided 93 to 99% control, which was equivalent to the

weed-free check. In previous research, cloransulam-methyl applied POST provided up to 88% control of 10 to 15 cm tall giant ragweed (Franey and Hart 1999). Norsworthy et al. (2011) reported 98% GR giant ragweed control when cloransulam-methyl was applied to 12 to 15 cm tall plants. In contrast, other studies have reported variable giant ragweed control with cloransulam-methyl (Taylor et al. 2002). In general, cloransulam-methyl was antagonized by the addition of glyphosate and control ranged from 80 to 92%. It is suggested that this decrease in control is due to rapid necrosis of the mature leaf tissue after the application of glyphosate which may reduce the absorption and/or translocation of cloransulam-methyl. Glyphosate plus fomesafen was generally more effective than fomesafen applied alone but control was variable at the later assessments (Table 4-4). Glyphosate plus fomesafen provided 84 to 94% control of giant ragweed in Illinois (Wiesbrook et al. 2001). In another study, fomesafen provided 64 to 86% control of three to five cm tall plants 6 WAA (Baysinger and Sims 1992). Norsworthy et al. (2011) reported 100% GR giant ragweed control with fomesafen in a greenhouse study. Chlorimuron-ethyl applied alone or with glyphosate provided 41 to 69 and 44 to 71% control, respectively. GR giant ragweed from Arkansas was controlled 68% with chlorimuron-ethyl applied in the greenhouse at 6 g a.i. ha⁻¹ (Norsworthy et al. 2011). Control with imazethapyr and imazethapyr plus bentazon applied alone or with glyphosate was variable and ranged from 25 to 82%. Hoss et al. (2003) reported up to only 46% giant ragweed control with imazethapyr applied POST at a lower rate of 70 g a.i. ha⁻¹.

Cloransulam-methyl reduced giant ragweed density 98% relative to the weedy check (Table 4-5). In a previous study, cloransulam-methyl reduced giant ragweed density up to 90% (Franey and Hart 1999). Glyphosate plus cloransulam-methyl reduced density 90%, but was not as effective as the stand alone treatment. In contrast, glyphosate applied alone reduced density

only 50%, and was equivalent to the weedy check. Fomesafen alone also failed to effectively reduce giant ragweed density and was equivalent to the weedy check. This is in contrast to Baysinger and Sims (1992) who reported up to 87% reduction in giant ragweed density when fomesafen was applied at a higher rate of 350 g a.i. ha⁻¹. Chlorimuron-ethyl, imazethapyr and imazethapyr plus bentazon alone and with glyphosate reduced density 55 to 63% and did not reduce density any more than glyphosate applied alone. This is consistent with previous research where chlorimuron-ethyl and imazethapyr reduced giant ragweed density 0 to 68 and 65 to 78%, respectively (Baysinger and Sims 1992).

Reduction in giant ragweed shoot dry weight correlated with control ratings (Table 4-5). Cloransulam-methyl alone and with glyphosate reduced shoot dry weight 95 to 99% and were once again the most effective treatments evaluated. Glyphosate alone reduced shoot dry weight 62% compared to the weedy check. Chlorimuron-ethyl, imazethapyr and imazethapyr plus bentazon applied alone or with glyphosate did not effectively reduce giant ragweed shoot dry weight, and were equivalent to glyphosate treatment alone (Table 4-5). Glyphosate plus fomesafen reduced shoot dry weight 80% and was an improvement over fomesafen alone.

Soybean yield data from L2, L3 and L5 could be combined, and L4 was analyzed separately. Soybean emergence at L4 was delayed due to shallow planting depth which resulted in lower yield compared to the other locations. GR giant ragweed reduced soybean yield up to 73% (Table 4-5). Poor giant ragweed control with glyphosate resulted in soybean yield equivalent to the weedy check. Chlorimuron-ethyl, fomesafen, imazethapyr and imazethapyr with bentazon applied alone or with glyphosate failed to adequately control giant ragweed and resulted in soybean yield equivalent to the weedy check (Table 4-5). In contrast, cloransulam-methyl alone resulted in soybean yield equivalent to the weed-free check. Glyphosate plus

cloransulam-methyl was the next best treatment but still resulted in 32 to 40% reduction in soybean yield. This further suggests that antagonism of cloransulam-methyl from glyphosate leads to reduced control of GR giant ragweed.

Giant ragweed biotypes from Ontario can survive doses of glyphosate in excess of 18 times the normal field dose. Growers will need to rely on alternative measures for the control of GR giant ragweed biotypes. The use of alternative herbicides with different modes of action could be a part of an integrated weed management program. Based on the results of this study, cloransulam-methyl applied POST can be an effective option for the control of GR giant ragweed. However, growers should be advised that some GR giant ragweed biotypes from Ontario have also evolved resistance to cloransulam-methyl (unpublished data). This research also suggests that the rapid necrosis after the application of glyphosate may antagonize cloransulam-methyl. Further research is needed to confirm multiple herbicide resistance in giant ragweed in Ontario.

Table 4-1. Location characteristics, agronomic information, and giant ragweed height at the time of treatment application for dose response and postemergence herbicides experiments conducted during 2011

Location	Nearest town	Soil Texture	Soil OM (%)	Soil pH	Soybean Cultivar	Planting Date	Planting Population (seeds ha ⁻¹)	Row Spacing (cm)	Treatment Application Date	Giant Ragweed Height (cm)
1	Windsor	Sandy clay	4.0	7.3	Dekalb 31-10	7-June	444,789	38	21-May	2-12
2	Windsor	Loam	2.8	6.9	Pioneer 92Y80	15-June	420,079	38	2-June	4-14
3	Belle River	Clay	3.3	6.8	Dekalb 31-10	7-June	444,789	38	3-June	2-25
4	LaSalle	Loam	2.6	7.5	Dekalb 31-10	13-June	467,029	38	21-May	1-8
5	Amherstburg	Clay loam	3.7	7.9	Pioneer 92Y80	8-June	568,342	19	20-May	1-9

Table 4-2. Dose response, segmented linear, and inverse exponential parameter values for giant ragweed control 1, 2, 4, and 8 WAA, density, and shoot dry weight and soybean yield for field dose response experiments conducted in 2011^z

<i>Dose response</i>	Location ^w	Regression parameters ^y (\pm SE)				Glyphosate dose (g a.e. ha ⁻¹) ^x		
		D	C	B	I ₅₀	ED ₅₀	ED ₈₀	ED ₉₅
<i>Giant ragweed control</i>								
1 WAA	1,2,3,4,5	93.4 (1.5)	0.0 (0.0)	1.1 (0.1)	1327 (70.6)	1508	6718	>43200
2 WAA	1,2,3,4,5	96.5 (1.4)	0.0 (0.0)	1.2 (0.1)	1140 (56.6)	1212	4332	37764
4 WAA	1	94.1 (5.2)	0.0 (0.0)	0.9 (0.1)	1443 (277.2)	1658	9991	>43200
	2,3,4,5	100.0 (0.0)	0.3 (2.2)	1.1 (0.1)	1113 (83.6)	1106	3890	15957
8 WAA	1	100.0 (0.0)	4.0 (3.4)	1.6 (0.4)	19974 (3042.7)	18982	>43200	>43200
	2,3,4,5	100.0 (0.0)	2.1 (2.9)	1.0 (0.1)	2536 (288.1)	2430	10086	>43200
Soybean yield	3,4,5	100.0 (0.0)	25.9 (4.6)	2.4 (0.9)	4557 (837.1)	3349	6931	13785
<i>Segmented linear</i>								
Soybean yield	2	a ₀ 10.2 (8.1)	b ₁ 2.1 (2.4)	br ₁ 7.8 (1.6)	j 4.8 (1.8)	5762	>43200	>43200
<i>Inverse exponential</i>								
Giant ragweed density	1	g 86.3 (39.1)	f 6.0 (38.7)	h 1.3 x 10 ⁻⁴	-	6734	18179	>43200
	2,3,4,5	101.8 (11.6)	0.0 (0.0)	1.2 x 10 ⁻⁴	-	6077	13908	25757
Giant ragweed shoot dry weight	1	80.5 (9.9)	0.0 (0.0)	1.3 x 10 ⁻⁴	-	3813	11143	22234
	2,3,4,5	83.3 (6.4)	6.2 (4.3)	6.7 x 10 ⁻⁴	-	953	2665	>43200

^zAbbreviations: WAA, week after application.

^yRegression parameters:

Dose response, D, upper limit; C, lower limit; B, slope of the line at I₅₀; I₅₀, rate required for 50% response between upper and lower limit.

Segmented linear, a₀, intercept of left segment; b₁, slope of the left segment; br₁, slope of the right segment; j, junction between left and right segment.

Inverse exponential, g, reduction in y from intercept to f; f, lower asymptote; h, slope of the line.

^xED₅₀, ED₈₀ and ED₉₅: Rate required to achieve 50, 80 and 95% giant ragweed control and soybean yield compared to the weed-free check, and 50, 80 and 95% reduction in giant ragweed density and shoot dry weight compared to the weedy check, respectively.

^wLocation: 1, Windsor; 2, Windsor; 3, Belle River; 4, LaSalle; 5, Amherstburg.

Table 4-3. Percent control of glyphosate-resistant giant ragweed at 1 and 2 WAA for various postemergence herbicides^z

Treatment	Rate (g ae/ai ha ⁻¹)	Control 1 WAA			Control 2 WAA	
		L1 and L4	L2 and L3	L5	L1, L4 and L5	L2 and L3
				(%)		
Weedy check		0 g	0 e	0 h	0 g	0 e
Weed-free check		100 a	100 a	100 a	100 a	100 a
Chlorimuron-ethyl ^{yw}	9	66 cde	63 b	44 g	60 e	68 bc
Cloransulam-methyl ^{xv}	17.5	78 bcd	66 b	50 efg	93 ab	90 a
Fomesafen ^u	240	80 bc	44 cd	86 b	66 de	27 d
Glyphosate	900	43 f	44 cd	43 g	45 f	50 c
Imazethapyr ^{xw}	100	55 ef	41 d	57 ef	73 cd	52 c
Imazethapyr + bentazon ^w	75 + 840	58 ef	52 bcd	48 fg	71 de	61 c
Glyphosate + chlorimuron-ethyl ^{yw}	900 + 9	64 cde	65 b	70 c	68 de	69 bc
Glyphosate + cloransulam-methyl ^{xv}	900 + 17.5	62 cdef	69 b	58 de	83 bc	83 ab
Glyphosate + fomesafen ^u	900 + 240	90 ab	61 bc	93 ab	86 b	50 c
Glyphosate + imazethapyr ^{xw}	900 + 100	60 def	62 b	67 cd	73 cd	68 bc
Glyphosate + imazethapyr + bentazon ^w	900 + 75 + 840	58 ef	60 bc	57 ef	66 de	66 bc

^zAbbreviations: L1, Windsor; L2, Windsor; L3, Belle River; L4, LaSalle; L5, Amherstburg; WAA, week after application.

^yIncluded non-ionic surfactant (0.2% vol/vol).

^xIncluded non-ionic surfactant (0.25% vol/vol.).

^wIncluded 28% UAN (2 L ha⁻¹).

^vIncluded 28% UAN (2.5% vol/vol.).

^uIncluded crop oil concentrate (0.5% vol/vol.).

^{a-h}Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P<0.05.

Table 4-4. Percent control of glyphosate-resistant giant ragweed at 4 and 8 WAA for various postemergence herbicides^z

Treatment	Rate (g ae/ai ha ⁻¹)	Control 4 WAA		Control 8 WAA	
		L1, L4 and L5	L2 and L3	L1, L4 and L5	L2 and L3
Weedy check		0 g	0 e	0 g	0 d
Weed-free check		100 a	100 a	100 a	100 a
Chlorimuron-ethyl ^{yw}	9	46 f	69 bc	42 ef	41 cd
Cloransulam-methyl ^{xv}	17.5	99 a	98 a	96 ab	93 ab
Fomesafen ^u	240	51 f	29 de	38 ef	11 d
Glyphosate	900	43 f	57 c	29 f	41 cd
Imazethapyr ^{xw}	100	78 cd	59 c	54 de	25 d
Imazethapyr + bentazon ^w	75 + 840	76 cde	53 cd	52 de	30 cd
Glyphosate + chlorimuron-ethyl ^{yw}	900 + 9	66 e	71 bc	44 ef	66 bc
Glyphosate + cloransulam-methyl ^{xv}	900 + 17.5	92 b	88 ab	81 bc	80 b
Glyphosate + fomesafen ^u	900 + 240	84 c	57 c	65 cd	32 cd
Glyphosate + imazethapyr ^{xw}	900 + 100	82 cd	69 bc	51 de	41 cd
Glyphosate + imazethapyr + bentazon ^w	900 + 75 + 840	74 de	69 bc	43 ef	35 cd

^zAbbreviations: L1, Windsor; L2, Windsor; L3, Belle River; L4, LaSalle; L5, Amherstburg; WAA, week after application.

^yIncluded non-ionic surfactant (0.2% vol/vol).

^xIncluded non-ionic surfactant (0.25% vol/vol.).

^wIncluded 28% UAN (2 L ha⁻¹).

^vIncluded 28% UAN (2.5% vol/vol.).

^uIncluded crop oil concentrate (0.5% vol/vol.).

^{a-g}Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P<0.05.

Table 4-5. Glyphosate-resistant giant ragweed density and shoot dry weight, and soybean yield for various postemergence herbicides^z

Treatment	Density Combined (g ae/ai ha ⁻¹)	Density Combined (no. m ⁻²)	Shoot dry weight Combined (g m ⁻²)	Soybean yield	
				L2, L3 and L5 (t ha ⁻¹)	L4
Weedy check		40 e	61.9 e	1.18 d	0.57 c
Weed-free check		0 a	0.0 a	3.73 a	2.10 a
Chlorimuron-ethyl ^{yw}	9	15 cd	20.7 cd	1.68 d	0.64 c
Cloransulam-methyl ^{xv}	17.5	1 a	0.3 b	3.29 ab	1.78 a
Fomesafen ^u	240	20 cde	32.3 d	1.34 d	0.50 c
Glyphosate	900	20 de	23.3 cd	1.53 d	0.59 c
Imazethapyr ^{xw}	100	18 cd	16.9 c	1.50 d	0.29 c
Imazethapyr + bentazon ^w	75 + 840	18 cd	20.1 cd	1.57 d	0.61 c
Glyphosate + chlorimuron-ethyl ^{yw}	900 + 9	18 cde	19.0 cd	1.86 cd	0.65 c
Glyphosate + cloransulam-methyl ^{xv}	900 + 17.5	4 b	3.1 b	2.53 bc	1.25 b
Glyphosate + fomesafen ^u	900 + 240	9 c	12.3 c	1.91 cd	0.58 c
Glyphosate + imazethapyr ^{xw}	900 + 100	18 cd	13.3 c	1.58 d	0.55 c
Glyphosate + imazethapyr + bentazon ^w	900 + 75 + 840	18 cd	13.7 c	1.54 d	0.39 c

^zAbbreviations: L2, Windsor; L3, Belle River; L4, LaSalle; L5, Amherstburg; WAA, week after application.

^yIncluded non-ionic surfactant (0.2% vol/vol).

^xIncluded non-ionic surfactant (0.25% vol/vol.).

^wIncluded 28% UAN (2 L ha⁻¹).

^vIncluded 28% UAN (2.5% vol/vol.).

^uIncluded crop oil concentrate (0.5% vol/vol.).

^{a-c}Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P<0.05.

5.0 Glyphosate-Resistant Giant Ragweed (*Ambrosia trifida* L.) Control in Dicamba-Tolerant Soybean (*Glycine max* L.)

5.1 Abstract

Glyphosate-resistant (GR) giant ragweed has been confirmed in Ontario, Canada. Giant ragweed is an extremely competitive weed and lack of control in soybean will lead to significant yield losses. Seed companies have developed new herbicide-resistant (HR) crop cultivars and hybrids which stack multiple herbicide-resistant traits. The objective of this research was to evaluate the efficacy of glyphosate and glyphosate plus dicamba tank mixes for the control of GR giant ragweed under Ontario environmental conditions in dicamba-tolerant soybean. Three field trials were established over a two year period (2010 and 2011) on farms near Windsor and Belle River, Ontario. Treatments included glyphosate (900 g a.e. ha⁻¹), dicamba (300 g a.e. ha⁻¹) and dicamba (600 g a.e. ha⁻¹) applied either preplant (PP), postemergence (POST), or sequentially in various combinations. Glyphosate applied PP, POST or sequentially provided 22 to 68, 40 to 47 and 59 to 95% control of GR giant ragweed and reduced shoot dry weight 26-80, 16-50 and 72-98%, respectively. Glyphosate plus dicamba applied PP followed by glyphosate plus dicamba applied POST consistently provided 100% control of GR giant ragweed. Dicamba-tolerant soybean yield correlated with GR giant ragweed control. This is the first report in Canada of weed control in dicamba-tolerant soybean, specifically for the control of GR giant ragweed. Results indicate that the use of dicamba in dicamba-tolerant soybean will provide an effective option for the control of GR giant ragweed in Ontario.

5.2 Introduction

The 1996 commercialization of GR soybean (*Glycine max* L.) revolutionized crop production (Feng et al. 2010). Growers were able to use glyphosate in crops and replace more expensive, selective herbicides that controlled a narrower weed spectrum (Green and Castle 2010). Since then, the adoption of GR crops has been rapid and is increasingly common in world agriculture (Feng et al. 2010). In the United States, 91% of the total soybean, and 68% of the total corn plantings were GR in 2009 (Reddy and Norsworthy 2010). Similar rates of adoption are evident in eastern Canada where the area planted with GR soybean cultivars reached 72% and the area planted with GR corn hybrids reached 90% in 2011 (Stratus, personal communication). Several benefits have driven the success of GR crops such as excellent weed control, excellent crop safety, simplicity of application, relatively low cost of weed control, reduced fuel costs and improved soil conservation (Feng et al. 2010; Nandula 2010).

Giant ragweed (*Ambrosia trifida* L.) is an annual weed that is native to North America and commonly found in regions of southern Canada as well as the midwestern and eastern portions of the U.S. (Abul-Fatih and Bazzaz 1980; Bassett and Crompton 1982; Hunt and Bazzaz 1980). It is a member of the Asteraceae family, and is well known for its allergenic pollen that is one of the main causes of hay fever (Bassett and Crompton 1982; Baysinger and Sims 1991). This species was previously known to occur in river valleys, meadows, roadsides, fence rows and drainage ditches and occasionally in low, cultivated flood plain fields (Alex 2011; Bassett and Crompton 1982; Johnson et al. 2007). More recently giant ragweed populations have appeared outside of their primary habitats in many fertile fields in southwestern Ontario, across the Corn Belt (Hartzler et al. 2002; Johnson et al. 2007) and increasingly in agronomic crop fields in the mid-South of the U.S. (Norsworthy et al. 2010; Steckel 2007).

The germination pattern of giant ragweed contributes to its prevalence in row crops. It is one of the earliest annual weeds to germinate in the spring which translates into an early competitive advantage over agronomic crops (Stoller and Wax 1973). Germination is unpredictable and populations emerge over an extended period beginning in March and continuing until late July. This has resulted in management challenges (Harrison et al. 2001; Johnson et al. 2007). For instance, early emerging plants are often too large at the time of the first glyphosate application while late germinating plants often emerge after the last glyphosate application.

Prior to widespread use of GR soybeans, some acetolactate synthase (ALS) inhibitors such as cloransulam-methyl, chlorimuron-ethyl and imazethapyr provided excellent control of giant ragweed (Baysinger and Sims 1992; Franey and Hart 1999; Taylor et al. 2002). By the mid 1990s, resistance to ALS inhibitors was already widespread and control became difficult (Johnson et al. 2007). After the introduction of GR soybeans, growers were able to effectively manage ALS-resistant giant ragweed in-crop with POST applications of glyphosate (Johnson et al. 2007; Stachler 2008; Taylor et al. 2002). However, many previously effective herbicides have failed to provide adequate giant ragweed control (Johnson et al. 2007).

Giant ragweed can be very competitive in soybeans resulting in large yield losses. After emergence, giant ragweed grows more rapidly than soybeans and can reach heights of up to six m. Giant ragweed interference resulted in greater than 90% soybean yield loss in field studies conducted in Ontario (unpublished data). Webster et al. (1994) reported a soybean yield reduction of up to 77% at a giant ragweed density of one plant per m⁻². Baysinger and Sims (1991) reported soybean yield loss of up to 92% at a density of 16 plants per 9 m of row when giant ragweed and soybeans emerged at the same time.

The wide spread adoption and repeated use of glyphosate has led to selection of weeds species that are naturally tolerant to glyphosate, late emerging weed species that emerge after the last application of glyphosate and weed biotypes that are resistant to glyphosate. GR giant ragweed was first reported in Ohio, USA in 2004 (Heap 2012; Stachler 2008). Since the initial documented case of GR giant ragweed, it has been confirmed in nine additional states in the U.S. Some populations of GR giant ragweed are also resistant to the ALS inhibiting herbicides (Heap 2012; Stachler 2008).

In 2008, a giant ragweed biotype near Windsor, Ontario, Canada was not controlled with glyphosate (Sikkema et al. 2009). Seed was collected, and greenhouse testing confirmed that the biotype from the Windsor population was resistant to glyphosate. This confirmed giant ragweed as the first weed species in Canada to evolve resistance to glyphosate. Since then, GR giant ragweed has been identified at 47 additional locations in southwestern Ontario and some populations have shown reduced sensitivity to the ALS inhibiting herbicide, cloransulam-methyl (Vink et al. 2011).

In response to the increased incidence of weed resistance to glyphosate and other herbicides, seed companies are now developing crop hybrids and cultivars with resistance to multiple herbicides such as glyphosate plus dicamba. Dicamba has been widely used for over 40 years, and is an effective herbicide for the control of most broadleaf weed species (Behrens et al. 2007). Dicamba and other dicamba-based herbicides are recommended for the control of giant ragweed in corn (OMAFRA 2011). These multiple HR crops will provide growers with new weed management tools, and if integrated with other weed management practices (effective residual herbicides, tillage) will help to preserve the utility of glyphosate and GR crops (Green and Castle 2010; Weller 2010). These multiple HR crops are not expected to be commercialized

until the middle of the decade. Limited information is available on herbicide efficacy in these new HR crops for the control of GR weed species. Therefore, the objective of this research was to evaluate the efficacy of dicamba for the control of GR giant ragweed in Ontario, in glyphosate and dicamba-tolerant soybean.

5.3 Materials and Methods

Three field experiments were conducted over a two-year period (2010 and 2011) on two Ontario farms with known GR giant ragweed infestations. In 2010, the trial was established at a site near Windsor, ON (N42 16.715°, W82 57.636°). In 2011, the trial was established at two sites; one near Windsor, ON and one near Belle River (N42 17.075, W82 45.110°), ON. The soil at Windsor was a sandy clay loam with 50% sand, 27% silt, 23% clay, 3.3 to 4.0% organic matter and pH of 6.5 to 7.3. The soil at Belle River was clay with 25% sand, 34% silt, 41% clay, 3.3% organic matter and pH of 6.8.

At Windsor, continuous reduced-tillage soybeans were grown for at least eight years prior to the establishment of this study. For most growing seasons, glyphosate alone was applied PP and POST at the recommended rate. The exceptions were in 2007 when glyphosate and cloransulam-methyl were applied PP followed by glyphosate POST, and in 2004 when s-metolachlor and metribuzin were applied preemergence (PRE) in identity preserved soybean. Prior to 2003, continuous identity preserved soybeans were grown and glyphosate alone was applied PP. In 2008, winter wheat and grass were planted in the autumn and the field was used as a buffer zone beside an airport runway for the 2009 growing season. Prior to giant ragweed emergence in the spring of 2010, glufosinate was applied as an over spray to control the existing stand of grass and wheat as well as mouse-eared chickweed (*Cerastium fontanum* L.). However,

control of the wheat and grass was not adequate and quizalofop p-ethyl was applied prior to soybean planting in the spring. In the autumn of 2010, the site was roto-tilled several times after harvest and glyphosate was applied to control mouse-eared chickweed seedlings. Cropping history at Belle River consisted of a corn, soybean, wheat rotation and glyphosate was applied each year since 2006. Site preparation at Belle River included disking in the autumn followed by no-tillage management in the spring. Giant ragweed was the predominant weed at Belle River, therefore an overspray to control other emerged weeds was not required.

The experiments were arranged in a randomized complete block design with three replications. Dicamba-tolerant soybeans (MON 87708, Monsanto Canada Inc., Guelph, Ontario, Canada, N1G 0B4) were seeded at the rate of approximately 556,000 seeds ha⁻¹ on May 27, 2010 and 444,789 seeds ha⁻¹ on June 7, 2011. Plots consisted of six rows of soybeans spaced 0.38 m apart that were six m long. Herbicides used included glyphosate (900 g ae ha⁻¹, Roundup WeatherMax®, 540 g a.e. L⁻¹, Monsanto Canada Inc., 900 One Research Road, Winnipeg, Manitoba, Canada, R3T 6E3), dicamba (300 g a.e. ha⁻¹, Banvel® II, 480 g a.e. L⁻¹, BASF Canada Inc., 100 Milverton Drive, Mississauga, Ontario, Canada, L5R 4H1) and dicamba (600 g a.e. ha⁻¹) applied either PP only, POST only, or sequentially (PP followed by POST) in various combinations. The rates selected for dicamba are the lowest and highest label rate registered in corn in eastern Canada. The rate selected for glyphosate is the recommended label rate for a single application registered in soybean in eastern Canada. Each trial included a weedy and weed-free check. Weed-free check plots were maintained with an application of glyphosate (1800 g a.e. ha⁻¹) plus dicamba (600 g a.e. ha⁻¹) applied PP followed by hand hoeing as required. Herbicide treatments were applied with a CO₂-pressurized backpack sprayer equipped with ULD 120-02 flat fan nozzles (Hypro, New Brighton, MN) calibrated to deliver 200 L ha⁻¹ of water at

210 kPa. Herbicide applications were made with a 1.5 meter boom with four nozzles spaced 50 cm apart over the center of the plot. PP treatments were applied 4 to 17 days before planting soybeans when the giant ragweed was up to 13 cm in height. POST treatments were applied when the soybeans reached the 1-2 trifoliolate vegetative growth stage. Giant ragweed height in the weedy check ranged from 10 to 92 cm and the density ranged from 5 to 144 plants m⁻² at the time of the POST application.

Visual estimate of soybean injury at 1, 2, and 4 weeks after the POST treatment application (WAA) and giant ragweed control at 1, 2, 4, and 8 WAA were rated on a scale of 0 to 100%, where a rating of 0 was defined as no injury/control and 100 was defined as plant death/total control. At 4 WAA, giant ragweed density and biomass (shoot dry weight) in each plot was determined by counting giant ragweed plants and by cutting the plants at the soil surface from two 0.25 m² quadrats. Plants were bagged by plot, dried at 60°C to constant weight, and the dry weights were recorded. At crop maturity, the soybeans from the center four rows were harvested with a small-plot combine and the weight and moisture was recorded. Soybean yields were adjusted to 13% moisture.

All data were subjected to ANOVA. Data were analyzed using the MIXED procedure of SAS (Ver. 9.1, SAS Institute Inc., Cary, NC). Variances were separated into the random effects of location (year and location), replication (within location), location by treatment interaction, and the fixed effect of herbicide treatment. Significance of random effects and their interaction with fixed effects was tested using the Z-test of the variance estimate, while the significance of fixed effects was tested using the F-test. For all variables there was a significant location by treatment interaction and the pooling of data was restricted to combinations of certain locations and is presented accordingly. Error assumptions of the variance analysis (random, independent

and homogenous) were confirmed by examining residual plots. Data were tested for normality using the Shapiro-Wilk statistic that was generated by the UNIVARIATE procedure in SAS. When necessary, a transformation (arcsine square root, square root) of the data was applied to meet the assumption of normality, and the transformation which generated the highest Shapiro-Wilk statistic was chosen. After interpretation, treatment means were transformed back to the original scale for presentation. Means were separated using Fisher's protected LSD at $P < 0.05$.

5.4 Results and Discussion

For control ratings, shoot dry weight and soybean yield, data from Windsor in 2010 and 2011 could be combined, and data from Belle River was analyzed separately. For giant ragweed density, data from Windsor 2010 and Belle River 2011 could be combined, and Windsor 2011 was analyzed separately. There was no soybean injury from the herbicides evaluated (data not shown).

Glyphosate plus dicamba applied PP provided 87 to 96% control of GR giant ragweed at Windsor compared to 98 to 100% control at Belle River 1, 2, 4 and 8 WAA (Table 5-1). Control with glyphosate applied PP was variable, and provided only 18, 15, 22 and 15% control at Windsor compared to 75, 80, 68 and 68% control at Belle River 1, 2, 4 and 8 WAA, respectively. Control with glyphosate plus dicamba applied POST was more variable than the PP treatments, and generally increased with the higher dose of dicamba ($600 \text{ g a.e. ha}^{-1}$). At Windsor, giant ragweed in the weedy check was up to 92 cm tall with a density of $144 \text{ plants m}^{-2}$ at the time of application. Control ranged from 46 to 63, 70 to 82, 76 to 88 and 78 to 95% at 1, 2, 4 and 8 WAA, respectively. This suggests that large giant ragweed at a high density may be difficult to control with dicamba at the lower rate ($300 \text{ g a.e. ha}^{-1}$). At Belle River, control ranged from 65 to

75, 86 to 89, 93 to 98 and 98 to 100% at 1, 2, 4 and 8 WAA, respectively. Glyphosate applied POST provided only 24, 37, 40 and 46% control at Windsor and 43, 45, 47 and 40% control at Belle River 1, 2, 4 and 8 WAA, respectively.

The sequential glyphosate treatment (glyphosate applied PP followed by glyphosate applied POST) is commonly used for weed control in Ontario soybean production and provided variable control of GR giant ragweed. At Windsor, control ranged from 54 to 67%, compared to Belle River where control ranged from 88 to 98%. The difference in control may be due to differences in the proportion of giant ragweed that are resistant to glyphosate at the two sites. In previous research conducted in Ontario, the proportion of GR biotypes in a surviving population was as high as 75% at Windsor, compared to only 14% at Belle River (unpublished data). The addition of dicamba to the sequential glyphosate application significantly improved control of GR giant ragweed, particularly at Windsor. Glyphosate applied PP followed by glyphosate plus dicamba applied POST at the low or high rate, provided 74 to 80, 86 to 88, 89 to 96 and 91 to 99% control at Windsor compared to 94 to 95, 99 to 98, 100 to 100 and 100 to 100% control at Belle River 1, 2, 4 and 8 WAA, respectively (Table 5-1). Glyphosate plus dicamba applied PP provided better GR giant ragweed control than when applied POST, especially at the earliest assessments (Table 5-1). Control ranged from 98 to 100% across all environments and rating dates, and can be attributed to smaller giant ragweed at the time of application (2-13 cm) compared to giant ragweed that was up to 92 cm tall at the POST application timing. Glyphosate plus dicamba applied PP followed by glyphosate plus dicamba applied POST provided control equivalent to the weed-free check regardless of the dicamba rate, across all environments and rating dates (Table 5-1).

Glyphosate applied PP resulted in giant ragweed density equivalent to the weedy check. In contrast, the addition of dicamba at the PP timing reduced giant ragweed density equivalent to the weed-free check (Table 5-2). Glyphosate, glyphosate plus dicamba (300), and glyphosate plus dicamba (600) applied POST reduced giant ragweed density up to 25, 64 and 92%, respectively (Table 5-2). Sequential applications of glyphosate resulted in giant ragweed density equivalent to the weedy check. Glyphosate applied PP followed by glyphosate plus dicamba applied POST reduced giant ragweed density 0-100% (Table 5-2). Glyphosate plus dicamba applied PP followed by glyphosate applied POST reduced giant ragweed density more consistently by 94-100% (Table 5-2). This implies that a high density of GR giant ragweed needs to be controlled with early applications of glyphosate plus dicamba rather than delaying application until the POST timing. Glyphosate plus dicamba applied PP followed by glyphosate plus dicamba applied POST reduced giant ragweed density equivalent to the weed-free check (Table 5-2).

Reduction in GR giant ragweed shoot dry weight correlated with the level of control. Glyphosate, glyphosate plus dicamba (300) and glyphosate plus dicamba (600) applied PP reduced giant ragweed shoot dry weight 26 to 80, 95 to 100 and 97 to 100%, respectively compared to the weedy check at Windsor and Belle River. Shoot dry weight reduction was poorer with POST application where glyphosate, glyphosate plus dicamba (300), and glyphosate plus dicamba (600) reduced giant ragweed shoot dry weight 16 to 50, 62 to 90 and 91 to 93% at Windsor and Belle River, respectively (Table 5-2). PP applications of glyphosate plus dicamba reduced giant ragweed shoot dry weight equivalent to the weed-free check, whereas POST applications were more variable. This once again suggests that an early application of glyphosate plus dicamba reduces giant ragweed shoot dry weight more effectively than POST applications.

Sequential applications of glyphosate reduced giant ragweed shoot dry weight 72 to 98%, whereas glyphosate applied PP followed by glyphosate plus dicamba applied POST reduced giant ragweed shoot dry weight equivalent to the weed-free check by 91 to 100% (Table 5-2). Glyphosate plus dicamba applied PP followed by glyphosate applied POST reduced giant ragweed shoot dry weight by 99 to 100% (Table 5-2). Glyphosate plus dicamba applied PP followed by glyphosate plus dicamba applied POST reduced giant ragweed shoot dry weight 100% across all environments (Table 5-2).

Untreated GR giant ragweed interference in soybean reduced soybean yield 92 and 50% at the Windsor and Bell River sites, respectively. All of the herbicide treatments evaluated increased soybean yield compared with the weedy check (Table 5-2). However, yield losses up to 85, 75 and 57% were observed compared to the weed-free check when glyphosate was applied PP, POST and sequentially, respectively. Weed control improvement with the addition of dicamba increased soybean yield. Glyphosate plus dicamba applied PP only and glyphosate plus dicamba applied POST only resulted in yields equivalent to the weed-free check (Table 5-2). Glyphosate applied PP followed by glyphosate plus dicamba applied POST or glyphosate plus dicamba applied PP followed by glyphosate applied POST increased soybean yield relative to the weedy check. Glyphosate plus dicamba applied PP followed by glyphosate plus dicamba applied POST resulted in yields equivalent to the weed-free check.

The results of this research are consistent with other studies in the literature. Stachler (2008) reported only 32% control of GR giant ragweed when glyphosate was applied at 840 g a.e. ha⁻¹. Johnson et al. (2007) reported 39% control with glyphosate applied POST at 1680 g a.e. ha⁻¹. In another study, Norsworthy et al. (2010) reported <50% control of GR giant ragweed 4 WAA in a greenhouse study, when glyphosate was applied at 870 g a.e. ha⁻¹ to four- to six- node

plants. In other studies, dicamba-based herbicides provided adequate control of giant ragweed. Soltani et al. (2011) reported up to 90% control with dicamba (600 g a.e. ha⁻¹) applied POST, 8 WAA. In the same study, dicamba/atrazine applied POST provided 82-94% control. This is consistent with Ferrell and Witt (2002) who reported up to 98% giant ragweed control with dimethenamid plus atrazine applied PRE followed by dicamba (100 g a.e. ha⁻¹) applied POST. In other studies, atrazine applied PRE provided limited control of giant ragweed (Soltani 2011; Woodyard et al. 2009) and dimethenamid is primarily used for annual grass control with limited activity on broadleaf weed species such as giant ragweed. Therefore, dicamba has traditionally been found to provide excellent control of giant ragweed (Johnston and Webb 1985). Other herbicides with a mode of action similar to that of dicamba such as 2,4-D, 2,4,5-T, 2,4-DB, MCPA and mecoprop have been shown to adequately control giant ragweed (Bassett and Crompton 1982; Robinson and Johnson 2010).

When a single herbicide is used as the basis for weed management, selection intensity will lead to the evolution of herbicide-resistant weeds (Shaner et al. 2011). Therefore, growers that adopt dicamba-tolerant soybean should not apply dicamba as a single weed management solution (Seifert-Higgins 2010). Synthetic auxin herbicides have been used for over six decades and resistance has been documented in 29 species, but none in *Ambrosia* (Heap, 2012). This suggests that the evolution of dicamba resistance in giant ragweed is low; however repeated use in consecutive years may lead to the selection of new resistant biotypes (Egan et al. 2011; Wright et al. 2011). The use of glyphosate and dicamba in dicamba-tolerant soybean must be complemented with an integrated weed management system that includes tillage or other mechanical/cultural weed control, herbicide rotations and sequences, and residual herbicide

treatments that promote sustainable long term weed control (Duke and Powles 2009; Wright et al. 2011).

In summary, glyphosate plus dicamba provided excellent control of GR giant ragweed in dicamba-tolerant soybean. Glyphosate applied alone provided unacceptable control. GR giant ragweed control was most consistent when glyphosate plus dicamba was applied PP followed by glyphosate plus dicamba applied POST. In some situations growers may want to apply glyphosate plus dicamba either PP only or POST only. At a site with heavy GR giant ragweed pressure, glyphosate plus dicamba applied PP will minimize early season competition with soybean and based on these results, only one application of dicamba can provide excellent control of GR giant ragweed. Prior to the introduction of GR crops, the evolution of GR weeds were limited because glyphosate was used for non-selective burndown of weeds before crop planting (Duke and Powles 2009). Therefore, to minimize selection pressure for the potential evolution of dicamba-resistant giant ragweed, dicamba could be applied PP only as a non-selective burndown. If dicamba is not applied at the PP timing, glyphosate plus dicamba applied POST at the highest label rate will provide better control of large giant ragweed. However, delaying control of GR giant ragweed until the POST application timing resulted in soybean yield loss of up to 29%. The results of this research suggest that the use of dicamba in dicamba-tolerant soybean will provide growers with an additional weed management tool for the control of GR giant ragweed in Ontario.

Table 5-1. Control of glyphosate-resistant giant ragweed at Windsor (2010/2011) and Belle River (2011) for various treatment combinations in dicamba-tolerant soybean^z

Treatment	Rate (g ae ha ⁻¹)	Timing	1 WAA		2 WAA		4 WAA		8 WAA	
			2010/2011	2011	2010/2011	2011	2010/2011	2011	2010/2011	2011
Weedy check			0 e	0 e	0 h	0 e	0 g	0 e	0 g	0 e
Weed-free check			100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
PP only treatments										
Glyphosate	900	PP	18 d	75 bc	15 g	80 c	22 f	68 c	15 f	68 c
Glyphosate + dicamba	900 + 300	PP	96 a	98 a	96 bc	100 a	90 bc	100 a	87 b	100 a
Glyphosate + dicamba	900 + 600	PP	95 a	100 a	96 abc	100 a	93 abc	100 a	93 abc	100 a
POST only treatments										
Glyphosate	900	POST	24 d	43 d	37 f	45 d	40 ef	47 d	46 e	40 d
Glyphosate + dicamba	900 + 300	POST	46 c	75 bc	70 e	86 bc	76 cd	93 b	78 cd	98 b
Glyphosate + dicamba	900 + 600	POST	63 bc	65 c	82 de	89 abc	88 bc	98 ab	95 abc	100 a
Sequential treatments										
Glyphosate fb	900	PP								
glyphosate	900	POST	60 bc	88 ab	67 e	93 ab	59 de	95 b	54 de	98 b
Glyphosate fb	900	PP								
glyphosate + dicamba	900 + 300	POST	74 b	94 a	86 cd	99 a	89 ab	100 a	91 abc	100 a
Glyphosate fb	900	PP								
glyphosate + dicamba	900 + 600	POST	80 b	95 a	88 cd	98 ab	96 ab	100 a	99 ab	100 a
Glyphosate + dicamba fb	900 + 300	PP								
glyphosate	900	POST	98 a	99 a	99 ab	100 a	99 ab	100 a	98 ab	100 a
Glyphosate + dicamba fb	900 + 600	PP								
glyphosate	900	POST	99 a	100 a	100 a	100 a	100 a	100 a	100 ab	100 a
Glyphosate + dicamba fb	900 + 300	PP								
glyphosate + dicamba	900 + 600	POST	99 a	100 a	99 ab	100 a	100 a	100 a	100 a	100 a
Glyphosate + dicamba fb	900 + 600	PP								
glyphosate + dicamba	900 + 300	POST	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Glyphosate + dicamba fb	900 + 300	PP								
glyphosate + dicamba	900 + 300	POST	99 a	100 a	100 ab	100 a	100 a	100 a	100 a	100 a
Glyphosate + dicamba fb	900 + 600	PP								
glyphosate + dicamba	900 + 600	POST	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a

^zAbbreviations: PP, preplant; fb, followed by; POST, postemergence; WAA, weeks after application.

^{a-h}Means followed by the same letter (a-h) within a column are not significantly different according to Fisher's Protected LSD at P < 0.05.

Table 5-2. Glyphosate-resistant giant ragweed density at Windsor/Belle River (2010/2011) and Windsor (2011), and shoot dry weight and soybean yield at Windsor (2010 and 2011) and Belle River (2011) for various treatment combinations in dicamba-tolerant soybean^z

Treatment	Dose (g ae ha ⁻¹)	Timing	Density		Shoot dry weight		Soybean yield	
			2010/2011 (no. m ⁻²)	2011	2010/2011 (g m ⁻²)	2011	2010/2011 (t ha ⁻¹)	2011
Weedy check			11.1 d	74.7 abc	646.8 e	145.4 d	0.23 e	1.75 f
Weed-free check			0.0 a	0.0 a	0.0 a	0.0 a	2.82 abc	3.52 abcd
PP only treatments								
Glyphosate	900	PP	8.9 d	178.0 c	477.4 de	29.7 c	0.42 e	3.21 d
Glyphosate + dicamba	900 + 300	PP	0.0 ab	9.3 ab	31.7 a	0.0 ab	2.50 bc	3.65 ab
Glyphosate + dicamba	900 + 600	PP	0.3 ab	6.0 ab	21.0 a	0.0 ab	2.98 abc	3.53 abcd
POST only treatments								
Glyphosate	900	POST	8.3 cd	128.0 c	325.6 cd	122.4 d	0.70 e	2.70 e
Glyphosate + dicamba	900 + 300	POST	4.0 c	96.0 abc	247.2 cd	14.0 bc	1.99 cd	3.34 bcd
Glyphosate + dicamba	900 + 600	POST	0.9 b	128.7 c	43.4 ab	13.1 bc	2.90 abc	3.28 cd
Sequential treatments								
Glyphosate fb	900	PP	5.5 cd	146.0 c	180.9 bc	3.1 abc	1.20 de	3.47 abcd
glyphosate	900	POST						
Glyphosate fb	900	PP	0.3 ab	102.0 bc	55.3 ab	0.0 ab	2.72 abc	3.52 abcd
glyphosate + dicamba	900 + 300	POST						
Glyphosate fb	900	PP	0.0 ab	123.3 c	27.2 a	0.0 ab	3.37 ab	3.63 ab
glyphosate + dicamba	900 + 600	POST						
Glyphosate + dicamba fb	900 + 300	PP	0.0 ab	4.7 ab	9.1 a	0.0 ab	3.27 abc	3.41 abcd
glyphosate	900	POST						
Glyphosate + dicamba fb	900 + 600	PP	0.0 ab	0.0 a	0.0 a	0.0 ab	3.86 a	3.75 a
glyphosate	900	POST						
Glyphosate + dicamba fb	900 + 300	PP	0.0 ab	0.0 a	0.0 a	0.0 ab	3.42 ab	3.68 ab
glyphosate + dicamba	900 + 600	POST						
Glyphosate + dicamba fb	900 + 600	PP	0.0 ab	0.0 a	0.0 a	0.0 ab	3.41 ab	3.59 abc
glyphosate + dicamba	900 + 300	POST						
Glyphosate + dicamba fb	900 + 300	PP	0.0 ab	0.0 a	0.0 a	0.0 ab	3.49 ab	3.44 abcd
glyphosate + dicamba	900 + 300	POST						
Glyphosate + dicamba fb	900 + 600	PP	0.0 ab	0.0 a	0.0 a	0.0 ab	3.54 ab	3.56 abcd
glyphosate + dicamba	900 + 600	POST						

^z Abbreviations: PP, preplant; fb, followed by; POST, postemergence.

^{a-f} Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P < 0.05.

6.0 General Discussion

6.1 Contributions

This is the first research on a glyphosate-resistant (GR) weed in Canada. A survey was conducted using non-random site selection methods, and GR giant ragweed was identified at 47 locations in addition to the first confirmed location at the Windsor airport. The distribution of GR giant ragweed was greater than originally thought with resistant biotypes confirmed in three different counties; Essex and Pelee Island, Chatham-Kent, and Lambton. The wide spread distribution and diversity among farms with resistance suggests that there were independent founding populations of GR giant ragweed. On the other hand, several resistant locations were identified in concentrated groups which suggest that both pollen flow and physical seed movement may contribute to the spread of GR giant ragweed. Based on growth room observations from the survey, giant ragweed biotypes displayed at least two different responses after application with glyphosate; rapid necrosis and non-rapid necrosis which suggests more than one mechanism of resistance. The survey has provided a foundation for future weed resistance surveys in Ontario, and has also created an overall awareness of the importance of herbicide resistance management.

Results from the field research determined that some GR giant ragweed biotypes have evolved a high level of resistance, but effective control options in soybean are available. In general, glyphosate would need to be applied in very high doses to provide effective control. This would not be environmentally sound, economical, or legal for Ontario farmers. Field control results showed that glyphosate plus 2, 4-D ester applied PP to soybean and POST to giant ragweed provided the most consistent control in the enhanced burndown study. Glyphosate plus

either saflufenacil or cloransulam-methyl were also effective options, but results were more variable compared to 2, 4-D ester. Based on the results of this research, farmers have changed their weed management tactics and have controlled GR giant ragweed with PP application of glyphosate plus 2, 4-D ester. This research has also contributed to the submission of a minor use registration for the application of glyphosate plus 2, 4-D ester applied PP to soybean. For full season control, glyphosate plus linuron applied PP to soybean provided the most consistent control. Glyphosate plus cloransulam-methyl applied at a higher rate for residual control was also effective but once again, the control was more variable. In some cases, cloransulam-methyl applied alone was more effective than the tank mix of glyphosate plus cloransulam-methyl. These results suggest that glyphosate may antagonize cloransulam-methyl due to rapid necrosis of the mature leaf tissue resulting in reduced uptake and translocation of cloransulam-methyl.

The field control studies have also identified herbicides that should not be used for GR giant ragweed control. Carfentrazone, chlorimuron-ethyl, glufosinate, clomazone, flumetsulam, imazethapyr and metribuzin all provided variable control. This is valuable information for farmers because poor control of GR giant ragweed can lead to significant yield losses in soybean.

Effective control of GR giant ragweed was achieved with dicamba in dicamba-tolerant soybean. Dicamba-tolerant soybean is not expected to be commercially launched until 2014 and this is the first research in Canada at a public institution to report on this new herbicide “tolerant” crop. Based on the results from three confined locations, glyphosate plus dicamba tank mixes applied either PP only or sequentially provided excellent control of GR giant ragweed. This research has shown that dicamba-tolerant soybean will be a valuable tool in an overall integrated weed management system for the control of GR giant ragweed.

6.2 Limitations

Giant ragweed seed collected from the various fields during the survey were grown in a growth room. Conditions in the growth room were much different than in a typical field setting in Ontario. Lighting in the growth room did not limit giant ragweed growth; however light conditions in a growth room are much less than natural sunlight that giant ragweed is exposed to in the field. Furthermore, giant ragweed in the growth room did not have to respond to variable conditions such as temperature and water availability. Giant ragweed plants in the growth room were also not influenced by shading from other plants such as a competing crop or other weed species.

Glyphosate was applied at 1800 g a.e. ha⁻¹ which is two times the recommended field labeled rate in Ontario. Herbicide resistance testing is usually based on a known discriminating dose for the weed species being examined. Researchers are divided on whether glyphosate efficacy is increased or decreased when applied under growth room conditions. For this study, it was not practical to determine a discriminating dose for all 102 seed samples collected. Therefore, the 2X rate of glyphosate was used to minimize the chance of a false positive. Previous research has reported higher giant ragweed mortality after application with glyphosate when plants were grown in non-sterile soil. For this research, giant ragweed plants were grown in potting mix that was presumably sterile and therefore the “frequency” of resistance reported could have been over estimated. On the other hand, the 2X rate of glyphosate caused mortality in some biotypes that were known resistant and this could underestimate the frequency of resistance.

Based on previous observations, giant ragweed may have a prolonged emergence period from March to July. Giant ragweed emerges much earlier in the spring compared to soybean

planting. For the POST herbicides experiment, the objective was to plant soybeans simultaneously with giant ragweed emergence. Glufosinate was applied as an over spray to control the early flush of giant ragweed and it was anticipated that a second flush would emerge simultaneously with soybean planting. Unfortunately, a second flush did not occur and the POST experiments were not evaluated in the first year of this study. Results for the POST experiment over two years would have provide more confidence in the data because there would have been a greater number of replications, and more diversity in locations used. For the POST trials in 2011, the herbicides used that would normally be applied POST in soybean were applied PP to the soybeans and POST to the giant ragweed. Therefore, giant ragweed was not influenced by a competing soybean crop at the time of application and may have responded differently to the herbicides.

For the enhanced burndown experiment, data collection was completed by 4 WAA. After data collection, an over spray of glyphosate plus fomesafen was applied to control any late emerging weed species and to better mimic a typical weed management program used by Ontario soybean producers. Glyphosate plus fomesafen suppressed giant ragweed in treatments that provided poor control. Therefore, treatments that originally provided poor control may have resulted in soybean yields that were higher than anticipated.

The results of this field research are specific to giant ragweed control. Herbicides that provided excellent control of giant ragweed may not control other weed species growing in the same field. Similarly, herbicides that provided poor control of giant ragweed may provide excellent control of other weed species. This research identifies options for GR giant ragweed control, but farmers should implement an integrated weed management program that includes

other methods of weed control in order to manage additional weed species that are specific to any given field.

6.3 Future Research

This is the first research on a GR weed in Canada. Glyphosate resistance is a new problem for Canadian growers, and this provides an opportunity for future research to build on the base line information from these studies. Studies should focus on further development of an integrated weed management strategy for the control of GR giant ragweed plus minimizing the selection intensity for additional GR weeds. Future studies should include new herbicide combinations that became available while this research was in progress. Sequential applications of PP/PRE herbicides followed by POST herbicides should be evaluated at locations with GR giant ragweed populations that emerge over a prolonged period. Future research could evaluate the effect of crop rotation, tillage and cover crops in combination with effective herbicides.

More research is needed to expand on some of the treatments evaluated in this study. The most effective preplant burndown treatments were 2, 4-D ester, saflufenacil and linuron. Dose response experiments with these herbicides would identify the biologically effective rate for the control of GR giant ragweed while minimizing the chance of soybean injury. Dicamba was also highly effective and shares a similar mode of action with 2, 4-D ester. Future research could evaluate which herbicide is more efficacious for long term control of GR giant ragweed. Cloransulam-methyl was also an effective herbicide but preliminary observations suggest that some giant ragweed biotypes have evolved multiple resistance. Future research should confirm whether giant ragweed has evolved multiple resistance to the group 2 and 9 herbicides in Ontario.

Results from these studies have identified new areas of interest in GR giant ragweed research. Several locations with GR giant ragweed were identified, but it is unclear whether glyphosate resistance is spreading or evolving independently at each location. Seedling emergence was unpredictable and may be related to soil type. Giant ragweed populations growing in sandy soils tended to emerge over a prolonged period whereas populations growing in clay soil tended to emerge in a concentrated group early in the spring. Seedling emergence in relation to soil type could be an important part of future research because it could help develop more specific management recommendations. Most GR giant ragweed responds to glyphosate with rapid necrosis of the mature leaf tissue. The unique phenotypic response presumably reduces the translocation of glyphosate, but further research is needed to explore this hypothesis. Research should also examine the mechanism of resistance in biotypes that do not respond with rapid necrosis.

Results from this research are specific to control in soybean. Previous research in Ontario has evaluated giant ragweed control in corn, but not at locations with GR biotypes. Most resistant locations were identified in Essex county where soybean and wheat are the primary field crops grown. Therefore, future research should evaluate the efficacy of herbicides commonly used for weed control in both wheat and corn in Ontario.

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Zelaya, I. A., Owen, M. D. K. and VanGessel, M. J. 2007. Transfer of glyphosate resistance: Evidence of hybridization in *Conyza* (Asteraceae). *Am. J. Bot.* **94**: 660-673.

8.0 Appendix 1: Dose Response Curves for Glyphosate-Resistant Giant Ragweed

Control 4 WAA

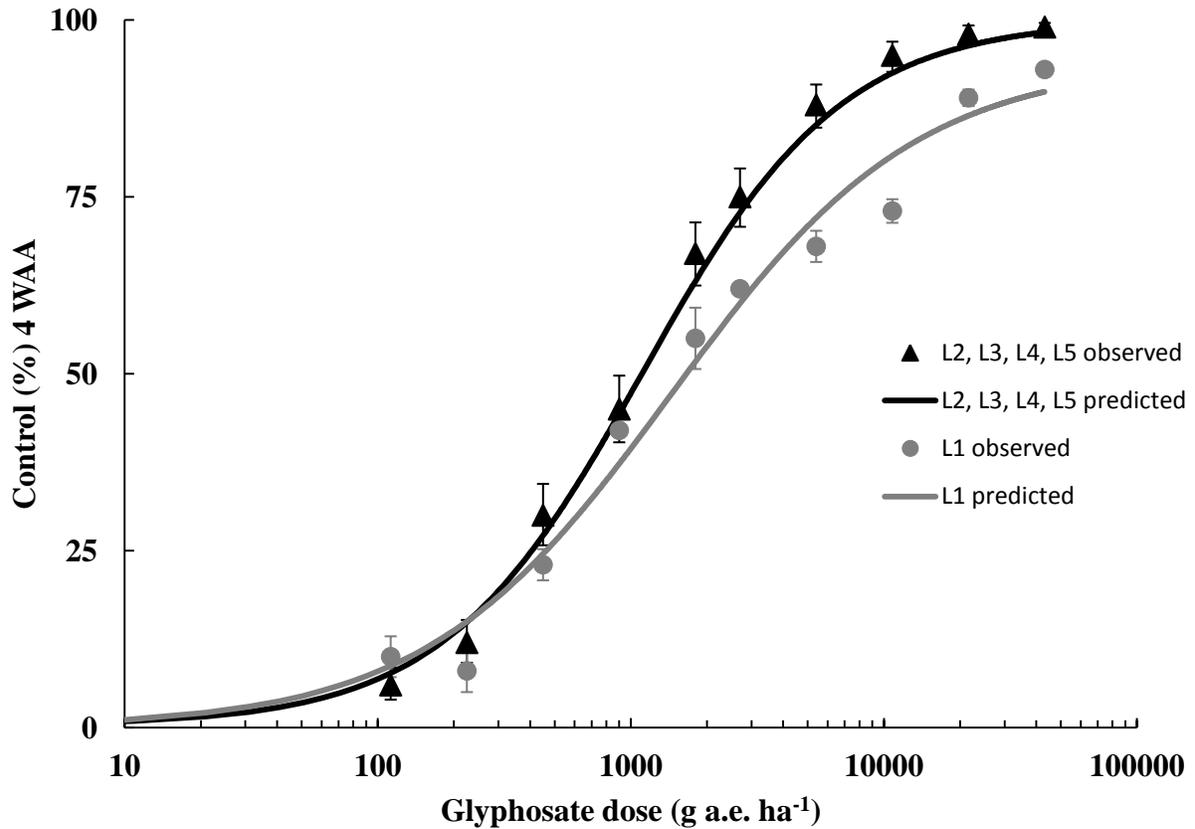


Figure 8-1. Effect of increasing glyphosate dose on glyphosate-resistant giant ragweed control at Windsor (L1) and Windsor (L2), Belle River (L3), LaSalle (L4) and Amherstburg (L5) combined for experiments conducted during 2011.

9.0 Appendix 2: SAS Code to Analyze Giant Ragweed Control Data

```
libname Joemsc "G:\MSc\statistics";
*burndown plus residual data;
*Env: 1-8 Runway (2010) = 1, Belleriver (2010) = 2, Windsor (2010) = 3, Runway (2011) = 4, Belle
River (2011) = 5, Windsor (2011) = 6, LaSalle (2011) = 7, Triangle (2011) = 8;
*Trt: 1-10, ;
*wc7, wc14, wc28, wc56, den, drwt, yieldton, yieldbu;

data first;
set Joemsc.burndownplusresidual;
title1 'burndown plus residual data';

*for control;
*if trt = 1 then delete;

*for den and drwt;
*if trt=2 then delete;

*if env = 1 then delete;
*if env = 2 then delete;
*if env = 3 then delete;
*if env = 4 then delete;
*if env = 5 then delete;
*if env = 6 then delete;
*if env = 7 then delete;
*if env = 8 then delete;

*title2 'wc28';
*analvar = wc28;

title2 'wc28 (log transformation)';
analvar = log(wc28+1);

*title2 'wc28 (squareroot transformation)';
*analvar = sqrt(wc28+0.5);

**Use following adjustment for arcsine square root trans, only for percentage data;
title2 'wc28(arcsine squareroot transformation)';
analvar1 = wc28;
if analvar1 = 100 then analvar1=100-0.05;
if analvar1 = 0 then analvar1=0+0.05;
analvar2 = analvar1/100;
analvar = arsin(sqrt(analvar2));
run;
proc sort data = first;
by env trt rep;
run;
```

```

**ANOVA
proc mixed covtest data = first;
classes env trt rep;
model analvar = trt /DDFM = satterth outp = second;
random env rep(env) trt*env;
*random rep;
parms/nobound;
lsmeans trt/pdiff;
run;

** Residual analysis;
proc plot;
plot resid*pred resid*trt resid*rep resid*env analvar*trt /vref = 0;
run;

proc univariate normal;
var resid;
run;

proc rank normal = blom out = two;
var resid;
ranks zvar;
run;

proc plot;
plot resid*zvar='*';
run;

proc sort;
*by trt;
*by env;
run;

proc summary mean stderr;
*by trt;
*by env;
var wc28 ;
output mean = mwc28 stderr = sewc28 ;
run;

proc print;
var mwc28 sewc28 ;
*by trt;
*by env;
run;

```

10.0 Appendix 3: SAS Code for Regression Analysis

```
libname Joemsc "G:\MSc\statistics";
*Biologically effective rate rate data;
*Env: 5 (Runway 2011 = 4, Belle River 2011 = 5, Windsor 2011 = 6, LaSalle 2011 = 7, Triangle 2011 =
8;
*Trt: 1-12;
*wc7, wc14, wc28, wc56, den, drwt, yieldton, yieldbu;

data first;
set Joemsc.biologicallyeffectiverate;
title1 'Biologically effective rate';

*for control;
*if trt = 1 then delete;

*no rate for weed free check;
*if trt = 2 then delete;

*for den and drwt;
*if trt = 2 then delete;

*if env = 3 then delete;
*if env = 4 then delete;
*if env = 5 then delete;
*if env = 6 then delete;
*if env = 7 then delete;
*if env = 8 then delete;

title2 'wc28';
analvar = wc28;

*title2 'wc28 (log transformation)';
*analvar = log(wc28+1);

*title2 'wc28 (squareroot transformation)';
*analvar = sqrt(wc28+0.5);

**Use following adjustment for arcsine square root trans, only for percentage data;
title2 'wc28(arcsine squareroot transformation)';
analvar1 = wc28;
if analvar1 = 100 then analvar1=100-0.05;
if analvar1 = 0 then analvar1=0+0.05;
analvar2 = analvar1/100;
analvar = arsin(sqrt(analvar2));
indvar = log(rate+1);
run;
```

```

proc sort data = first;
by rate env rep;
run;

**ANOVA to determine which locations could be combined;
proc mixed covtest data = first;
classes env rate rep;
model analvar = rate /DDFM = satterth outp = second;
random env rep(env) rate*env;
*random rep;
parms/nobound;
lsmeans rate/pdiff;
run;

**dose response for control ratings, pyield data;
/*
proc nlin;
bounds c>= 0;
bounds d<= 100;
parameters
d = 100
c = 0
i50 = 1200
b = 2;
if rate = 0 then predict = c;
else predict = c+(d-c)/(1 + exp(-b*(indvar-log(i50))));
model analvar = predict;
run;
*/

**dose response for descending curve - pdensity and pdry weight;
/*
proc nlin;
bounds c>=0;
parameters
d = 100
c = 0
i50 = 1800
b = 2;
if rate = 0 then predict = d;
else predict = c+(d-c)/(1+exp(b*(indvar-log(i50))));
model analvar = predict;
run;
*/

```

```

**inverse exponential - pdensity and pdry weight;
/*
proc nlin;
bounds f>= 0;
parameters
f = 0
g = 80
h =.001;
predict = f + g*(exp(-h*rate));
model analvar = predict;
run;
*/

**segmented linear for pyield at Windsor
/*
**a = left intercept, b1 = slope of left segment;
**br1 = slope of right segment, j = junction of two lines;
**use indvar = log rate + 1;
proc nlin method = marquardt;
bounds a0>= 0;
parameters a0 = 0
           b1 = 4
           br1 = 7
           j = 5.5;
*left segment;
if indvar<= j then do;
  model analvar = a0+b1*indvar;
  der.a0 = 1;
  der.b1 = indvar;
  der.br1 = 0;
  der.j = 0;
end;
*right segment;
if indvar>j then do;
*else do;
model analvar = a0+b1*j+br1*(indvar-j);
  der.a0 = 1;
  der.b1 = j;
  der.br1 = indvar-j;
  der.j = b1-br1;
end;
run;
*/

```