Characterization of ethametsulfuron-methyl and group 2 herbicide resistance in ALS resistant wild mustard (*Sinapis arvensis* L.) populations in Manitoba.

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by

Jody Elaine Dexter

In Partial Fulfillment of the

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Characterization of ethametsulfuron-methyl and group 2 herbicide resistance in ALS resistant wild mustard (*Sinapis arvensis* L.) populations in Manitoba.

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Of

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ABSTRACT

Dexter, J.E., M.Sc., The University of Manitoba, 2004. Characterization of ethametsulfuron-methyl and group 2 herbicide resistance in ALS resistant wild mustard (*Sinapis arvensis* L.) populations in Manitoba. Major Professor: Dr. Rene Van Acker, Department of Plant Science.

Wild mustard (*Sinapis arvensis* L.) is a common and competitive weed of field and horticultural crops and occurs across all of the Canadian provinces. Since 1989, wild mustard has been effectively controlled in argentine canola (*Brassica napus* L.), polish canola (*Brassica rapa* L.) and commercial brown mustard (*Brassica juncea* L.) by ethametsulfuron-methyl. In 2000, spraying with ethametsulfuron failed to control 20 geographically separate wild mustard populations in conventional canola crops in southern Manitoba. Escaping patches of wild mustard were sampled in August of 2000 and ethametsulfuron herbicide resistance was confirmed at the whole plant level in a greenhouse trial. Subsamples of four acetolactate synthase (ALS) inhibitor resistant wild mustard populations (and a known susceptible population) were selected for indoor, whole-plant, dose response experiments to characterize the patterns and levels of cross-resistance to several ALS inhibitor herbicides and to the group 4 herbicide 2,4-D. ALS inhibitor herbicides used in these indoor dose-response experiments included ethametsulfuron, thifensulfuron, and imazethapyr. Furthermore, the insecticide malathion, which is a known inhibitor of Cytochrome P450 enzymes, was tank-mixed with ethametsulfuron in an additional dose-response experiment. In 2002, one ALS inhibitor resistant wild mustard population was evaluated in a separate field experiment to determine the efficacy of group 2 herbicides in competition with a competitive canola crop. The results of the greenhouse experiments show that that patterns of resistance to

ethametsulfuron varied among biotypes with one biotype showing a very high level of resistance. This same biotype also demonstrated a moderate level of resistance to imazethapyr and thifensulfuron. A different biotype demonstrated a high level of cross resistance to thifensulfuron at the whole plant level. The addition of malathion to ethametsulfuron either completely or partially restored susceptibility in 3 out of 4 biotypes tested suggesting that the mechanism for resistance in these biotypes may be related to Cytochrome P450 mediated metabolism of ethametsulfuron. Furthermore, all ALS resistant inhibitor wild mustard populations were equally susceptible to herbicide treatments with 2,4-D at the whole plant level. The results of the field experiment did not agree with the results of the greenhouse experiments as they suggested that one particular biotype may be resistant to ALS inhibitor herbicides by an altered target site rather than by enhanced metabolism. The differences in the results between the field experiment and the growth room experiment stresses the importance of assessing the response of reputed resistant wild mustard populations in the field in a competitive crop, particularly if the resistance levels are low because growing conditions for plants in pots in the growth room are quite different from those in the field. Furthermore, many herbicides, particularly ALS inhibitor herbicides tend to be more active in the growth room than in the field.

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CHAPTER 1

INTRODUCTION

Wild mustard (*Sinapis arvensis* L.) is a common and competitive weed of field and horticultural crops and occurs across all of the Canadian provinces (Friesen and Shebeski 1960). Although wild mustard is effectively controlled in all herbicide tolerant canola (HTC) cropping systems, wild mustard is difficult to control in conventional Brassicaceae crops (Blackshaw et al 1987). Since 1989, wild mustard has been effectively controlled in argentine canola (*Brassica napus* L.), polish canola (*Brassica rapa* L.) and commercial brown mustard (*Brassica juncea* L.) by ethametsulfuron-methyl and by other group 2 herbicides such as thifensulfuron-methyl and imazethapyr (Veldhuis et al 2000).

In 2000, spraying with ethametsulfuron failed to control 20 geographically separate wild mustard populations in southern Manitoba. Escaping patches of wild mustard were sampled in August of 2000 and ethametsulfuron herbicide resistance was confirmed at the whole plant level in a greenhouse trail. Most of the resistant samples were collected in an area between Morris and Roland Manitoba, although resistant populations were also confirmed around High Bluff (near Portage La Prairie), Crystal City and Altamont, Manitoba. There is a strong possibility that there are more resistant wild mustard populations in Manitoba.

The goal of this research project is to monitor and characterize levels and patterns of resistance for wild mustard populations in Manitoba. A subsample of seeds of four acetolactate synthase (ALS) inhibitor resistant wild mustard populations (and a known susceptible population) were selected for indoor, whole-plant dose response experiments

to characterize the patterns and levels of cross-resistance to several ALS inhibitor herbicides and to the group 4 herbicide, 2,4-D. ALS inhibitor herbicides used in these indoor dose response experiments included ethametsulfuron, thifensulfuron and imazethapyr. Furthermore, the insecticide malathion, which is a known inhibitor of Cytochrome P450 enzymes was tank mixed with ethametsulfuron in an additional dose response experiment. In a separate indoor, whole-plant, dose response experiment, a known 2,4-D resistant wild mustard population was used with the susceptible and resistant ALS inhibitor resistant wild mustard populations to determine if the latter resistant wild mustard populations were also resistant to group 4 herbicides at the whole plant level. In 2002, one ALS inhibitor resistant wild mustard population was evaluated in a separate field experiment to determine the efficacy of group 2 herbicides in competition with a competitive canola crop.

CHAPTER 2

LITERATURE REVIEW

1.1 Wild Mustard

Introduction

Wild mustard, *Sinapis arvensis* L. is a member of the *Cruciferae* family (Warwick et al. 2000). It is a weed of field and horticultural crops and it occurs across all of the Canadian provinces (Friesen and Shebeski 1960). Currently, wild mustard ranks as the eleventh most abundant weed in Manitoba (Leeson et al. 2002).

Sinapis arvensis is effectively controlled in all herbicide tolerant canola (HTC) cropping systems. However, the number of herbicides available for wild mustard control in conventional *Brassicaceae* crops is limited. Since 1989, wild mustard has been effectively controlled in rapeseed (*Brassica napus* L.), argentine canola (*Brassica napus* L.), polish canola (*Brassica rapa* L.) and commercial brown mustard (*Brassica juncea* L.) by Muster herbicide (ethametsulfuron-methyl), formerly DPX A7881 (Veldhuis et al. 2000). Wild mustard may also be controlled by other group 2 herbicides such as thifensulfuron methyl and imazethapyr. For optimum weed control, early herbicide application is recommended but because of wild mustard's sporadic release from dormancy throughout the growing season late emerging individuals can reduce crop yield and quality, even with repeated control measures (Goudey et al. 1986).

Biology

Wild mustard is an annual broadleaved plant that is readily killed by frost. It is common in spring planted annual crops and is virtually absent from land that has not been recently disturbed (Mulligan and Bailey 1975). Seedlings initially form a rosette

and later develop into an erect plant up to 100 cm in height (Debreuil 1996). Stems are typically simple or branched, hairy, and purple at the junction with the main stem. In mature plants, the lower leaves are petiolate and lobed but the upper leaves are broadly triangular shaped, sessile, deeply lobed and often purplish in the leaf axial (Warwick et al. 2000).

Wild mustard has bright yellow flowers (Mulligan and Bailey 1975), each with 4 petals, 1.5 cm across with spreading sepals (Warwick et al. 2000). The flowers are borne in racemose clusters at the end of stems and branches (Debreuil 1996). Plants of wild mustard are self-incompatible and mostly depend on insects to perform cross-pollination. The insects are attracted to the odoriferous flowers due to their high reflectance in the yellow and ultraviolet wavelengths. The large ultra-violet free spot in the center of the corolla serves as a guide to the sexual parts of the flower (Mulligan and Bailey 1975).

After flowering, wild mustard forms distinctive pods. Fruit pods are 3-5 cm in length and are usually hairless with a short thick stalk. Wild mustard differs from other mustards in the *Cruciferae* family by having one to two seeds in the beak of the pod in addition to those in the valve (Warwick et al. 2000). Although wild mustard is an out-crossing species, it is morphologically and cytologically quite uniform throughout the Canadian prairies (Mulligan and Bailey 1975).

Wild mustard is a prolific seed producer capable of returning large quantities of seed to the soil (Blackshaw et al. 1987). For example, plants of wild mustard grown in cultivated fields may produce up to 18 seeds per pod and 3,500 seeds per plant. Blackshaw et al. (1987) reported that wild mustard plants grown in cultivated fields in competition with common lambsquarters (*Chenopodium album* L.) and spring rapeseed

(*Brassica napus*) produced 5700-30,100 seeds m^{-2} with weed densities ranging from 10-80 plants m^{-2}. Competition for available light and water rather than nutrients was found to play a major role in interference, where wild mustard was the strongest competitor for both, followed by *B. napus* and *C. album* (Blackshaw et al. 1989).

The mature seeds of wild mustard can be distinguished from those of cultivated Brassica's by their relatively small size (1.5-2.0 mm in diameter), completely round shape, and black slightly greasy testa. Van Acker and Oree (1999) measured the seed return of wild mustard and wild oat (*Avena fatua* L.) left uncontrolled in canola (*B. napus*). In comparison, to wild oat, wild mustard had greater return of viable seeds. Maximum viable seed return was 1300 seeds m^{-2} for wild oat and 3300 seeds m^{-2} for wild mustard (Van Acker and Oree 1999). Forcella et al. (1996) reported an average wild mustard seed return of 2475 seeds m^{-2} in a cultivated cornfield that received no herbicide treatment. Seeds of wild mustard were completely dispersed before corn harvest in warm years, but in cooler years, the plants retained one-third of their seeds and were later dispersed via combines during harvest (Forcella et al. 1996).

Some seed of wild mustard is capable of germination as soon as it is mature but for the most part, germination of wild mustard seed is irregular. A 1934 report in Mulligan and Bailey (1975) indicated that 20% of freshly harvested seeds from a Saskatchewan wild mustard population germinated within a 4 to 20 day period. Seed germination among Canadian populations suggests inter-population differences in levels and patterns of seed dormancy (Warwick et al. 2000).

Seed longevity of up to 75 years has been reported for wild mustard (Mulligan and Bailey 1975). Barralis et al. (1988) compared the longevity of seeds of 17 annual

weed species in plots of monocultures of winter wheat and spring barley. Over a 5-year period, an average of 10% of wild mustard seedlings emerged from the annual weed seedbank. Barralis et al. (1988) also reported that *S. arvensis* seed viability decreased linearly from 94 to 78% when wild mustard seed replenishment was prevented. Hails et al. (1997) found that wild mustard seeds buried in nylon mesh bags exhibited much greater seed survival than oilseed rape at a burial depth of 15 cm relative to a 2 cm burial. In Morris MN, Warnes and Andersen (1984) determined that after 7 growing seasons, approximately 50% of known wild mustard seeds remained in the annual weed seedbank in treatments in which the soil had not been disturbed. In contrast, less than 3% wild of the mustard seeds remained in a treatment that involved plowing three times a year with additional plowing throughout the growing season. However, this 3% was equivalent to 2.4 million seeds/ha in the plow layer (Warnes and Andersen 1984). In Manitoba, Bullied et al.(2003) reported that conventional tillage systems promote earlier emergence than conservation tillage systems both in terms of thermal and chronological time. Differences in soil disturbance between the tillage systems influence the vertical location of the weed seedling recruitment zone thereby influencing wild mustard emergence periodicity (Bullied et al. 2003).

Genetic studies conducted by Garbutt and Witcombe (1986) on crosses between dormant and non-dormant lines of a *S. arvensis* biotype isolated from the UK indicated both maternal and embryonic components of seed dormancy. A single locus with two alleles was found to control the maternal component. The dormant allele *I* was dominant to the non-dormant allele *i*. Genetic control of the embryonic component was not identified (Garbutt and Witcombe 1986).

Edwards (1968) determined that wild mustard seed dormancy is regulated by an inhibitor produced by the embryo under low oxygen concentrations. A layer of mucilages and phenols present in the seed coat retards the diffusion of oxygen into the embryo and permits its formation. This substance diffuses to the meristems preventing root elongation (Edwards 1968).

In the greenhouse, Goudey et al. (1986) evaluated the effect of temperature, light, and various nitrogenous compounds on germination of wild mustard seeds. Independent treatments of light, nitrogen and temperature resulted in a maximum wild mustard seed germination of 51%. However, a combination of KNO_3 plus NH_4Cl with irradiation at 20 C resulted in a germination rate of 92%. These results suggest that applications of nitrogen fertilizers in combination with surface tillage to expose the seeds to light could promote germination of dormant wild mustard seeds in the field (Goudey et al. 1986). In Minnesota, Buhler (1997) compared the effects of tillage and light on emergence of 13 annual weed species. *S. arvensis* emergence was similar to that of other small-seeded broadleaf weeds in requiring some exposure to light, with highest densities of 152, 143, 90 and 53 plants m^{-2} following tillage in the light, light/dark, no light and no tillage, respectively.

In field trails, Wright et al. (1999) evaluated the influence of two different soil moisture regimes (10% and 70% of field capacity) on the competitive ability and seed dormancy of wild mustard in spring wheat. Under dry conditions, the competitiveness of *S. arvensis* is reduced and the potential of wild mustard to produce persistent seeds is diminished. For example, Wright et al. (1999) reported that plants of wild mustard grown in dry soil produce smaller and less dormant seeds than those which are grown

under a higher moisture regime. Edwards (1980) similarly reported that drought conditions decreased wild mustard seed production and plant density. Wall et al. (1991) found that pea losses due to wild mustard competition were less in 1998 compared to 1987 and 1989 due to below average precipitation. Blackshaw et al. (1987) reported that wild mustard threshold densities at which economic losses in canola yield occurred were greater in dry years than in wet years.

Wild mustard seedling emergence is correlated with soil temperature (Edwards 1980). In field trails conducted over a three year period, Edwards (1980) reported that wild mustard seedlings begin to emerge when the mean weekly soil temperature was greater than 4.4 C up to a depth of 10cm. In the same study, it was also determined that the annual emergence of wild mustard is 2.5% of the total seed in the weed seed bank and that the average annual seedbank death and decay of wild mustard is 17.9%. The ability of wild mustard seed to germinate and establish when favorable growing conditions arise ensures its long-term survival and contributes to its weedy nature (Wall et al. 1991).

Weedy Nature

Due to its tall stature, robust growth habit and large root system, wild mustard is a highly competitive weed (Debreuil et al. 1996). According to the Federal Seeds Act and Regulations of Canada, *Sinapis arvensis* is a noxious weed. This limits the number of wild mustard seeds permitted in commercial seed to a specified minimum (Mulligan and Bailey 1975). More than 5% by weight of wild mustard in commercial brown mustard, canola and rapeseed results in these harvested crops receiving a sample grade at the primary elevator (Blackshaw 1989b). Pedigreed canola seed growers must comply with a

zero tolerance levels regarding wild mustard seed contamination as stipulated by the Canada Seeds Act, 1986 (Debreuil 1996).

Wild Mustard as a Contaminant of Cruciferae Crops

Canola is one of the major crops grown in western Canada (McMullan et al. 1994). Uncontrolled populations of wild mustard can reduce canola yield and seed quality (Swanton and Chandler 1989). For example, Blackshaw et al. (1987) reported that wild mustard densities of 10 plants m^{-2} can reduce canola yields by 20%. Contamination of canola with wild mustard seed increases the levels of erucic acid in the extracted oils as well as the glucosinolate content of the remaining meal (Swanton and Chandler 1989).

Wild mustard is a source of infection by insect, nematode, fungus and bacterial pests in cruciferae crops, particularly *Brassica napus* and *Brassica rapa* (Warwick et al. 2000). White rust (*Albugo candida* (Pers.) O. Ktze) is an economically important disease of polish canola (*Brassica rapa*). The pathogen causing white rust can infect wild mustard, and in favorable conditions allow for proliferation of the disease (Downey and Rimmer 1993). Moreover, early germinating plants of wild mustard can nurture overwintered adult crucifer flea beetles until such time as the canola emerges later in the growing season (Philip and Mengeren 1989).

1.2 Auxin-type Herbicides for Wild Mustard Control

Auxin-type herbicide groups

Currently, the auxin-type herbicides that are used in western Canada are grouped by their chemical structure and include benzoic acids, phenoxyalkanoic acids, aromatic carboxymetyl derivatives, quinoline carboxylic acids and pryridine derivatives (Cobb

1992). The most widely used groups are the phenoxyalkanoic (phenoxy) acids benzoic acids which include 2,4-D (phenoxy) and dicamba (3,6-dichloro-2-methoxybenzoic acid) (benzoic acid) respectively. In comparison to dicamba, 2,4-D is highly effective and is widely used to control wild mustard. However, dicamba is more effective than 2,4-D on other problematic weeds like wild buckwheat and green smartweed (Debreuil 1996).

History

The first of the auxin-type herbicides to be used in commercial grain production were the phenoxyalkanoic acids, 2,4-D and MCPA. In 1941, both herbicides were synthesized independently and were subsequently kept secret until the end of the Second World War (Peacock 1978; Cobb 1992). In 1945, the American Chemical and Paint Company first marketed 2,4-D in the United States for agricultural use. One year later MCPA was marketed as a 1% active dust by the Imperial Chemical Company (Peacock 1978). The introduction of these products revolutionized modern agriculture (Debreuil 1996).

With introduction of 2,4-D and MCPA an inexpensive, effective and selective chemical weed killer was available to the farmer for the first time. Farming became more productive with less of a reliance on labor to control weeds. Today, the phenoxy herbicides 2,4-D and MCPA are still among the world's most effective and widely used herbicides (Cobb 1992; Devine et al. 1993).

Herbicide Properties

Auxin-type herbicides are synthetic analogues of natural plant hormones (Ashton and Crafts 1981; Cobb 1992; Devine et al. 1993). Synthetic auxins stimulate 1-aminocyclopropane carboxylic acid synthase (a plant enzyme) which induces ethylene

evolution and uncontrolled growth in susceptible plants. In some plants, ethylene evolution is linked with auxin-induced epinasty; however, this does not occur in all species (Barnwell and Cobb 1989).

Auxin-type herbicides are typically foliar-applied, and are absorbed through the leaf cuticle into the apoplast. Norris (1974) examined cuticles from 8 different species (7 leaf cuticles and 1 tomato fruit cuticle) and determined that there was no correlation between cuticle thickness and penetration of 2,4-D. For example, the cuticle of the tomato fruit was thickest, yet 2,4-D penetrated it the most easily. From further analysis Norris (1974) concluded that penetration into the plant by 2,4-D was more directly related to the composition of cutin and wax of the cuticle.

Peniuk et al (1993) reported rapid penetration of both 2,4-D and dicamba into leaves of phenoxy- resistant and phenoxy- susceptible populations of wild mustard. Using radiolabelled herbicides, Peniuk et al (1993) determined that there was greater than 95% penetration within 12 hours of application for both populations.

Once through the cuticle, auxin-type herbicides penetrate the plasma lemma where they are readily translocated to the meristematic tissues via the phloem (Ashton and Crafts 1981; Devine 1989). Absorption and translocation of dicamba was measured by Chang and Vanden Born (1968) in four species, including wild mustard, under controlled environmental conditions. Chang and Vanden Born (1968) reported that dicamba was quickly absorbed into the leaf and translocated from the leaf to meristematic tissue in wild mustard. The authors also reported that the relative susceptibility of the four species was correlated with the amount of dicamba absorbed and translocated (Chang and Vanden Born 1968).

The site of action of auxin-type herbicides is purported to be an auxin binding protein (Debreuil 1996). However, the details of the cause-and-effect sequence after an auxin-type herbicide binds to a plasmalemma auxin-binding protein are not fully known (Devine et al. 1993) because the current understanding of auxin and its effects on plant physiology is incomplete (Debreuil 1996). Auxin exists within the plant at very low doses and different tissues have various amounts. The phytotoxic action of auxinic herbicides takes place as a result of their ability to mimic endogenous auxin. The high concentrations of exogenous auxin as a result of herbicide treatment create an imbalance relative to intracellular auxin concentrations, disrupting normal growth patterns. Eventually, the ongoing mobilization of metabolic reserves for excessive growth leads to the loss of cellular integrity, cellular functions, and repair capacities. Symptoms of auxin imbalance are stem and petiole elongation and curling, stem and petiole thickening and the formation of irregular adventitious roots (Debreuil 1996). Eventually plant tissues begin to desiccate and/or disintegrate. Therefore, the herbicidal effect of auxin–type herbicides is to supply an overdose of auxin to the plant, causing death (Devine et al. 1993).

Phenoxy Herbicides and Wild Mustard

Wild mustard has been controlled effectively with the phenoxy herbicides, 2,4-D and MCPA since the early 1950's. As such, wild mustard is no longer considered a problem in the United Kingdom (Edwards 1980; Richardson 1980). Although phenoxy herbicides have reduced the wild mustard problem in western Canada, the weed remains a major problem in crops such as canola which are sensitive to these herbicides (Thomas 1984).

Wild mustard is most susceptible to phenoxy herbicides at early stages of development. Burrows and Olson (1955) observed a high mortality of wild mustard when it was sprayed at the four-leaf stage (5 to 12 cm in height) with 280 (g ai ha^{-1}) of 2,4-D. Burrows and Olson (1955) observed that efficacy of the herbicide was substantially lower at later leaf stages. In a different experiment, 280 g ai ha^{-1} rate applied at the four-leaf stage was more effective than a 420 g ha^{-1} rate at the late five-leaf stage (22-30 cm in height) (Burrows and Olson 1955). Currently it is recommended that for wild mustard control in field crops 2,4-D be applied at 420 g ai ha^{-1} at the 2 to 4 leaf stage (Manitoba Agriculture and Food 2003).

1.3 Acetolactate Synthase Inhibitor Herbicides for Wild Mustard Control

Introduction

Group 2 herbicides are non-competitive inhibitors of acetolactate synthase (ALS) (Beckie et al. 2001). There are 5 different chemical families of ALS inhibitors. These include the sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidnyl thiobenzoates and sulfonylaminocarbonyltriazolinones (Beckie et al. 2001). The use of the two major classes (the sulfonylureas and imidazolinones) has grown to a 1991 market value of $1.3 billion worldwide. This popularity is due to very low dose rates (2-75 g ai ha^{-1}), wide crop tolerance, sound environmental properties, low mammalian toxicity and high efficacy (Brown 1990).

Inhibition of ALS leads to rapid cessation of plant cell division and growth (Schloss 1990). Acetolactate synthase (ALS) is required for the biosynthesis of two acetohydroxyacids, acetolactate and acetohydroxybutyrate (Saari et al. 1994), which are key intermediates in the synthesis of the branched chain amino acids leucine, isoleucine

and valine (Devine et al. 1991). Shaner et al. (1991) suggests that an important consequence of ALS inhibition in plants may be the accumulation of one of its substrates, alpha-ketobutyrate. There is good biochemical and genetic evidence that sodium butyrate (which may be derived from an alpha-ketobutyrate through metabolic reduction) arrests plant cell division in interphase, a cellular response similar to that caused by sulfonylurea herbicides (Brown 1990).

Although there are up to 6 isozymes of ALS in microorganisms, there appears to be only one type of ALS in plants (Brown 1990). Most diploid species have a single ALS locus with the exception of corn (*Zea mays* L.) and tobacco (*Nicotiana tabaccum* L.P) which have 2 loci and *Brassica* species have been reported to have numerous loci. Although the *in vivo* oligomeric structure of ALS in higher plants has not been fully characterized, the mature protein has approximately 575 amino acids and is highly conserved across plant species (Guttieri et al. 1996).

The Discovery of the Sulfonylurea and Imidazolinone Herbicides

Sulfonylureas

G. Levitt discovered the sulfonylurea herbicides in the late 1970s. Upon their discovery, the mechanism of action of the sulfonylureas was unknown. Reasoning that microorganisms and plants may share sensitivity to the sulfonylurea herbicides, R. A. LaRossa discovered that the growth of bacteria was inhibited on minimal media but not on complete media. The components of the complete media that obviated the sensitivity of bacteria by inhibition to the sulfonylurea herbicides were the branched chain amino acids. The enzyme ALS, appeared to be the enzyme responsible for the basis of herbicide selectivity (Schloss 1990).

These studies on microorganisms were later extended to plants by T.B. Ray. Experiments using whole pea (*Pisium sativum* L.) plants on solid agar medium and excided pea-root tips in liquid culture showed that growth inhibition by chlorsulfuron could be fully reversed by supplementing the medium with branched chain amino acids. As in the studies with prokaryotes, partially purified ALS from plants was markedly inhibited by chlorsulfuron and other sulfonylureas at concentrations which are also herbicidal (Schloss 1990).

Unequivocal evidence for this site of action for the sulfonylureas in plants comes from genetic studies. Using plant cell culture techniques, preferred lines of sulfonylurea-resistant tobacco (*Nicotiana tabaccum* L.P) callus were selected and from these cultures, mutant tobacco plants were regenerated. The regenerated mutant tobacco plants were 100-500 times more tolerant of chlorsulfuron than their wild-type progenitors and were shown to have an ALS enzyme much less sensitive to inhibition by sulfonylurea herbicides. Genetic analysis showed that the sulfonylurea resistant phenotype resulted from a single semi-dominant nuclear gene mutation. Apart from confirming the site of action of the sulfonylurea herbicides in plants, these studies also demonstrated that there was no second specific site of sulfonylurea herbicidal activity (Brown 1990).

Imidazolinones

The imidazolinone herbicides were discovered by a random screening test in the 1970s by scientists at the American Cyanamid Company. Due to their nature of discovery, the mechanism of action of the imidazolinones was unknown. Research on the mode of action of the imidazolinone herbicides led to the conclusion these herbicides solely inhibit ALS (Stidham and Singh 1991).

Initial experiments on the imidazolinone herbicides were first conducted on the ALS enzyme extracted from corn roots. When ALS activity was measured over an extended assay period (4 h) in the presence of various concentrations of imazethapyr, inhibition was found to increase with time, a feature typical of many tight ALS binding inhibitors. Treatment of susceptible maize cell cultures with imazapyr resulted in reduced levels of leucine, valine, and isoleucine. However, when these cells were supplemented with these three amino acids, all symptoms of herbicide injury were alleviated (Stidham and Singh 1991).

Additional enzyme inhibition ALS by the imidazolinone herbicides were conducted on extracts of ALS from excised corn leaves. When ALS is extracted from corn leaves that were treated with imazapyr, the amount of extractable ALS activity is drastically reduced in comparison to the untreated control. This effect was observed with other imidazolinones as well. For example, 8 hours after application, the amount of extractable ALS activity in corn plants treated with imazaquin was 60% lower relative to the ALS in untreated plants and 1 day after treatment; the quantity dropped an additional 20% relative to the ALS in the untreated control. The effect was found to be specific for ALS, since other enzyme levels were unaffected after imidazolinone treatment (Stidham and Singh 1991).

Thifensulfuron-methyl for Broadleaved Weed Control

Thifensulfuron-methyl (methyl 3-(((4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino-carbonyl) aminosulfonyl)-2-thiophenecarboxylate formerly DPX-M6316, is a sulfonylurea herbicide for post emergence (POST) broad spectrum broadleaf weed control. At a POST application rate of 4.3 g ai ha^{-1}, thifensulfuron-methyl controls or

suppresses several broadleaf species including *Chenopodium album*, *Abutilon theophrasti* Medik, *Amaranthus* spp. and *Xanthium pensylvanicum* Wallr. These four weed species exhibit up to 50% growth reduction at mean application rates ranging from <0.5 to 1.2 g ai ha^{-1} (Brown et al. 1990).

Thifensulfuron-methyl is characterized by having a very high herbicidal activity, with use rates ranging from 4 to 40 g ai ha^{-1}. However, thifensulfuron-methyl differs from most other sulfonylurea herbicides in several respects. Thifensulfuron-methyl is a short residual herbicide, by virtue of its high susceptibility to microbial degradation in the soil. It is also not subject to significant re-cropping restrictions and fields treated with thifensulfuron-methyl can be replanted with rotational crops as soon as 45 days after treatment. Thifensulfuron-methyl is also unique in that it exhibits commercial levels of tolerance in several major crop species such as soybeans (*Glycine max*, cv "Williams") and corn (*Zea mays*) (Brown et al. 1990).

Soybeans exhibit significant tolerance to thifensulfuron-methyl relative to sensitive weed species such as velvetleaf (*Abutilon theophrasti* Medic.). At each injury level, 10-100 times higher application rates of thifensulfuron-methyl are required to injure soybeans to the same extent as sensitive broadleaf weed species (Brown et al. 1990).

Several possible mechanisms for soybean tolerance to thifensulfuron-methyl have been investigated. There is no correlation between foliar uptake and plant sensitivity to herbicide treatment. Brown et al. (1990) observed that uptake by tolerant soybeans is nearly as great as or greater than uptake by sensitive weed species such as redroot pigweed (*Amaranthus retroflexus* L.) and common lambsquaters (*C. album*) (Brown et al.

1990). Sweeter et al. (1982) also concluded that wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) tolerance to chlorsulfuron was not due to differences in foliar uptake between tolerant and sensitive plant species. These conclusions are supported by mechanistic studies showing that sulfonylurea herbicide uptake and translocation depend on an acid trapping mechanism that is driven by the energy-dependent pH gradient between symplastic (neutral/alkaline) and apoplastic (acidic) compartments and is controlled by the physical properties of the herbicide (Sweeter et al. 1982).

All higher plants maintain similar pH gradients across their cellular membranes (including phloem sieve elements). This means that tolerant crops and sensitive weed species are physiologically equivalent in this respect. Also, there is considerable overlap in physical properties among many sulfonylurea structures without corresponding overlap in crop selectivity's. Therefore, it is unlikely that soybean selectivity to thifensulfuron-methyl results, primarily, from differential uptake and translocation (Brown et al. 1990).

Soybean tolerance to thifensulfuron-methyl results from its rapid metabolic deesterification to thifensulfuron acid. For example, soybeans metabolize thifensulfuron-methyl with a half-live of 4-6 hours while very sensitive species, including velvetleaf, lambsquarters and redroot pigweed, metabolize this herbicide much more slowly with half- lives greater than 36 hours (Brown et al. 1990). In order for rapid herbicide metabolism to account for plant tolerance, the metabolic products must be herbicidally inactive. Thifensulfuron acid has been shown to be inactive in greenhouse studies and inactive against ALS (Cotterman and Saari 1994).

Thifensulfuron-methyl and metsulfuron-methyl are also rapidly metabolized by wheat, accounting for its tolerance to these herbicides. Unlike thifensulfuron-methyl,

metsulfuron-methyl is subject to the same metabolic pathway as chlorsulfuron in wheat, namely aryl hydroxylation and glucose conjugation. Instead, thifensulfuron-methyl is rapidly metabolized by four independent routes in wheat. The three major reactions include aurea-bridge cleavage, deesterification and sulfonamide bond cleavage. Each of the primary products of these reactions are both herbicidally inactive and inactive against ALS. A fourth, and relatively minor, pathway directly inactivates the herbicide without conjugation to glucose by oxidative *O*-demethylation of the triazine methoxy subsitutent. Clearly, wheat can catalyze a number of metabolic transformations of sulfonylurea herbicides, but aryl hydroxylation is kinetically favored, given the phenyl rings of metsulfuron-methyl and chlorsulfuron, while the thiophene ring of thifensulfuron-methyl does not serve as a substrate for oxidative activity (Brown et al. 1990).

Imazethapyr for Broadleaved Weed Control

The imidazolinones are slow, tight-binding uncompetitive inhibitors of ALS. Imazethapyr is an imidazolinone herbicide and is used to control a variety of annual grass and broadleaf weeds (Stidham and Singh 1991). Imidazolinone tolerance in some plant species may be conferred by an altered form of ALS which is less sensitive to herbicide inhibition. For example, imidazolinone tolerance in wheat is inherited as a single dominant gene. When this gene is present in its homozygous state, it confers tolerance to imazethapyr through an altered site of action (Newhouse et al. 1992). In contrast, herbicide absorption and translocation plays a minor role in imidazolinone activity (Baerg and Barrett 1996)

Imazethapyr (2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl)-3-pyridinecarboxylic acid) is a member of the imidazolinone class of acetohydroxy

acid synthase inhibitors. Treatment of corn with imazapyr, imazaquin (2-(4,5-dihydro-4-methyl-4-(1-methylethyl-5-oxo-1h-imidazol-2-yl)-3-quinolincarboxlic acid), or imazethapyr results in less extractable ALS activity. The most common basis of imazethapyr crop selectivity is herbicide metabolism to nontoxic derivatives (Baerg and Barrett 1996). Rapid metabolism of imazethapyr has been reported in tolerant species such as soybeans and peanuts (*Arachis hypogaea* L.) (Ballard et al. 1995). Imazethapyr is metabolized faster in soybean and peanut roots than shoots in comparison to susceptible species such as redroot pigweed and sicklepod (*Senna obtusiolia* L.) (Bearg and Barrett 1996). In greenhouse experiments, Bearg and Barrett (1996) reported that the fresh weights of peanut and soybean were reduced by 7 and 4% respectively, by 140 g ai ha^{-1} of foliarly applied imazethapyr, while fresh weights of susceptible sicklepod and redroot pigweed were reduced 50 and 44% respectively, by the same herbicide treatment. In previous studies, greenhouse grown soybean was not injured until 112 g ai ha^{-1} of imazethapyr was applied, and soybean fresh weight was reduced less than 50% by 1120 g ai ha^{-1} of imazethapyr (Ballard et al. 1995).

Rapid metabolism of imazethapyr has been reported in common ragweed (*Ambrosia artemisofolia* L.) and giant ragweed (*Ambrosia trifidia* L.). Two metabolites of imazethapyr have been observed in both weed species. The two metabolites have been identified as the alpha-hydroxyethyl analog of imazethapyr and its glucose conjugate, based on reverse phase high-performance liquid chromatography (HPLC) with synthetic reference compounds (Ballard et al. 1995). These metabolites were previously described by Shaner and Mallipudi (1991) in soybeans and common cocklebur (*Xanthium stramarium L.* XANST). Shaner and Mallipudi (1991) have proposed that the metabolic

pathway of imazethapyr in these plant species is due to alkyl hydroxylation of the ethyl substituent on the pyridine ring followed by glucose conjugation. Although the hydroxylated metabolite is slightly less active than the parent compound and is relatively immobile in the plant, it has some herbicidal activity (Shaner and Mallipudi 1991). Therefore, differential tolerance to imazethapyr is associated with the relative capacity of the plant to conjugate the hydroxylated metabolite with glucose because the hydroxyimazethapyr metabolite will inhibit ALS activity (Ballard et al. 1995).

The metabolism of imazethapyr in soybeans is similar in corn and it has been suggested that imazethapyr is converted to hydroxyimazethapyr via cytochrome P450 monooxgenases. On the basis of HPLC profiles, Mallipudi et al. (1994) concluded that the major metabolic pathway for imazethapyr in corn is oxidative hydroxylation at the alpha-carbon atom of the ethyl side chain substituent on the pyridine ring to yield the alpha-hydroxyethyl analog of imazethapyr. However, in comparison to soybean, corn forms very limited amounts of the glycosyl conjugate (Baerg and Barrett 1996). In corn, hydroxylation primarily acts alone as the mechanism of detoxification and for this reason, it has to be very rapid. If hydroxylation occurs rapidly enough, imazethapyr will be immobilized at the site of herbicide application. This greatly reduces the phytotoxicity of imazethapyr by limiting the amount of compound that reaches the meristematic regions of the plant (Mallipudi et al. 1994).

Cowpea (*Vigna unguiculata* L.) is another crop which also rapidly metabolizes imazethapyr to polar and nonpolar metabolites. For example, only 41% of absorbed imazethapyr was recovered in the parent form 4 hours after herbicide treatment. The amount of parent imazethapyr continued to decrease rapidly over a 20 h period and

during this time, the amount of polar metabolites increased (Baerg and Barrett 1996). It has been suggested that imazethapyr metabolism in cowpea follows the same pathway as soybeans. It is also possible that soybean and cowpea may share the same cytochrome P450 monooxygenase to catalyze the hydroxylation of imazethapyr to hydroxyimazethapyr before it is conjugated to glucose by a glucose tranferase (Baerg and Barrett 1996).

Ethametsulfuron-methyl for Broadleaf Weed Control

Ethylmetsulfuron-methyl (methyl 2((((4-ethoxy-6-methylamino-1,3,5 triazine-2-yl) amino) carbonyl)amino) sulfonyl) benzoate, formerly DPX A7881 is the active ingredient in Muster herbicide (Litchner et al. 1995). A unique aspect of this herbicide is its use to selectively remove *Brassicaceae* weeds from *Brassicaceae* crops (Veldhuis et al. 2000). Before the registration of ethametsulfuron-methyl in 1990, selective control of *S. arvensis* in canola was possible only by using metribuzin (4-amino-6 (1,1-dimethylethyl)-3(methylthio)-1,2,4-triazin-5(4H)-one) and cyanazine (2((4-chloro-6-(ethylamino) 1,3,5-triazin-2-yl) amino) methyl propanenitrile) in triazine resistant varieties. In the absence of herbicides, the triazine resistant varieties naturally yield 20-30% less than triazine susceptible cultivars (Blackshaw 1989a) and more susceptible to lodging and delayed maturity (Swanton and Chandler 1989). In conventional canola cropping systems, growers largely rely on ethafluralin (N-ethyl-N-(2-methyl-3-propenyl)-2,6-dinitro-4-(trifluoromethyl) benzenamine) or triflurin (2,6-dinitro-n,n-dipropyl-4-(triflourimethyl) benzenamine) for broadleaf weed control. However, these herbicides do not control weeds of the *Cruciferae* family such as wild mustard, field pennygrass (*Thlaspi arvense* L.) and flixweed (*Descurainia sophia* L.) which frequently occur in

canola fields. Additionally, these herbicides must be soil applied and incorporated to prevent excessive losses through volatilization and photodegradation thus negating their use in reduced tillage systems (Blackshaw 1989a).

In Manitoba, ethametsulfuron-methyl is recommended for postemergence control of several broadleaf weeds in canola at dosages of 14.8 and 22.2 g ai ha^{-1} (Wall 1995). Postemergence applications of 16-64 g ai ha^{-1} ethametsulfuron-methyl under greenhouse conditions causes negligible injury to *B. napus* L. 'Westar', while *C. album*, *S. arvensis* and *A. retroflexus* exhibit 80-100% injury 16-18 days after application. ED_{50} values (dose required to reduce growth by 50%) determined from total plant dry weights in response to foliar-applied ethametsulfuron-methyl were 148 and 0.85 g ai ha^{-1} for commercial brown mustard and wild mustard respectively. In field trials, postemergence application rates of ethametsulfuron-methyl at 20-30 g ai ha^{-1} readily control *T. arvense*, *D. Sophia* and *P. persicaria* L. without injury to the oilseed rape crop (Blackshaw et al. 1989a). Blackshaw et al. (1989b) reported that ethametsulfuron-methyl increased canola yield without altering oil content, 1000 kernel weight or green seed content of canola. Swanton and Chandler (1989) reported that ethametsulfuron-methyl at 10-15 g ai ha^{-1} selectively controlled wild mustard in canola, increasing yields and decreasing levels of glucosinolates in the meal.

There is little or no difference in ethametsulfuron-methyl absorption and translocation among plant species. Hall et al. (1992) reported that approximately 90 and 86% of ethametsulfuron-methyl was absorbed 8 hours after treatment (HAT) and 8 and 10% of the recovered radioactivity was translocated out of the leaves 72 hours after treatment (HAT) in commercial brown mustard and wild mustard respectively.

Blackshaw et al. (1989a) reported that wild mustard is more susceptible to Muster herbicide when it is applied at the earlier stages of development rather than at later stages of development. In greenhouse experiments, foliarly applied ethametsulfuron-methyl at 20 to 30 g ai ha^{-1} controlled 90% of wild mustard seedlings when applied at the two leaf stage, but only controlled 75% of the wild mustard seedlings when ethametsulfuron-methyl was applied at the 6^{th} leaf stage (Blackshaw, 1989a). In a separate experiment, Litchner et al. (1995) observed that at the 6^{th} leaf stage of weed development, 60 g ai ha^{-1} of ethametsulfuron-methyl was required to reduce wild mustard fresh weight biomass by 60%.

The relative tolerance of different plant species to ethametsulfuron-methyl is not due to differences in ALS sensitivity (Litchner et al. 1995). Crop selectivity to ethametsulfuron-methyl results from rapid metabolic inactivation of xenobiotics in the tolerant crop species (Brown 1990). This tolerance mechanism is consistent with those previously identified for other sulfonylurea herbicides in crops including soybeans, wheat and corn. For example, ethametsulfuron-methyl is metabolized with a half-life of 2.5 hours in tolerant oilseed rape whereas *Amaranthus* spp. metabolize this herbicide more slowly with half-lives ranging from 5 to 14 hours (Litchner et al. 1995). Similarly, Hall et al. (1992) found that ethylmetsulfuron-methyl metabolism was 2-14 times slower in sensitive wild mustard than in tolerant rapeseed and commercial brown mustard. Tolerance of oilseed rape to ethametsulfuron-methyl is unusual in that this crop is generally quite sensitive to other sulfonylurea herbicides such as chlorsulfuron, metsulfuron-methyl and triflusulfuron-methyl (Litchner et al. 1995).

Two metabolites of ethametsulfuron-methyl were identified by Litchner et al. (1995) by cochromatography and by mass spectrometry in isolated canola seedlings. These two metabolites include *O*-desethyl ethametsulfuron-methyl and *N*-desmethyl-*O*-desethyl ethametsulfuron-methyl. The initial metabolite, *O*-desethyl ethametsulfuron-methyl was found to be inactive against ALS in both oilseed rape and wild mustard (ALS I_{50}>1000 ppb) and completely inactive against a range of crop and weed species when applied preemergence or postemergence at 50 g ai ha^{-1} in greenhouse tests. This result is consistent with the testing of numerous sulfonylurea analogs which have shown that a free hydroxyl group in the 4 or 6 positions of the triazine or pyrimidine moieties leads to complete loss of herbicidal activity (Litchner et al. 1995).

Dissipation of Sulfonylurea and Imidazolonine Herbicides in the Soil

Thifensulfuron and Ethametsulfuron methyl

Chemical hydrolysis and microbial degradation are the primary mechanisms of sulfonylurea degradation in the soil. Deesterification is the first step in the transformation of most pesticides in the soil. However, only a few examples of isolated microorganisms capable of this hydrolysis have been reported. The mechanism of action of isolated microorganisms able to transform thifensulfuron-methyl and ethametsulfuron-methyl concerns the hydroxylation of methyl or phenyl groups (Cambon et al. 1998). Previous work has shown that in alkaline soils, thifensulfuron-methyl and ethametsulfuron-methyl are highly susceptible to microbial degradation especially with increasing soil temperature and moisture (Wall 1995). In neutral or acidic soils with accelerated rates of chemical hydrolysis, microbial degradation plays a lesser important role for these particular sulfonylurea herbicides (Beckie and McKercher 1989).

All sulfonylurea herbicides are subject to chemical hydrolysis of the sulfonylurea bridge. Sulfonylurea chemical hydrolysis is controlled by ionization of the sulfonylurea bridge. All sulfonylureas are weak acids having p*K*a values ranging from 3.3 to 5.2. Ionization is centered on the sulfonamide nitrogen and at any normal soil pH, there is an equal mixture of the neutral and anionic forms of the molecule. Since the neutral sulfonylurea molecule is much more lipophilic than its anionic form, soil sorption, water solubility and soil mobility are controlled by pH. However, the most important effect of pH and consequent ionization results from the fact that the neutral form of the sulfonylurea bridge is 250 to 1000 times more susceptible to hydrolysis than the anionic form. In the hydrolysis reaction, water attacks the carbonyl carbon of the neutral sulfonylurea bridge, cleaving the sulfonylurea into the herbicidally inactive arylsulfonamide and aminoheterocyclic halves of the molecule. The anionic bridge form is markedly deactivated toward hydrolysis because the negative charge is distributed throughout the bridge, reducing the electrophilic nature of the carbonyl carbon. Thus, pH controls the rate of sulfonylurea bridge hydrolysis through its effect on ionization, with faster hydrolysis typically occurring at acidic pH values (Cambon et al. 1997).

Thifensulfuron-methyl and ethametsulfuron-methyl are also subject to significant microbial degradation in all soils but an accelerated rate of breakdown is observed in alkaline soils. For example, the time in days required to degrade 50% (DT_{50}) thifensulfuron-methyl in non-sterile soil ranges from 0.75 to 3.5 days (pH 7.8 to 5.7 respectively) (Brown et al. 1997) while the DT_{50} of ethametsulfuron methyl ranges from 33 to 167 days (pH 7.6 to 5.5 respectively) (Beckie and McKercher 1989). Although ethametsulfuron methyl is much more persistent in the soil (Beckie and McKercher

1989), thifensulfuron-methyl and ethametsulfuron-methyl degradation will vary depending on soil type, textural class, organic matter, soil moisture and temperature (Brown et al. 1997).

Thifensulfuron methyl is one of several short soil residual sulfonylurea herbicides used in agronomic systems that require rotational cropping flexibility, and fields treated with this herbicide may be replanted to any rotational crop 45 days after treatment or sooner. This short soil residual characteristic of thifensulfuron-methyl is due to rapid microbial degradation to thifensulfuron acid. Thifensulfuron acid is herbicidally inactive against ALS and long term laboratory and field studies have shown that thifensulfuron acid subsequently degrades via several pathways, including evolution as CO_2 (Brown et al. 1990).

In contrast to thifensulfuron methyl, re-cropping restrictions apply for many crops following ethametsulfuron-methyl application. In a study conducted by Wall (1995), injury to buckwheat (*Fagopyrum esculentum* Moench.), dry bean (*Phaseolus vulgaris* L.), potato (*Solanum tuberosum* L.) and sunflower (*Helianthus annuus* L.) increased with ethametsulfuron-methyl dosage. Injury symptoms were similar among crop species and consisted primarily of stunting and foliar chlorosis in newly developed leaves. However, in contrast to potato, buckwheat and sunflower, navy bean was the only crop for which yields were unaffected by ethametsulfuron-methyl residues. This indicates that buckwheat, sunflower and potato are more sensitive to ethametsulfuron-methyl soil residues than navy bean and should not be planted in the cropping year following herbicide application (Wall 1995).

Imazethapyr

Imazethapyr is an imidazolinone herbicide that has both soil and foliar activity. Imazethapyr can be applied preplant incorporated (PPI), preemergence (PRE) or postemergence (POST), to control grass and broadleaved weeds. Imazethapyr's biological activity and behavior in the soil is largely dependent upon availability in soil solution, which, in turn is strongly affected by pH dependent adsorption (Jensen et al. 1995) and desorption from soil colloids (Flint and Witt 1997). Soil adsorption increases below pH 6, which decreases the phytotoxicity of the herbicide but increases its persistence. Aerobic microbial degradation accounts for most soil losses, but photolysis may also be important under some climatic conditions. There is little leaching of imazethapyr under field conditions (Jensen et al. 1995).

Imazethapyr is sufficiently persistent to injure sensitive rotational crops. For example, in the US Midwest, corn has been injured by imazethapyr 1 year after application. In contrast, there has been little indication of crop damage from imazethapyr residues in Atlantic Canada, even on highly sensitive potatoes (Jensen et al. 1995). Herbicide dissipation and herbicide carryover is influenced by soil type, application method, soil water content and temperature, tillage systems and precipitation (Curran et al. 1992).

Flint and Witt (1997) reported that imazethapyr degrades faster at temperatures optimal for soil microbial activity up to 75% of soil water field capacity. For instance, imazethapyr persists about two times longer at 15 C compared to 30 C and this is reflected by its half-life of 53 days (there is often a two to three fold increase in half-life

with a 10 C decrease in temperature). Longer herbicide persistence at lower soil temperatures is typical of herbicides degraded by microorganisms (Flint and Witt 1997).

Persistence of imazethapyr can vary on the same soil type, depending on the method of herbicide application and on the climatic conditions. Incorporating herbicides into the soil increases persistence by reducing photochemical degradation, volatilization, or both (Curran et al. 1992). Jensen et al. (1995) reported that half-lives of imazethapyr were 19, 47 and 63 days following POST, PRE and PPI herbicide applications respectively. Curran et al. (1992) observed that PPI applications of imazethapyr persist longer than PRE applications if mobilizing rains are delayed following application. In comparison to other imidazolinone herbicides such as imazapyr and imazaquin, imazethapyr persistence is less influenced by low soil water content due to its low water solubility (60 vs 1400 ppm by weight, respectively) (Curran et al. 1992).

1.4 Herbicide Tolerant Canola for Wild Mustard Control

Approximately 76% of the 5.6 million ha of canola in western Canada were seeded to Herbicide Tolerant Canola (HTC) in 1999. Three major HTC are widely used in western Canada and include varieties resistant to glufosinate (transgenic), glyphosate (transgenic) and imidazolinone herbicides (selected via cell culture). All of these HTC's offer the possibility of improved weed management in canola via a broader spectrum of weed control and/or greater efficacy on specific persistent weed species (Harker et al. 2000).

Glufosinate tolerant canola allows the use of glufosinate ammonium as a POST herbicide, thus providing an alternative for weed control in canola production. Glufosinate tolerant canola was developed using a disarmed non-pathogenic

Agrobacterium tumefaciens vector by introducing two bacterial genes into *Brassica napus*. The vector contained the T-DNA region of an Agrobacterium plasmid from which the virulence and plant-disease causing genes were removed and replaced with genes encoding for glufosinolate ammonium tolerance and kanamycin resistance. The kanamycin gene was not of agronomic importance as it was only used to select for modified canola plants (Canadian Food Inspection Agency 1995a).

Phosphinothricin (PPT) is the active ingredient in glufosinate ammonium and it inhibits the enzyme glutamine synthetase. Glufosinate resistance in canola is conferred by a single bar gene that codes for phosphinothricin acetyltransferase (PAT). This enzyme detoxifies phosphinothricin by acetylation into an inactive compound, thereby preventing the buildup of lethal levels of ammonia. The PAT gene was originally isolated from *Streptomyces viridochromogenes*, an aerobic soil actinomycete (Canadian Food Inspection Agency 1995a).

Glyphosate is the active ingredient in Roundup® herbicide. Research scientists at Monsanto Canada Inc. developed glyphosate tolerant canola from the variety 'Westar' by introducing two Roundup-Ready genes on a disarmed non-pathogenic *Agrobacterium tumefaciens* vector. When these genes are present in combination, they provide tolerance to Roundup herbicide. The first gene expresses a bacterial derived version of a plant enzyme involved in the shikimate biochemical pathway for the production of the aromatic amino acids tyrosine, phenylalanine, and tryptophan. The plant version of this enzyme, enolpyruvylshikimate phosphate (EPSP), is sensitive to glyphosate. Inhibition of EPSP by glyphosate suppresses plant growth and eventually results in plant death. The second gene, also bacterial derived, expresses an enzyme that degrades gylphosate,

thereby deactivating the herbicidal effect. The coding sequence of this gene has been altered to enhance the efficiency of glyphosate degradation, compared to the original bacterial version. Each of the Roundup ready genes was co-introduced on a chloroplast transit peptide into the chloroplast, the site of both the shikimate pathway and glyphosate mode-of-action (Canadian Food Inspection Agency 1995b).

Imazethapyr is the active ingredient in Pursuit herbicide. Pioneer Hi-Breed International developed three lines of *Brassica napus* tolerant imazethapyr. Imazethapyr is currently registered in Canada as a POST herbicide for the control of various broadleaved and grass weeds. These three *B. napus* lines do not exhibit significant symptoms of herbicide injury when treated with pursuit at normal field application rates and will allow the use of imazethapyr as a POST herbicide on canola crops. In the genome of *B. napus* ALS is encoded by five genes, two of which are constitutively expressed and are assumed to encode the primary ALS activities necessary for plant growth and development. Modifications to the ALS enzyme in various plant species including *B. napus* can result in herbicide tolerant phenotypes and typically consist of one amino acid substitution, sufficient to alter the binding site for imazethapyr such that the herbicide no longer inactivates the ALS enzyme. The modifications in the ALS enzyme in all three lines of *B. napus* tolerant to imazethapyr occur at different and unlinked loci (Canadian Food Inspection Agency 1995c) and resistance is inherited as a semidominant trait (Harker et al. 2000).

Field experiments conducted by Harker et al. (2000) across five western Canadian locations in 1996 and 1997 determined that by using herbicides "designated" for HTC compared to a more "standard" treatment of sethoxydim plus ethametsulfuron, can lead

to substantial yield gains as a result of superior herbicide performance and can provide significant weed control advantages. In 3 of the 10 site years, glyphosate in Quest canola and imazaethapyr in 45A71 canola provided a yield advantage over the standard treatment. The yield advantages were restricted to the Lacombe and Lethbridge, AB sites and ranged from 13 to 39% increases over sethoxydim plus ethametsulfuron treatments. Among the HTCs, weed control was usually greatest with glyphosate, followed by imazethapyr and then glufosinate. For example, in 1996 at Lethbridge AB, glyphosate was more efficacious than sethoxydim plus ethametsulfuron on more dominant weed species such as redroot pigweed and wild mustard while stork's bill, the least dominant weed species, was controlled better with ethametsulfuron in 45A71 canola. Similarly in 1996, densities of wild mustard, wild oat and false cleavers were better controlled with glyphosate in Quest canola at Morden, MB and Lacombe, AB respectively while at Brandon, MB and Scott, SK respectively, densities of wild oat in 1996 and lambsquarters in 1997 were better controlled with sethoxydim plus ethametsulfuron and imazethapyr in 45A71 canola (Harker et al. 2000).

1.5 Herbicide Resistance

In response to herbicide treatment there are three types of plant responses that are recognized. These include susceptibility, tolerance and resistance. Susceptibility is the lack of capacity to withstand herbicide treatment so that the plant is damaged by herbicides. In contrast, tolerance and resistance describe a situation whereby a plant withstands an herbicide treatment (Holt and Lebaron 1990).

Tolerance is defined as a low degree of resistance that is rate dependent. Such a response is considered to result from the naturally occurring variability that exists within

a species and is present before the first use of a chemical. Tolerance is a term that is often used to describe crop responses to herbicides which are due to naturally occurring mechanisms (Holt and Lebaron 1990).

Resistance is defined by the Herbicide Action Committee as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide which is normally lethal to the wild type (Poston et al. 2000). According to Jasieniuk et al. (1996), the development of herbicide resistance in weeds is an evolutionary process. In response to repeated herbicide treatment with a particular class or family of herbicides, weed populations change in their genetic composition resuting in an increased frequency of resistant alleles and therefore, resistant individuals. In this way, weed populations become adapted to the intense selection pressure imposed by herbicides (Jasieniuk et al. 1996).

Evolution of Herbicide Resistant Weeds

In order for herbicide resistance to occur, genetic variation must be present in a susceptible weed population (Jasieniuk et al. 1996). The major source of genetic variation in an area where herbicide resistance has not been previously detected is likely to be due to gene mutation(s) (Tranel and Wright 2002). In general, gene mutations conferring resistance to a specific herbicide class are are believed to occur spontaneously are not believed to be induced by herbicide application. Mutations at some loci that encode herbicide sites of action may confer resistance. A newly arisen resistance mutation will be present in the heterozygous state. Thus, its probability of survival in a population will depend on its degree of dominance. If the gene mutation is dominant, the plant will survive herbicide treatment but if the mutation is recessive, the plant carrying

the mutation will be susceptible to herbicide treatment (Jasieniuk et al. 1996). Typical spontaneous mutation rates in biological organisms have been cited as 1×10^{-5} or 1×10^{-6} gametes per locus per generation (Merrell 1981). Actual rates of mutation to herbicide resistance are presently unknown for any weed species (Jasieniuk et al. 1996).

Gene flow, via seed or pollen from a field with herbicide resistant plants to an adjacent or nearby field with susceptible plants may provide an initial source of resistance genes, similar to mutation, for the evolution of herbicide resistance. Generally, rates of gene flow are believed to be higher than rates of mutation and therefore would result in a higher frequency of plants resistant to a particular herbicide prior to its initial application (Jasieniuk et al. 1996).

The relative importance of gene mutation versus gene flow as a source of resistance genes in a susceptible weed population is not known. Gene mutation is likely to result in the initial appearance of herbicide resistance in a particular area whereas gene flow is more likely to bring about its spread among populations in a region. Until recently, the role of gene flow in the spread of herbicide resistance among weed populations has not been extensively studied. One reason for this is that rates of gene flow between plant populations in natural environments were once believed to be too low to carry much evolutionary significance. On average, levels of gene flow are estimated to be less than 1% among plant populations that are separated by a few hundred meters and at least two orders of magnitude less between plant populations 1.5 km apart. In many highly self-fertilizing species, gene flow rates between plant populations are expected to be so low that they approach rates of mutation (Jasieniuk et al. 1996).

Experiential data on levels of gene flow between herbicide resistant weed species are lacking. However, pollen movement of resistance genes within populations has been measured in diclofop-methyl resistant Italian ryegrass (*Lolium multiflorum* Lam.) and sulfonylurea resistant kochia (*Kochia scoparia* L. Schrad). Outcrossing of resistant pollen to susceptible plants was 1% at a distance of 6.84 m in Italian ryegrass and 1.4% at 28.9 m in kochia. Thus, it possible to establish new herbicide resistant weed populations in fields which were previously susceptible through gene flow (Jasieniuk et al. 1996).

Although pollen dispersal has generally been assumed to be the major mechanism of inter-population gene flow in plants, there is evidence that seed dispersal plays a far greater role in the establishment of herbicide resistant weed populations. Many weed species are highly self-fertilizing and pollen flow among resistant and susceptible weed populations is minimal. Weed seed movement due to agricultural implements can be substantial. In particular, grain harvesting equipment, may be an important mechanism of weed seed dispersal among fields. For example, roughly 3% of the seed of wild-proso millet (*Panicum miliaceum* L.) in one field was moved to a second field by combine harvesters. Movement of harvesting equipment from one field to another may similarly disperse seeds of herbicide resistant weeds into susceptible populations (Jasieniuk et al. 1996).

Inheritance of ALS Resistance

Resistance to ALS inhibitory herbicides is determined by nuclear inheritance. Transmission of resistant ALS alleles occurs through ovules and pollen (Jasieniuk et al. 1996). By means of nuclear inheritance, herbicide resistance is conferred by an allele that is partially dominant to the susceptibile allele. According to Jasieniuk et al. (1996)

when a favored allele is rare, it will spread much faster in a weed population than if it is recessive because heterozygotes will manifest at least part of the homozygote phenotype under selection pressure. Furthermore, a rare dominant resistance allele that is more likely to become established in a weed population following herbicide treatment than a recessive allele following herbicide treatment (Jasienuik et al. 1996).

To-date, ALS herbicide resistance has been thought to be determined by major genes. The predominance of major gene inheritance for this resistant trait can be attributed to two factors. First, ALS inhibitors interfere with a single enzyme in a metabolic pathway and are highly target site specific. Therefore, gene mutations of the gene encoding for the enzyme may alter a plant's sensitivity to the herbicide and result in herbicide resistance. Secondly, repeated herbicide application imposes strong selection pressure, causing 95-99% mortality in the majority of susceptible individuals in a weed population. Adaptation is only possible if resistance genes are present in a weed population and only if the resistance genes have a sufficiently large phenotypic effect to allow the survival of a few individuals in a single generation (Jasienuik et al. 1990).

Selection

A unique feature of the evolution of herbicide resistance in weeds, in contrast to most evolutionary processes occurring in natural plant populations, is the intensity of selection imposed by herbicides. If genetic variation for resistance is present due to mutation or gene flow, repeated herbicide applications will normally result in a rapid increase in the frequency of resistant individuals until they dominate the population. Selection pressure is the most important factor determining the rate of enrichment of herbicide resistance in a weed population (Saari et al. 1994). Selection is a function of

the frequency of usage, rate, efficacy on targeted weeds and persistence (Gressel and Segel 1982). The higher the intensity of selection imposed by a particular herbicide on a given weed population, the faster the expected rate of evolution and spread of resistance (Saari et al. 1994). Herbicides that have a long-term soil residual activity and are applied frequently with a highly specific mechanism of action will impose a more intense selection pressure against susceptible weed seedlings for the evolution of resistance (Jasieniuk et al. 1996).

Selection pressure is measured by the relative fitness of genotypes in the presence of a selective factor such as herbicide treatment. Population processes that determine the relative fitness of phenotypes are survivorship (demography of seeds, seedlings and mature plants) and fecundity (pollen and seed production). When an herbicide is used, its selection pressure (reduced survivorship of susceptible individuals) overwhelmingly increases the relative fitness of the resistant genotype. Only when herbicide selection pressure is removed will population dynamics be determined by differences in all the processes that contribute to the fitness of each biotype (Maxwell et al. 1990).

Population geneticists measure selection pressure by the relative fitnesses of genotypes in the presence of a selective factor such as herbicide treatment. Fitness can be defined as a measure of the number of gametes or progeny contributed by a particular genotype to the next generation. Therefore, fitness is a measure of the survival and reproduction of each genotype in the population under selection (Holt and LeBaron 1990). Relative fitness is a quantitative measure of the reproductive success of the weaker genotype compared to the more fit genotype. Relative fitness of genotypes is calculated by assigning a fitness value of 1 to the genotype with the highest gametic or

progeny contribution. The values that correspond to their reproductive success would then be subsequently assigned to susceptible and resistant genotypes. The difference between the relative fitness of the most prolific genotype and the least fit genotype is termed the selection coefficient. The selection coefficient is directly proportional to the intensity of selection against the less fit genotype. In the context of herbicide resistance evolution in weeds, a relative fitness value of 1 would be assigned to fully resistant genotypes in the presence of herbicide. Values corresponding to their relative reproductive success would then be assigned to the partially susceptible and resistant genotypes. The magnitude of the selection coefficient indicates the intensity of selection favoring resistant genotypes (Jasienuik et al. 1996).

Fecundity

Despite the importance of selection pressure imposed by herbicides on the rate of resistance evolution, accurate quantitative estimates of the selection intensity imposed by herbicides in terms of plant responses are lacking. Although herbicide selection intensity can be estimated by indicators of plant mortality, the reduction in seed return is a more accurate estimate of selection pressure. The reason for this is that seed production closely reflects the change in relative numbers of susceptible versus resistant weed individuals in the next generation. Seed production also takes into account the influence of susceptible individuals that miss herbicide treatment and contribute progeny to the next generation. These susceptible individuals reduce selection pressure and slow the rate of resistance evolution (Jasieniuk et al. 1996).

"Knockdown studies" according to Gressel and Segel (1978) refer to estimates of herbicide efficacy based on observation of initial mortality measured as relative

differences in weed densities or shoot dry matter. The assumption that a particular phenotype (resistant or susceptible) has a higher fitness value on the basis of shoot dry matter production alone is likely subject to considerable error. In order to accurately predict rates of increase or decline in resistance due to herbicide rotation or due to the complete cessation of use of a particular herbicide group, measurements of relative fitness must incorporate the differential survival and fecundity of susceptible and resistant plants (Debreuil 1996).

Gressel and Segel (1978) include seed production as a component of their estimate of selection pressure by measuring "effective kill". Jasienuik et al (1996) defines "effective kill" as the percentage decrease in seed yield at the end of a growing season due to treatment by an herbicide. Values are obtained for both susceptible and resistant plants and it was proposed by Gressel and Segel (1978) that seed return measurements could be used to quantify selection pressure of an herbicide. Selection pressure is calculated as the ratio of the fraction of resistant plants that survive herbicide treatment to the corresponding fraction of susceptible plants (Jasieniuk et al. 1996). Therefore, selection pressure is defined as (1-effective kill of resistant plants)/(1-effective kill of susceptible plants) (Debreuil 1996).

Mechanisms of Herbicide Resistance

There are 3 mechanisms by which plants have become resistant to formerly phytotoxic herbicides. These include a modification to the site of action of the herbicide, a change in the absorption and translocation of the herbicide so that it does not reach the target site of action and a change in the ability of the plant to metabolize the herbicide. Two of these mechanisms appear to account for the resistance of weeds to ALS

inhibitors. These are changes at the site of action and changes in the ability of plants to metabolize herbicides. The predominant mechanism has been a change in ALS so that it is no longer sensitive to the inhibitors (Shaner 1991).

Target Site Resistance

Target site resistance involves a reduction in the sensitivity of herbicide target site to inhibition by herbicides. Gene mutations at some loci, particularly those encoding herbicide sites of action may confer target site resistance (Jasienuik et al. 1996). In North America, selection with sulfonylurea herbicides has resulted in target site resistance with no other concurrently selected resistance mechanisms (Saari et al. 1994).

Rigorous studies have not been performed on the initial frequency of ALS inhibitor resistance mutations in weeds, but model studies often cite the frequency of target site ALS inhibitor resistance as $1x\ 10^{-5}$ or 1×10^{-6} gametes per locus per generation or less (Saari et al. 1994). The frequency of spontaneous chlorsulfuron resistance and imidazolinone resistance ALS mutations in mouse ear cress (*Arabidopsis thaliana* L. Heynh) is estimated to be approximately 1×10^{-8}. A similar frequency has been reported for imidazolinone resistant *A. thaliana* mutants using imazapyr as the selection agent (Saari et al. 1994).

The gene encoding ALS of several plant species has been sequenced and changes that confer resistance have been identified and mapped (Sibony et al. 2001). Target site resistance to all ALS inhibitors in all weed biotypes investigated to-date has been caused by one of five amino acid substitutions that, when changed, confer resistance to ALS inhibitors (Sibony et al. 2001). Three of these five amino acids ($Alanine_{122}$, $Proline_{197}$, $Alanine_{205}$) are located near the amino-terminal end of ALS and the other two amino

acids (Tryptophan$_{574}$ and Serine$_{653}$) are located near the carboxy-terminal end. These five amino acids are highly conserved among plant species and have been found at equivalent positions in nearly all cases. Common ragweed and common cocklebur, however, have an alanine rather than a serine residue at position 653 (Tranel and Wright 2002).

Several other single amino acid substitutions are sufficient to convert ALS from an herbicide sensitive to an herbicide resistant enzyme. In most cases, a single nucleotide difference resulting in a substitution of an amino acid of the ALS enzyme has been observed in at least one of the 5 highly conserved regions of the ALS gene. These regions have been designated as domains A, B, C, D and E and are 12-57 base pairs long. Mutations in domain A are most common. For example, Proline 173 (Pro$_{173}$) is contained in a highly conserved region of the ALS amino acid sequence known as domain A. Point mutation in the codon for Pro$_{173}$ confers resistance to prickly lettuce (*Lactuca serriola* L.), Arabidopsis (*Arabidopsis thaliana* L.), tobacco (*Nicotiana tabaccum L.*), and rapeseed (*Brassica napus*) (Guttieri et al. 1995). Evaluation of the DNA sequence in the region encompassing domain A in 10 chlorsulfuron-resistant *Kochia scoparia* biotypes from across North America indicated that 7 of the 10 biotypes had mutations in Pro$_{173}$. All six possible single point mutations conferring amino acid substitutions were identified in 7 of these resistant biotypes while 3 of the resistant biotypes did not have point mutations in Pro$_{173}$ the latter were analogous to the wild type (Guttieri et al. 1996).

In addition to the fact that different amino acid substitutions in ALS have been identified in resistant weed populations, multiple substitutions have been identified for at least two amino acids. In fact, eight different amino acid substitutions for Proline$_{197}$ (Pro$_{197}$) have been reported in herbicide resistant weed populations. Thus, there is a

relatively large amount of flexibility in the herbicide binding site of the ALS enzyme, without or with minimal consequences to normal catalytic function of the enzyme. A likely explanation for this is that the herbicide binding site of ALS is different from its active site, although the two sites are in close proximity (Tranel and Wright 2002).

Cross Resistance

Cross resistance occurs when a plant is resistant to multiple herbicides with the same mode of action. ALS inhibitor resistant weed biotypes are often cross resistant to herbicides within the same chemical family as the given selection agent, but exhibit varying patterns of cross resistance to herbicides in other chemical classes of ALS inhibitors (Poston et al. 2000). Although several amino acid substitutions will result in resistance to ALS inhibitors; the magnitudes of resistance to different ALS-inhibiting herbicides vary widely among substitutions. Since the five different chemical classes of ALS inhibitors bind to the ALS enzyme differently, different weed resistant biotypes may exhibit variable patterns of cross resistance (Friesen et al. 1993).

Although exceptions exist, resistance caused by an altered ALS can be classified into three types on the basis of cross resistance. These three types include sulfonylurea and triazolopyrimidine sulfonanilide resistance, pyrimidnylthiobenzoate and imidazolinone resistance and sulfonylurea, imidazolinone, pyrimidnylthiobenzoate and triazolopyrimidine sulfonanilide resistance (Tranel and Wright 2002).

Resistance to one compound of a particular class of ALS-inhibiting herbicides does not guarantee cross-resistance to all other members belonging to the same chemical family. This is particularly true of the imidazolinone (IMI) and sulfonylurea (SU) herbicides for which differential resistance (or lack thereof) has been reported in several

resistant weed biotypes (Hall and Devine 1990). For example, $Alanine_{122}$ or $Serine_{653}$ substitutions result in IMI but not SU resistance, whereas substitutions of Pro_{197} usually result in SU but not IMI resistance (Tranel and Wright 2002). For instance, chlorsulfuron resistant *A. thaliana* is cross-resistant to sulfometuron-methyl, but only marginally resistant to imazamethabenz and to imazapyr. In some cases, low to moderate levels of IMI resistance have also been observed in weed biotypes with the Pro_{197} substitution, but resistance is typically less than 10 fold and inconsistent among various IMI herbicides (Saari et al. 1994). For example, several biotypes of *D. innoxia* L. resistant to chlorsulfuron are also cross resistant to imazapyr, imazaquin and sulfometuron-methyl, while 6 of 8 biotypes of chlorsulfuron-resistant *C. reinharatii* are cross resistant to imazaquin but not to imazethapyr (Hall and Devine 1990).

Effects of ALS Target-Site Resistance on Plant Fitness

Given the large number of different resistant ALS alleles, not all have been investigated for fitness effects. Depending on the genetic background, the number and expression pattern of mutations among ALS loci, fitness costs caused by resistance will vary (Tranel and Wright 2002). Holt and Thill (1994) concluded that resistance-conferring mutations in ALS have subtle effects on plant growth and development but do not reduce plant fitness. In comparisons with resistant and susceptible kochia plants, Holt and Thill (1994) concluded that there were no significant differences in terms of the number of seed produced, biomass production or competitiveness. In contrast, a susceptible biotype of prickly lettuce (*Latuca sativa* L. Bibb) produced 30% more aboveground biomass relative to a biotype with target site resistance to ALS inhibitors, although the two biotypes were similar in terms of seed production and competitiveness

(Alcocer-Ruthling et al. 1992). However, these plant fitness studies may have used R and S biotypes that are not genetically similar. Thus, the observed differences in fitness may have been caused by genetic polymorphisms rather than the actual resistance mutation. Eberlein et al. (1999) minimized this confounding factor by comparing R and S biotypes of lettuce (*Latuca sativa* L. Bibb) derived from five generations of backcrossing. In this case, the R allele came from prickly lettuce and the two lettuce lines were greater than 96% similar. Two conclusions obtained from comparison of these two lines were that specific activity of ALS was higher in the susceptible than in the resistant biotype and that ALS from the R biotype was less sensitive to feedback inhibition by branched chain amino acids, resulting in greater amino acid accumulation (Eberlein et al. 1999). Fitness differences between the two lines were not described in this study; however, it seems likely that under certain environmental conditions such physiological differences could confer at least subtle fitness differences (Tranel and Wright 2002).

One of the few reports of a substantial fitness cost of an ALS inhibitor resistance gene comes from a study with Arabidopsis. Plants transformed with the *Csr-1* allele (which contains an asparagine for Pro_{197}) as well as the originally selected mutant line produced about 35% fewer seeds than the control plants, when grown in the field. Reduced fitness was attributed to the production of fewer siliques, rather than fewer seeds per silique. No differences among Arabidopsis lines were observed for biomass production. It was concluded that the observed fitness difference were specifically caused by the *Csr*-1 allele and not by any other genetic differences (Bergelson et al. 1996).

Herbicide Uptake and Translocation

Limitations in uptake of a herbicide or its movement to the site of action are often cited as possible mechanisms of resistance. In weeds, neither uptake nor translocation has yet been found to be a major contributor to ALS resistance. For example, uptake and translocation of foliarly applied ^{14}C-ethametsulfuron-methyl was nearly identical in resistant commercial brown mustard and susceptible wild mustard 72 hours after foliar application (Hall et al. 1992). Similarly, uptake and translocation of ^{14}C-chlorsulfuron in *K. scoparia* applied to a selected leaf demonstrated that the amount of chlorsulfuron translocated out of the leaf after 24 hours did not differ between resistant and susceptible biotypes and was only slightly greater after 48 hours for the resistant kochia biotype. However, this difference was not of sufficient magnitude or correct direction to be responsible for resistance (Saari et al. 1990). Reduced sensitivity of plants to ALS inhibitors due to lower rates of either uptake or translocation from the site of action plays only a minor role or no role at all compared to the primary mechanism of either metabolic inactivation or target site resistance (Saari et al. 1994).

Metabolic Inactivation

Another mechanism of resistance to ALS inhibitors is rapid metabolic alteration of the active herbicide to non-phytotoxic compounds to prevent lethal herbicide levels from reaching ALS (Kemp and Caseley 1991). Increased ability to detoxify xenobiotics has been widely reported as a mechanism of insecticide resistance. Insecticide detoxification is often mediated by cytochrome P450 (cyt P450) dependent monooxygenases (cyt P450). Cyt P450s are membrane bound enzyme systems

containing heme-coordinated divalent cations which facilitate the oxidation of a wide variety of substrates using reducing power from NADH or NADPH. There is increasing evidence for the occurrence of many different isozymes of cyt P450s in plant tissues that differ in level and range of substrates specificity as well as the level of inducibility or inhibition by exogenous chemicals (Christopher et al. 1994).

Microsomal cyt P450s capable of metabolizing herbicides have been isolated from several crop species and are often associated with herbicide insensitivity. Wheat microsomal cyt P450s with the capacity to metabolize diclofop-methyl, triasulfuron and chlorotoluron have been reported (Christopher et al. 1994). Other grasses such as barley and oats (*Avena sativa* L.) rapidly metabolize chlorsulfuron, the active ingredient in "Glean" herbicide killer, to a polar herbicidally inactive product. This metabolite has been identified as the *O*-glycoside of chlorsulfuron in which the phenyl ring has undergone hydroxylation followed by conjugation with a carbohydrate moiety (Sweeter et al. 1982). Maize is another crop in which cyt P450-dependent metabolism is important for herbicide insensitivity. Maize microsomal cyt P450s are able to metabolize primisulfuron, but tolerance of maize to this sulfonylurea herbicide is impaired by the use of some organophosphurous insecticides (Baerg et al. 1996). For example, in excised corn leaves, the addition of malathion to primisulfuron increased the metabolic half-life of primisulfuron. In microsomal preparations, malathion was shown to inhibit cytochrome P450-dependent primisulfuron phenyl- and pyrimidine-ring hydroxylation (Kreuz and Fonne-Pfister 1992).

Sensitive weed species that have been studied typically metabolize an active herbicide very slowly, if at all (Saari et al. 1994). Therefore, just as metabolic

inactivation is the mechanism by which crops are intrinsically resistant to certain herbicides; it also appears to be the mechanism responsible for herbicide selectivity in some tolerant weed species (Christopher et al. 1994). For example, a diclofop-methyl resistant *L. ridigium* biotype (SR4/84) shows non-target site cross resistance to chlorsulfuron and other wheat selective ALS inhibitors. In this weed biotype, chlorsulfuron is metabolized two to four times more rapidly in shoots and four times more rapidly in roots in resistant versus susceptible biotypes of *Lolium rigidium*. While chlorsulfuron metabolism is present in both the roots and shoots of the resistant biotype, the rate of metabolism in the roots plays an initial role in determining the sensitivity of *Lolium rigidium* to chlorsulfuron (Cotterman and Saari 1992). Sensitivity to chlorsulfuron in other grasses, including *Seraria viridis* L. Beauv. is related to how slowly chlorsulfuron is degraded in roots and this was found to be independent of the degradation rate in the shoots. The major metabolite produced in the diclofop-methyl resistant *Lolium rigidium* biotype has been identified as the glucose conjugate of hydroxy-chlorsulfuron. Minor metabolites include triazine amine and sulfonamide. All of these metabolites have been shown to be herbicidally inactive against ALS (Saari et al. 1994).

Jeffers et al. (1996) described a resistant *S. arvensis* biotype discovered in Alberta that was highly resistant to ethametsulfuron-methyl, slightly resistant to metsulfuron, but not resistant to any other sulfonylurea herbicides. On the basis of the lack of cross-selectivity of this biotype, it was suggested that resistance of *S. arvensis* to ethametsulfuron-methyl might be due to enhanced metabolism rather than an altered form of ALS (Jeffers et al. 1996).

Enhanced metabolism of ethametsulfuron-methyl in *S. arvensis* was later confirmed by Veldhuis et al. (2000). Veldhuis et al. (2000) reported that 48 hours after treatment (HAT), 17% of the recovered radioactivity remained as ethametsulfuron-methyl in the R wild mustard biotype but more than 70% of ^{14}C-ethametysulfuron-methyl remained in the susceptible biotype 72 HAT. Furthermore, the dosages of ethametsulfuron-methyl that inhibited wild mustard growth by 50% were >100 and <1 g ai ha^{-1} for resistant and susceptible wild mustard biotypes respectively under controlled environmental conditions. Although there was no qualitative difference in the metabolism in the resistant and susceptible biotypes, there was a quantitative difference between the biotypes. They reported that ethametsulfuron-methyl and a short-lived metabolite eluted at 31 and 29 min. This metabolite was further metabolized to two stable metabolites with retention times of 22 and 21 minutes (Veldhuis et al. 2000). Litchner et al. (1995) determined that the two latter metabolites are non-phytotoxic breakdown products of ethametsulfuron-methyl and has identified them as *O*-desmethylethametsulfuron-methyl and *N*-desmethyl-*O*-desethylethametsulfuron-methyl by cochromatography with an authentic reference standard.

Veldhuis et al. (2000) also confirmed that resistance to ethametsulfuron methyl in wild mustard was not due to differences in the target site or due to differences in absorption or translocation. In greenhouse experiments, both resistant and susceptible wild mustard biotypes were found to be equally sensitive to ethametsulfuron-methyl and chlorsulfuron as ALS enzyme activity was inhibited to the same extent regardless of dose. Uptake of ethametsulfuron-methyl was also found to be similar in resistant and susceptible wild mustard biotypes at all harvest times. For example, 72 HAT

approximately 83 and 78% of ethametsulfuron-methyl was absorbed by the treated leaves of the resistant and susceptible plants respectively. Furthermore, three days after treatment approximately 90, 5 and 2% of the applied ^{14}C-ethametsulfuron-methyl was found in the treated leaves, foliage and roots of each wild mustard biotype respectively. These results discount differences in absorption and translocation as possible resistance mechanisms in wild mustard to ethametsulfuron-methyl (Veldhuis et al. 2000).

Exploring Mechanisms of Herbicide Resistance with pesticide "restorer agents"

Strategies for controlling herbicide resistance in weeds depend currently upon the use of herbicide rotations and mixtures and changing agronomic and cultural practices. These methods are mainly preventative and are designed to delay the development of herbicide resistant weed populations by reducing the weed seed bank (Kemp and Caseley 1991).

Once weed resistance to herbicides is established in a field, alternative herbicides have to be used. Advances in our understanding of the modes of action of herbicides, their metabolism and mechanism of selectivity and synergistic interaction with adjuvants indicate that it may be possible to combat some forms of herbicide resistance more directly by the use of pesticide synergists (Kemp and Caseley 1991). In this sense these added products act as pesticide "restorer agents" and will be referred to as such in this thesis.

The cyt P450 inhibitors 1-aminobenzotriazole (ABT), piperonyl butoxide (PBO), malathion and tetcyclasis can enhance the phytotoxicity of certain herbicides by decreasing the rate of herbicide metabolism by plant microsomal cyt P450s. For example, Veldhuis et al. (2000) reported that the rate of ethametsulfuron-methyl

metabolism in a resistant biotype of wild mustard was reduced when PBO was added to ethametsulfuron-methyl as a pesticide restorer. Approximately 18 HAT, 58% and 37% of the recovered radioactivity remained as ^{14}C-ethametsulfuron-methyl in the PBO-treated and untreated leaves respectively. Furthermore, 18 HAT, 36% and 49% of the recovered ^{14}C-ethametsulfuron-methyl was found as nonphytotoxic breakdown products of ethametsulfuron-methyl in PBO-treated and untreated leaves respectively (Veldhuis et al. 2000).

Christopher et al. (1990) reported that the cyt P450 inhibitors ABT, PBO and tetcyclasis can enhance the phytoxtoxicity of chlorotoluron detoxification by wheat microsomal cyt P450s. The resistance spectrum of the *L. rigidum* biotype SLR 31 resembles that of wheat in that each species is resistant to chlorsulfuron, triasulfuron and metsulfuron-methyl, but both are sensitive to sulfometuron-methyl. Similar patterns of resistance for wheat and rigid ryegrass are also observed for the imidazolinone herbicides. Both species are resistant to imazamethabenz via enhanced metabolism, but are sensitive to the nonselective herbicide imazapyr (Christopher et al. 1990).

Maize is another crop in which cyt P450 dependent metabolism is important for herbicide insensitivity. Maize microsomal cyt P450s are able to metabolize primisulfuron to non-phytoxic compounds but the addition of the cyt P450 inhibitor malathion, inhibits primisulfuron metabolism. Malathion also increases chlorsulfuron toxicity in the chlorsulfuron resistant *L. rigidium* population SLR31. ^{14}C-chlorsulfuron metabolism in SLR31 was reduced from 83.5% 9 HAT in the absence of malathion to 13.3% in its presence. It has been suggested that chlorsulfuron metabolism in *L. rigidum* SLR 31 may be catalyzed by the same cyt P450 monooxygenase that also catalyzes the

oxidation of malathion. This would lead to competition between malathion and chlorsulfuron for the binding site of the detoxifying enzyme thereby lowering the level of chlorsulfuron resistance in the *L. rigidum* biotype (Christopher et al. 1994).

Like SLR31, rigid ryegrass population WLR1 is resistant to the wheat selective ALS inhibiting herbicides chlorsulfuron, triasulfuron, metsulfuron and imazamethabenz but WLR 1 tolerates higher dosages of these herbicides in comparison to SLR31. Similarly, resistant ryegrass biotypes SLR31 and WLR1 differ markedly in their response to sulfometuron-methyl and imazapyr. Application of sulfometuron-methyl controls the susceptible and the cross-resistant biotype SLR31 at doses greater than 16 g ai ha^{-1} but gave little control of WLR1 even at 64 g ai ha^{-1}. Similarly, doses >50 g ai ha^{-1} of imazapyr controlled the susceptible biotype and SLR31 but only gave 75% control of WLR1 (Christopher et al. 1990). Therefore, WLR1 exhibits resistance to both wheat selective and nonselective sulfonylurea and imidazolinone herbicides, whereas SLR31 is resistant only to the wheat-selective compounds. Although enhanced metabolism may be involved in chlorsulfuron resistance in biotype WLR1, Christopher et al. (1990) suggests that WLR1 may possess a mutant ALS that is less sensitive to inhibition by imazapyr and sulfometuron-methyl herbicides. This would explain the differences in the resistance spectrum of SLR31and WLR1 (Christopher et al. 1990).

It is possible that the enzymatic system responsible for chlorsulfuron degradation in rigid ryegrass *(Lolium rigidium)* is also effective on other herbicides (Tardif and Powles 1999). However, there are indications from studies on another rigid ryegrass population (WLR2) that the enzymes involved in herbicide metabolism can be relatively specific and do not show much cross selectivity (Preston et al. 1996). Results of whole

plant dose response experiments indicate a clear difference in response to cyt P450 inhibitors between metabolism based chlorsulfuron-resistant SLR31 and a metabolism based chlorotoluron-resistant WLR2. Malathion was able to increase the chlorsulfuorn toxicity in SLR31, while PBO and tetcyclasis increased chlorotoluron toxicity for biotype WLR2. The mechanistic basis for this difference has yet to be determined. One likely explanation is that the enzymes metabolizing chlorotoluron in *Lolium rigidium* WLR2 can be inhibited by ABT, tetcyclasis and PBO, but not by malathion, while enzymes metabolizing chlorsulfuron in SLR31 can be inhibited by malathion but not by other cyt P450 inhibitors (Christopher et al. 1994).

1.6 Weed Biotypes Resistant to ALS Inhibitor Herbicides

Even though ALS inhibitors were relatively recently introduced, the greatest number of reported herbicide resistant weed biotypes are resistant to this group of herbicides (Beckie et al. 2001). Only five years after the initial use of an ALS inhibitor herbicide, the first herbicide resistant weeds appeared and their incidence has steadily increased both in the number of sites and species. A common feature of many weed biotypes resistant to ALS inhibitors is that their selection often involved reliance on ALS inhibitors for their control. ALS resistance in some weed biotypes is not limited to a few isolated populations, but rather is so widespread and common as to pose a real threat to the continued use of this group of herbicides (Saari et al. 1994). For example in Illinois, so much of the waterhemp (*Amaranthus rudis* Sauer) is resistant to ALS inhibitors that these herbicides are no longer recommended for waterhemp control (Tranel and Wright 2002). Similarly, widespread occurrence of ALS resistant kochia in the intensive wheat production areas of the United States and Canada has limited the use of chlorsulfuron

(Guttieri et al. 1995). Ironically, it is the high efficacy that causes quick selection for the resistant biotypes; the same characteristic that enables ALS inhibitor herbicides to be used at very low dose rates (Saari et al. 1994).

Weeds resistant to ALS inhibiting herbicides are a great concern for several reasons. First, ALS-resistant biotypes do not show reduced fitness compared to susceptible members of the species. Thus, resistant individuals remain in the same proportion in the population even after the selection agent is removed, eliminating ALS inhibitors as an effective weed control option in fields in which resistant individuals reside. Secondly, resistance to ALS inhibiting herbicides is conferred by a single, semidominant, nuclear gene, allowing the survival of both homozygous and heterozygous plants. Therefore, ALS alleles may be spread through pollen in addition to seeds, increasing the movement of resistance to adjacent areas (Lovell et al. 1996). Thus, the genetics of ALS resistance partially accounts for the high frequency of occurrence of weeds resistance to ALS inhibitors relative to other herbicide groups (Tranel and Wright 2002).

Resistance to ALS inhibitors is common in many broadleaf weeds in western Canada and adjoining States and includes biotypes of chickweed (*Stellaria media* L. Vill.), kochia (*Kochia scoparia* L.), eastern black nightshade (*Solanum ptycanthum* L.), Russian thistle (*Salsola iberica* Sennen and Pan), prickly lettuce (*Lactuca serriola* L.), common cocklebur (*Xanthium stramarium* L.) and *Amaranthus* spp. In grass weeds, Group 2 herbicide resistance has been documented in three biotypes of wild oat (*Avena fatua*), perennial ryegrass (*Lolium perenne* L.) and giant foxtail (*Setaria faberi* Herrm. SETFA).

The first ALS inhibitor resistant weed biotype, chlorsulfuron resistant prickly lettuce, was identified in a field of winter wheat in Northern Idaho in 1987. This field had been annually treated with sulfonylurea herbicides for 5 years (Mallory-Smith et al. 1990). The basis of resistance in this biotype was due to an altered ALS that was much less sensitive to inhibition by chlorsulfuron. The resistance trait was found to be controlled by a single nuclear gene with incomplete dominance (Eberlein et al. 1997). DNA sequence analysis identified a point mutation in domain A encoding a histidine for Pro_{197} substitution in the resistant biotype (Eberlein et al. 1997). The resistant lettuce biotype was also cross resistant, at the whole plant level to imazapyr and imazethapyr but not to imazaquin (Guttieri et al. 1996).

Previous studies have shown 100% amino acid sequence homology in Domain A in susceptible biotypes of several weed species (Guttieri et al. 1995), suggesting that there is a strong selection pressure to conserve the wild-type amino acid sequence. This suggests that there may be a physiological cost to altering the consensus amino acid sequence of the ALS enzyme (Guttieri et al. 1996).

Eberlein et al. (1997) evaluated the effect of target site resistance on ALS activity in a sulfonylurea resistant prickly lettuce biotype with a $Proline_{173}$ to histidine substitution in Domain A. In addition to causing changes in ALS inhibitor sensitivity, amino acid substitutions in the ALS gene might also result in altered catalytic properties (Saari et al. 1992). ALS catalyzes the formation of acetolactate and the formation of acetohydroxybutyrate. Pyruvate will compete with 2-oxobutrate to determine whether acetolactate or acetohydoxybutyrate is formed. Eberlein et al. (1997) reported that the K_m (pyruvate) of ALS isolated from resistant and susceptible biotypes of prickly lettuce

were similar. This result suggests that the gene mutation for chlorsulfuron resistance does not impair pyruvate binding. Acetolacate production was also inhibited by 2-oxobutyrate in both biotypes of prickly lettuce suggesting that the mutation does not interfere with 2-oxobutyrate binding. In the same study, Eberlein et al. (1997) also observed that ALS activity in both biotypes of prickly lettuce was feed-back inhibited by valine, leucine and isoleucine. However, the ALS from resistant plants was 12 to 30% less sensitive to inhibition by the individual amino acids than the ALS isolated from susceptible plants. Due to the differential response to branched chain amino acids, the specific mutation site as well as the substituted amino acid may affect ALS structure and function (Eberlein et al. 1997). In this study, the specific ALS activity extracted from the resistant prickly lettuce biotype was 57% less than the specific activity of the S enzyme. In contrast, specific activities of chlorsulfuron resistant and susceptible biotypes of chickweed, kochia, and Russian thistle (*Salsola iberica* L.) were similar. Eberlein et al. (1997) concluded that the lower observed specific activity of ALS in the R prickly lettuce biotype was either due to the detrimental effects of mutation for resistance on ALS expression, function or stability, or it may have been coincidental; the consequence of using R and S biotypes with unknown genetic backgrounds (Eberlein et al. 1997).

In 1999, plants of *Solanum ptycanthum* resistant to ALS inhibitor herbicides were identified in a Wisconsin soybean field. The selection of *S. ptycanthum* cross-resistance to these herbicides occurred in an area of the field that had received 3 annual applications of imazethapyr. Greenhouse experiments conducted by Volenberg et al. (2000) confirmed that plants of *S. ptycanthum* at the three- to four-leaf-stage were 150 and 120 fold resistant to imazethapyr and imazamox and 5.9 fold resistant to primisulfuron in

comparison to the susceptible biotype. Resistance was associated with an insensitive ALS (Volenberg et al. 2000).

The majority of the reported incidences of sulfonylurea herbicide resistance are in *Kochia scoparia*. In all resistant kochia biotypes, the mechanism of resistance is reported to be a modified ALS that is less sensitive to herbicide inhibition. Friesen et al. (1993) reported chlorsulfuron resistance in a kochia biotype grown on an industrial site where chlorsulfuron was repeatedly applied over several seasons. This biotype was also confirmed to be resistant to other ALS inhibiting herbicides in growth room experiments. Friesen et al. (1993) reported that resistant plants were 2 to 180 fold more resistant to imazethapyr and five other sulfonylurea herbicides than susceptible plants, as measured by the ratio of dosages required to inhibit shoot dry matter accumulation by 50%. Similarly, *in vitro* assays indicated that from 3 to 30 times more herbicide was required to inhibit the ALS enzyme from resistant plants than from susceptible plants (Friesen et al. 1993).

Foes et al. (1999) reported a kochia biotype resistant to both triazine and acetolacate synthase-inhibiting herbicides from McDonough County, Illinois. Whole plant phytoxicity assays indicated that the biotype was resistant to atrazine, imazethapyr, thifensulfuron and chlorsulfuron. In comparison to the susceptible kochia biotype, resistance to these herbicides ranged from 500- to >28,000 fold in the resistant biotype. In *in vivo* ALS enzyme assays, the resistant kochia biotype also displayed high levels of resistance (2,000 to 9,000 fold) to ALS-inhibiting herbicides, indicating that resistance to these herbicides was site-of-action mediated. Resistance to the ALS and triazine-inhibiting herbicides in the resistant kochia biotype was conferred by a leucine for

tryptophan substitution at residue 570 of ALS and a glycine for serine at residue 264 of the D1 protein respectively (Foes et al. 1999).

Guttieri et al. (1995) compared the DNA sequence of an 83-base pair region of ALS for 10 chlorsulfuron resistant and three chlorsulfuron susceptible kochia biotypes. Point mutation in the codon for the proline residue at position 173 in Domain A of the ALS enzyme was observed in seven of ten resistant biotypes. Among these seven resistant biotypes, mutations to threonine, arginine, serine, leucine, glutamine, and alanine were identified. The mechanism of resistance was determined for the three resistant biotypes that did not have a mutation in Domain A. All three biotypes were resistant to chlorsulfuron due to a modified ALS, which indicates that chlorsulfuorn resistance in kochia is due to multiple resistance alleles (Guttieri et al. 1995).

Sulfonylurea herbicide resistance has been confirmed in Russian thistle (*Salsola iberica*), common chickweed (*Stellaria media*) and perennial ryegrass (*Lolium perenne*). Herbicide resistance in each of the three weed biotypes is due to an altered ALS that is less sensitive to inhibition by ALS inhibiting herbicides. In comparison to the three susceptible weed biotypes, the herbicide concentrations required to inhibit ALS activity by 50% were 4- to 50-times higher for the three resistant weed biotypes for five sulfonylurea (metsulfuron methyl, chlorsulfuron, thifensulfuron methyl, sulfometuron methyl, and triasulfuron) herbicides tested. Similarly, the post-emergent rates of three sulfonylureas (sulfometuron methyl, chlorsulfuron, and triasulfuron) and one imidazolinone (imazapyr) herbicide required to reduce dry weight accumulation by 50% (GR_{50}) were significantly greater for each of the resistant weed biotypes relative to the susceptible biotypes. The largest degree of resistance to sulfometuron methyl and

chlorsulfuron at the whole plant level was observed in perennial ryegrass where the GR_{50} for the resistant biotype increased from >300- to >100,000 fold. While not all of these herbicides are recommended for the agronomic control of the three weeds studied, this experiment demonstrated that selection for resistance with one ALS inhibiting herbicide can affect the response to other ALS inhibiting herbicides, even those with a dissimilar chemical structure (Saari et al. 1992).

ALS inhibitor herbicides have also failed to control some biotypes of common cocklebur (*Xanthium stramarium* L.) (Lee and Owen 2000). One of these resistant biotypes, isolated from Mississippi, arose from 3 years of banded applications of imazaquin. The basis of resistance in this biotype was determined to be a point mutation in the codon for an $Alanine_{133}$ to threonine substitution. This substitution is analogous to the alanine to threonine substitution in imidazolinone tolerant corn. Like imidazolinone tolerant corn, ALS isolated from the cocklebur biotype was cross resistant to pyrimidinyl oxybenzoate but not to chlorsulfuron or flumetsulam. A second imazaquin-resistant common cocklebur biotype was isolated from a field in Missouri that had received multiple applications of imazaquin over 4 years. Three unique point mutations encoding amino acid substitutions were identified in this resistant biotype. These amino acid substitutions included an $Asparagine_{522}$ to serine, $tryptophan_{552(551)}$ to leucine and $Glycine_{269}$ to Histidine. ALS isolated from the Missouri cocklebur biotype demonstrated resistance to imazaquin, pyrimidinyl oxybenzoate, chlorsulfuron and flumetsulam. Due to the similar pattern of cross resistance observed in Pioneer 3180 IR corn (imidazolinone tolerant corn), the $tryptophan_{552(551)}$ to leucine substitution was identified as the cause of herbicide resistance (Guttieri et al. 1996).

Two biotypes of *Stellaria media* from Stony Plain, Alberta, Canada have been identified as being highly resistant to chlorsulfuron. Resistance to chlorsulfuron in *S. media* is due to an altered ALS that is much less sensitive to chlorsulfuron inhibition. Similar results were obtained with triasulfuron, metsulfuron methyl and ethametsulfuron. However, the levels and patterns of cross-resistance varied, indicating that the alteration in ALS that confers chlorsulfuron resistance does not confer the same level of resistance to other sulfonylurea herbicides. The resistant biotypes were highly cross resistant to ethametsulfuron methyl and sulfometuron-methyl, but expressed a reduced level of cross-resistance to triasulfuron. Both biotypes also exhibited cross-resistance at both the whole plant and enzyme levels to D489 (N-(2,6-dichlorophenyl)-5,7-dimethyl-1,2,4-triazolo (1,5a) pyrimidine-2-sulfoamide), a triazolopyrimidine herbicide, while the susceptible biotype was highly sensitive to the herbicide. ALS activity of the resistant biotype was reduced by less than 10%, while ALS activity of the susceptible biotype was reduced by 90% in the presence of 0.1 micromolar D489 (Hall and Devine 1990).

A false cleavers (*Galium spurium* L.) population that survived treatment with triasulfuron/bromoxynil in 1996 was been identified in central Alberta, Canada, in a field that had been treated with ALS inhibitors in 3 of the previous 6 years. In greenhouse studies, this resistant biotype was found to be highly resistant to triasulfuron, thifensulfuron/tribenuron and sulfometuron and moderately resistant to imazethapyr. In addition, multiple resistance was identified to the auxin-type herbicide quinclorac (Gr_{50} value >6.7) but not to fluroxypyr (Gr_{50} value 1) or MCPA. Although other auxin type herbicides had been previously used on the field, quinclorac had never been applied. The specific mechanism of resistance to quinclorac is unknown. Analysis of ALS extracted

from the resistant biotype and a susceptible biotype from a nearby location indicated that resistance to ALS inhibitors was due to an altered target site. ALS I_{50} values for triasulfuron, metsulfuron, chlorsulfuron, thifensulfuron and imazethapyr were 36, 34, 92, 96 and 14 times higher, respectively, for the resistant biotype compared to the susceptible biotype (Hall et al. 1998).

Amaranthus spp. are extremely sensitive to many ALS-inhibiting herbicides and possess many characteristics often associated with herbicide-resistant weed biotypes such as a competitive growth habit and prolific seed production. Within the past 6 to 7 years, biotypes of *Amaranthus palmeri* S Wars., *Amaranthus retroflexus* L., *Amaranthus powellii* S Wats., *Amaranthus rudis* Sauer and *Amaranthus hybridus* L. resistant to various ALS inhibitors have been reported. In all instances, repeated use of ALS-inhibiting herbicides was documented (Poston et al. 2000).

In 1996, imidazolinone resistance was confirmed in four biotypes of *Amaranthus hybridus* from Marion, MD. In greenhouse experiments, postemergent applications of imazethapyr reduced shoot growth by 93% in the S population while shoot dry weight reductions in R populations ranged from 9 to 32%. In comparison to the susceptible population, resistant populations were 730 to 1,350 fold more tolerant to imazethapyr based on resistance ratios. These resistant biotypes were not found to be cross-resistant to the sulfonylurea herbicides chlorimuron and thifensulfuron. In the field, these resistant populations of *A. hybridus* were not effectively controlled with imazaquin (<8%) but were effectively controlled with chlorimuron (72%) and with other sulfonylurea herbicides such as thifensulfuron and nicosulfuron (99%) (Poston et al. 2000).

In the fall of 1996, a common waterhemp (*Amaranthus rudis* L.) biotype that was not controlled by triazine or ALS inhibiting herbicides was isolated from a field in Bond County, IL. This biotype was confirmed to be resistant to atrazine and three ALS-inhibiting herbicides in greenhouse and laboratory experiments. Based on whole plant dose response, the resistant biotype required over 1,000 times more imazethapyr to reduce growth by 50% relative to the susceptible biotype. Cross-resistance to thifensulfuron and flumetsulam, was also detected. Based on *in vivo* enzyme assays, ALS in the resistant biotype required more than 20 kg ha^{-1} of atrazine to inhibit growth by 50%. Chlorophyll fluorescence assays revealed that 10 M did not affect photosynthesis in the resistant biotype, whereas 100 nM of atrazine inhibited photosynthesis in the susceptible biotype. Regions of the genes encoding ALS and D1 proteins were sequenced to determine the molecular basis for herbicide resistance. ALS resistance was conferred by a leucine for tryptophan substitution at residue 569 of ALS, while triazine resistance was conferred by a glycine for serine substitution at residue 264 in the D1 protein (Foes et al. 1998).

Imazethapyr-resistant biotypes of palmer amaranth (*Amaranthus palmeri*) were studied by Sprague et al (1997) to determine the magnitude of resistance and cross-resistance to three acetolactate synthase inhibiting herbicides. Resistant biotypes of Palmer amaranth demonstrated >2,800-fold resistance to imazethapyr relative to susceptible biotypes. The concentration required for 50% *in vivo* inhibition of ALS activity was at least 13,000 times greater for resistant biotypes of Palmer amaranth compared to the susceptible biotype. Resistant biotypes also demonstrated cross-resistance to thifensulfuron and chlorimuron at both the whole plant and enzyme levels,

indicating that a less sensitive ALS enzyme conferred ALS inhibitor resistance in this case (Sprague et al. 1997).

In 1997, farmers in Ontario, Canada reported that some ALS-inhibiting herbicides failed to provide adequate control of certain *Amaranthus* spp. Growth room experiments were conducted by Ferguson et al. (2001) to confirm ALS-inhibitor resistance in populations of Powell Amaranth (*Amaranthus powellii*) and redroot pigweed (*Amaranthus retroflexus*). Twenty-two out of 35 seed samples were able to grow in the presence of soil-applied imazethapyr and thifensulfuron. Resistance to ALS inhibitors was confirmed in five and nine populations of redroot pigweed and Powell amaranth respectively. Within each species, the herbicide rate required to reduce plant dry weight by 50% (GR_{50}) was compared between the resistant and susceptible populations. For imazethapyr, resistance factors ranged from 33 to 168 and from 4.2 to 3,438 for redroot pigweed and Powell amaranth respectively. A high level cross-resistance to thifensulfuron was found in both populations of each species, with resistance factors ranging from 270 to 2,416 (Poston et al. 2000).

Certain populations of giant foxtail (*Setaria faberii*) in the north-central United States demonstrated a high level of resistance to acetolactate synthase inhibitors. Three biotypes of giant foxtail isolated from Minnesota, Wisconsin, and Illinois have been reported to be highly resistant to the sulfonylurea and imidazolinone herbicides. In the greenhouse, dose-response experiments using three-to four-leaf giant foxtail plants confirmed cross-resistance to nicosulfuron and imazethapyr in the Minnesota, Wisconsin, and Illinois biotypes to nicosulfuron and imazethapyr. Based on ED_{50} values (the effective dose that reduced shoot dry biomass by 50% compared to the non-treated

plants), the Minnesota, Wisconsin, and Illinois accessions were >320, >750, and >670-fold resistant to imazethapyr respectively and >1,900, 1,900- and 80-fold resistant to nicosulfuron respectively compared to the susceptible population. Genetic studies indicated that resistance to ALS inhibitor herbicides was due to an insensitive ALS enzyme in which resistance was conferred by a single, nuclear, semidominant allele (Volenberg et al. 2001).

Three wild oat (*Avena fatua)* populations resistant to fenoxaprop-p, imazamethabenz, flamprop were identified from the northwest agricultural region of Manitoba, Canada. These populations were identified after producer reports of failure of imazamethabenz to provide satisfactory wild oat control in the field. Although these wild oat populations had previously been exposed to other ALS and ACCase inhibiting herbicides, imazamethabenz had never been applied. In growth room experiments, resistant plants were 7.2 and 8.7 times more resistant to imazamethabenz and flamprop, than susceptible plants respectively, as measured by the ratio of dosages required to inhibit shoot dry matter by 50% (GR_{50}). Although the three wild oat populations did not differ significantly in levels of resistance to imazamethabenz nor to flamprop, they did significantly differ in their level of response to fenoxaprop-P (Friesen et al. 2000). Three additional populations of *A. fatua* with multiple herbicide resistance from other areas of Manitoba were identified in a field experiment in 1996. It is unlikely that altered target sites are the mechanism of multiple herbicide in these *A. fatua* populations. Current understanding of herbicide action indicates different target sites for each of flamprop, imazamethabenz, and fenoxaprop-P. Population genetics theory indicates that it would be very rare for three different target site mutations to develop in a plant population over

a short period of time, particularly in the absence of selection for flamprop and imazamethabenz (Friesen et al. 2000).

Multiple herbicide resistance in *A. fatua* is not rare. Approximately 40 additional populations of *A. fatua* with multiple herbicide resistance have been identified in all agricultural regions of Manitoba. The evolution of herbicide resistance in the absence of a direct selection agent is potentially a very serious problem. Producers with multiple herbicide resistant *A. fatua* are left with a very limited number of herbicides for selective wild oat control in crops that are commonly grown in western Canada (Friesen et al. 2000).

1.7 Characterizing Group 2 (ALS) resistant wild mustard biotypes in Manitoba

Wild mustard readily outcrosses. As a result, there is a potential for rapid spread of resistance by both pollen and seed movement from a field with herbicide resistant plants to a nearby or adjacent field with susceptible plants. Therefore, gene flow and the resultant increase in initial frequency of resistance genes would reduce the time that is required to reach a specific level of resistance within a field once a herbicide is applied (Jasienuik et al. 1996). This has important financial implications for growers because canola samples with greater than 5% wild mustard contamination are downgraded to sample grade (Blackshaw et al. 1989b) and wild mustard is a very competitive and highly persistent weed (Warwick et al. 2000).

In 2000, 30% of canola acres in western Canada were seeded with conventional canola varieties. In 1993, Alberta researchers reported metabolic resistance of wild mustard to ethametsulfuron, the active ingredient in Muster (Veldhuis et al. 2000). Recently, spraying with Muster herbicide failed to reduce wild mustard populations on

many acres of conventional canola in Manitoba. Numerous growers in southern Manitoba noticed persistent wild mustard infestations after one and in some cases, after two applications of Muster (ethametsulfuron-methyl) herbicide. In Manitoba, suspicious patches of wild mustard in conventional canola fields were sampled by Dupont employees. Some level of herbicide resistance to ethametsulfuron was found in 17 out of the 23 samples (Lyle Friesen, personal communication). Most of the resistant samples were collected in an area between Morris and Roland MB, although resistant populations were also confirmed around High Bluff (near Portage La Prairie), Crystal City and Altamont, Manitoba. There is a strong possibility that there are more ethametsulfuron resistant wild mustard populations in Manitoba.

The goal of this project is to characterize the levels and patterns of resistance for four wild mustard populations in Manitoba that are suspected to be resistant to ALS inhibitor herbicides. In collaboration with Dupont field research staff we will collect seed from suspected resistant populations in Manitoba. We will test these and previously collected samples in growth room dose response experiments to characterize the levels and patterns of ALS resistance (i.e. resistant to which ALS inhibitors and what levels of resistance). We will use three ALS inhibitor herbicides (ethametsulfuron-methyl, thifensulfuron-methyl and imazethapyr) with and without the use of malathion, a known cytochrome P450 inhibitor, to determine the possible mechanism of herbicide resistance in each wild mustard biotype. In a separate dose response experiment, we will test these ALS resistant wild mustard populations with a known group 4 resistant wild mustard population to test for multiple herbicide resistance to 2,4-D. It is expected that wild mustard populations that are resistant only to Muster (ethametsulfuron–methyl) should

show expected differences with the addition of malathion as a pesticide restorer, but the populations which possess an altered ALS target enzyme due to point mutations at certain loci should not. It is also not expected that these biotypes will show multiple herbicide resistance to 2,4-D. By broadly characterizing the levels and patterns of resistance to group 2 inihibitor herbicides we will be able to devise strategies for the containment and prevention of further resistance occurrences. We will also be able to determine which herbicides effectively control specific wild mustard populations and recommend to producers effective herbicidal or non-herbicidal control strategies for these wild mustard populations.

CHAPTER 2

Materials and Methods

Seed Source. The four acetolactate synthase (ALS) resistant wild mustard populations used in this study originated from farmers fields in MB. The farmers had reported that these ALS resistant wild mustard populations were able to survive two or more commercial applications of Muster herbicide. Populations UMWM02-01 and UMWM02-17 originating from Birch River, MB and Altamont, MB, respectively, were suspected to be resistant to a broad spectrum of sulfonylurea herbicides. Populations UMWM02-05 and UMWM02-08 originating from Lowe Farm and Roland, MB, respectively, were suspected to be resistant to only Muster herbicide. All resistant wild mustard populations were collected by Dupont researchers in August of 2000 after the pods had fully ripened and the seeds were considered to be mature. According to researchers at Dupont, the resistant wild mustard plants were not pure breeding with respect to the resistance trait as each population was not subsequently increased in isolation to ensure that the resistant populations were near-homozygous for the resistance trait. The phenoxy resistant wild mustard population (UMWM04-04) and the susceptible wild mustard population (UMWM00-00) that were used in this study originated from a field in Gilbert Plains, MB and Portage La Prairie, MB respectively. The susceptible biotype was collected by researchers from the University of Manitoba in August 2000.

All seed was stored at the University of Manitoba in the seed storage room at 5 C. Each wild mustard population was kept separate labeled paper envelopes and placed in a dark metal drawer for storage.

Greenhouse experiments. In order to obtain uniform emergence patterns among populations, UMWM00-00 seed was pre-germinated 24 hours before the seed of UMWM02-05, UMWM02-08, UMWM02-17 and UMWM04-04. Population UMWM02-01 was the only population which did not need to be pre-germinated and it was seeded directly into the prepared pots 24 hours before the pre-germinated seeds of the UMWM00-00, UMWM02-05, UMWM02-08, UMWM02-17 and UMWM04-04 populations were planted.

Pre-germination of wild mustard seeds was done in petri dishes lined with two Whatman No.1 filter papers. For each experiment run, six petri dishes were allocated per wild mustard population and labeled with a permanent black marker. Five mL of 100 ppm giberellic acid solution was added to each Petri-dish along with approximately 80 seeds of wild mustard. The petri dishes for each population were then kept in separate plastic tubs lined with paper towels moistened with water. Tubs were covered in a plastic and paper bag and placed in a dark drawer at room temperature for 72 hours. Germination was considered to have occurred when the radicle broke the seed coat.

The base of each fifteen cm plastic pot was lined with 2 cm of peat and then filled with a 1:1:1 clay soil/sand/peat potting mixture. The soil, sand and peat used to make this potting mixture were supplied by the University of Manitoba. The soil mix was covered with a 2 cm layer of Metro-Mix® 220 Growing Medium (W.R. Grace and Co. of Canada Ltd., Ajax, Ontario). The growing medium consists of a premixed combination of vermiculite, perlite, sphagham peat moss, granite sand and nutrients such as nitrogen (50.7 ppm), phosphorous (7.36 ppm) and potassium (83.3 ppm). The growing medium has a slightly acidic pH in the range of 5.5 to 6.5, ideal for most plant species.

After each pot was covered with the 2 cm layer of Metro-Mix®, 5 pre-germinated seeds or 5 seeds in the case of UMWM02-01, were planted at a 1 cm depth using a pair of clean tweezers. Each pot was labeled with a plastic stake denoting the population. Shortly after seedling emergence, the pots were thinned to a total of 2 plants per pot to reduce the effects of competition.

In 2003, suspicions were raised regarding potential herbicide residues in a particular batch of soil used to make the potting mixture. In two dose response experiments, the susceptible wild mustard population and the majority of the resistant wild mustard populations either failed to germinate or did not develop past the cotyledon stage. Although the soil was not tested for soil active herbicides, the susceptible and the resistant wild mustard populations were showing signs of susceptibility to either Glean (chlorsulfuron) or Odyssey (imazethapyr:imazamox) herbicide. In order to ensure that future potting mixtures were free from contamination; new soil was supplied by a local farmer. In 2002, the field from which the new soil was acquired had been cropped to sunflowers and it was confirmed that no soil residual herbicides had been applied to the field in the past five years. According to the Canadian System of Soil Classification, the soil found at the farm site was classified as a Black Chernozemic clay soil. In February 2003, 28 medium sized burlap potato bags were filled with top soil from the farm site. The top soil was obtained by using a pick axe and shovel. After the bags were filled, the soil samples were transported back to the University of Manitoba and 3 bags of soil were left to thaw in the greenhouse for approximately 48 hours. The soil was sieved and then mixed with the sand and peat to make the 1:1:1 clay soil/sand/peat potting mixture. The base of each fifteen cm plastic pot was lined with 2 cm of peat and then filled with a

1:1:1 clay soil/sand/peat potting mixture. The soil mix was covered with a 2 cm layer of Metro-Mix® 200 Growing Medium . Each pot was directly seeded with 5 pre-germinated seeds of UMWM02-00 at a 1 cm depth to test for the presence or absence of residual herbicides. All of the pre-germinated wild mustard seeds developed into normal, mature seedlings and did not show signs of herbicide injury due to soil residual herbicides.

The soil obtained from the farm site was used in all subsequent greenhouse experiments. This new soil contained many kochia and lambsquarters seeds. In order to ensure that the wild mustard seeds were planted into a relatively weed–free seed bed, the pots were filled with the potting mixture 1 week prior to planting the wild mustard populations. During this week, the pots were watered daily to encourage the kochia and lambsquarters seeds to germinate. After the kochia and lambsquarters seedlings had germinated and emerged, the seedlings were removed by hand.

All studies were conducted in a growth chamber set at 20/15C day/night temperatures with a 16/8 hour day/night regime and an irradiance of 480 u Em^{-2} s^{-1} photosynthetic photon flux density. To avoid etiolated seedlings, light banks in the growth room were lowered to just above the pots or seedling canopy. Plants were initially watered with soluble fertilizer (20-20-20) and then every third day after that with water alone. Approximately 16 days after planting (DAP), the herbicide treatments were applied when the wild mustard plants were at the 3-4 leaf stage and 10- 12 cm in diameter.

Herbicide rates (dosages) for each herbicide were calculated prior to spraying for each dose response experiment. Currently it is recommended that for wild mustard control in field crops, 2,4-D should be applied at 420 g ai ha^{-1} at the 2 to 4 leaf stage

(Manitoba Agriculture and Food 2003). It is also recommended that thifensulfuron-methyl, ethametsulfuron-methyl and imazethapyr be applied at 15, 7.5 and 15 g ai ha^{-1} at the same leaf stage as 2,4-D in field crops to obtain an optimal level of wild mustard control (Manitoba Agriculture and Food 2003).

In order to ensure that a log-logistic model could be fitted to the data, the herbicide dosages were were adjusted prior to repeating each dose response experiment to ensure that the data could be fitted to a log-logistic model (Seefeldt et al. 1995). The herbicide dosages for each dose response experiment are summarized in Table 1.

Table 1. Herbicide dosages used in the ethametsulfuron, ethametsulfuron with malathion, imazethapyr and thifensulfuron dose response experiments for wild mustard populations UMWM00-00 (susceptible), UMWM02-01, UMWM02-17, UMWM02-05, UMWM02-08 and UMWM04-04.

Wild Mustard Population UMWM00-00											
Ethametsulfuron[b] Experiment			Ethametsulfuron[b] with malathion[a] Experiment			Imazethapyr[b] Experiment		Thifensulfuron[b] Experiment		2,4-D[b] Experiment	
Run #1	Run #2	Run #3	Run #1	Run #2	Run #3	Run #1	Run #2	Run #1	Run #2	Run #1	Run #2
g ai ha^{-1}			g ai ha^{-1}			g ai ha^{-1}		g ai ha^{-1}		g ai ha^{-1}	
0	0	0	0	0	0	0	0	0	0	0	0
2	2	1	1	1	0.05	0.5	0.1	0.5	0.05	13	3
4	4	3	2	2	0.1	1	0.25	1	0.1	25	13
8	8	6	4	4	0.5	2	0.5.	2	0.25	50	25
15	15	15	8	8	1	4	1	4	0.5	100	50
30	30	30	15	15	2	8	2		1	150	100
		60	30	30	4		4		2		150
					8				4		

Table 1 continued.....

Wild Mustard Population UMWM02-01

Ethametsulfuron[b] Experiment			Ethametsulfuron[b] with malathion[a] Experiment			Imazethapyr[b] Experiment		Thifensulfuron[b] Experiment		2,4-D[b] Experiment	
Run #1	Run #2	Run #3	Run #1	Run #1	Run #2	Run #1	Run #2	Run #1	Run #2	Run #1	Run #2
g ai ha^{-1}			g ai ha^{-1}			g ai ha^{-1}		g ai ha^{-1}		g ai ha^{-1}	
0	0	0	0	0	0	0	0	0	0	0	0
8	4	3	8	13	3	0.5	0.1	4	0.5	13	3
15	8	6	15	25	13	1	0.25	8	1	25	13
30	15	15	30	50	25	2	0.5.	15	2	50	25
60	30	30	60	100	50	4	1	30	4	100	50
	60	60	120	150	100	8	2	60	8	150	100
	120	120	0		150		4		15		150
					120				30		

Wild Mustard Population UMWM02-17

Ethametsulfuron[b] Experiment			Ethametsulfuron[b] with malathion[a] Experiment			Imazethapyr[b] Experiment		Thifensulfuron[b] Experiment		2,4-D[b] Experiment	
Run #1	Run #2	Run #3	Run #1	Run #1	Run #2	Run #1	Run #2	Run #1	Run #2	Run #1	Run #2
g ai ha^{-1}			g ai ha^{-1}			g ai ha^{-1}		g ai ha^{-1}		g ai ha^{-1}	
0	0	0	0	0	0	0	0	0	0	0	0
8	8	NA	8	8	0.05	0.5	0.1	4	0.5	13	6
15	15	NA	15	15	0.1	1	0.25	8	1	25	13
30	30	NA	30	30	0.5	2	0.5.	15	2	50	25
60	60	NA	60	60	1	4	1	30	4	100	50
120	120	NA	120	120	2	8	2	60	8	150	100
					4		4		15		150
					8				30		

Wild Mustard Population UMWM02-05

Ethametsulfuron[b] Experiment			Ethametsulfuron[b] with malathion[a] experiment			Imazethapyr[b] experiment		Thifensulfuron[b] Experiment		2,4-D[b] Experiment	
Run #1	Run #2	Run #3	Run #1	Run #2	Run #3	Run #1	Run #2	Run #1	Run #2	Run #1	Run #2
g ai ha^{-1}			g ai ha^{-1}			g ai ha^{-1}		g ai ha^{-1}		g ai ha^{-1}	
0	0	0	0	0	0	0	0	0	0	0	0
2	2	1	1	2	0.5	0.5	0.1	0.5	0.05	13	3
4	4	3	2	4	1	1	0.25	1	0.1	25	6
8	8	6	4	8	2	2	0.5.	2	0.25	50	13
15	15	15	8	15	4	4	1	4	0.5	100	25
		30		30	8		2		1	150	50
		60		60	15		4		2		100
									4		150
									8		

Table 1 continued....

Wild Mustard Population UMWM02-08											
Ethametsulfuron[b] Experiment			Ethametsulfuron[b] with malathion[a] Experiment			Imazethapyr[b] Experiment		Thifensulfuron[b] Experiment		2,4-D[b] Experiment	
Run#1	Run #2	Run #3	Run #1	Run #2	Run #3	Run #1	Run #2	Run #1	Run #2	Run #1	Run #2
g ai ha^{-1}			g ai ha^{-1}			g ai ha^{-1}		g ai ha^{-1}		g ai ha^{-1}	
0	0	0	0	0	0	0	0	0	0	0	0
2	2	1	1	2	0.5	0.5	0.1	0.5	0.05	13	3
4	4	3	2	4	1	1	0.25	1	0.1	25	6
8	8	6	4	8	2	2	0.5.	2	0.25	50	13
15	15	15	8	15	4	4	1	4	0.5	100	25
30	30	30	15	30	8	8	2	8	1	150	50
		60		60	15		4	15	2		100
					30				4		150
									8		
Wild Mustard Population UMWM04-04											
Ethametsulfuron[b] Experiment			Ethametsulfuron[b] with malathion[b] experiment			Imazethapyr[b] experiment		Thifensulfuron[b] Experiment		2,4-D[b] Experiment	
Run #1	Run #2	Run #3	Run #1	Run #2	Run #3	Run #1	Run #2	Run #1	Run #2	Run #1	Run #2
g ai ha^{-1}			g ai ha^{-1}			g ai ha^{-1}		g ai ha^{-1}		g ai ha^{-1}	
c	c	c	c	c	c	c	c	c	c	0	0
c	c	c	c	c	c	c	c	c	c	13	3
c	c	c	c	c	c	c	c	c	c	25	6
c	c	c	c	c	c	c	c	c	c	50	13
c	c	c	c	c	c	c	c	c	c	100	25
c	c	c	c	c	c	c	c	c	c	150	50
c	c	c	c	c	c	c	c	c	c		100
c	c	c	c	c	c	c	c	c	c		150

[a]Malathion mixed at the rate of 1500 g ai ha^{-1} with ethametsulfuron herbicide
[b]Agral 90 was added to all spray solutions at a rate of 0.2% v/v.
cBiotype UMWM04-04 was excluded from these dose response experiments and served as the group 4 resistant check

All pots in each dose response experiment in the growth chamber were arranged in a complete randomized design pre and post spray. Dose response experiments conducted with Refine (thifensulfuron), Pursuit (imazethapyr) and 2,4-D were repeated twice while dose response experiments conducted with Muster (ethametsulfuron) alone and Muster with 1500 g ai ha^{-1} of malathion were repeated 3 times. In the first dose response experiments with Muster, Refine, Muster with malathion, and Pursuit each

herbicide treatment was replicated 3 times. In the second (or third) runs of these experiments, each herbicide treatment was replicated 6 times. For the first 2,4-D dose response experiment, each herbicide treatment was replicated 4 times while in the second, each herbicide treatment was replicated 5 times.

Herbicides were applied using a moving-nozzle cabinet sprayer equipped with a 80015 flat-fan nozzle that delivered 117 L ha^{-1} spray solution at 275 kPa in a single pass over the foliage. To determine shoot biomass at the time of herbicide application, 12 plants (6 pots) of each population were harvested, oven dried for 48 hours at 80C and subsequently weighed. Sixteen days after herbicide application, shoot biomass was determined for all treated plants. All the plants were harvested by cutting off the shoots at soil level with a pair of scissors. Each pot was assigned 1 or 2 envelopes depending on the results. One envelope was assigned if the result was consistent (either plants survived herbicide treatment or both plants did not survive herbicide treatment), 2 envelopes per pot were assigned if the results were inconsistent (1 plant survived herbicide treatment and the other plant did not). Each envelope was denoted with the number of plants, the herbicide treatment and the wild mustard population. Envelopes and contents were oven dried for 72 hours at 80C and then subsequently weighed.

Field experiment. A field experiment was conducted in 2002 at Lowe Farm, Manitoba to assess the response of UMWM02-05 (an indigenous population) to ethametsulfuron (Muster), ethametsulfuron (Muster) with 1500 g ai ha^{-1} of malathion, thifensulfuron (Refine) and imazethapyr (Pursuit). The soil at the experimental site was a Black Chernozemic clay soil and in 2001, the experimental area had been seeded with Roundup® Ready canola. The experimental site was established on land which had not

been sprayed with glyphosate herbicide that year. The experiment was arranged as a randomized complete block design with three replications. Individual plots were 2.5 m x 7 m. Eleven randomized treatments included an untreated control, ethametsulfuron at 7.5, 15, 30 and 60 g ai ha^{-1}, ethametsulfuron at 7.5, 15, 30 and 60 g ai ha^{-1} tanked mixed with 1500 g ai ha^{-1} malathion, thifensulfuron-methyl at 15 g ai ha^{-1} and imazethapyr at 15 g ai ha^{-1}. The adjuvant Agral 90 was added at 0.2% v/v to all herbicide treatments.

All herbicide treatments were applied on June 27, 2002 using a bicycle-wheel plot sprayer equipped with flat fan nozzles delivering 107 L ha^{-1} of spray solution at 275 kPa. At the time of herbicide application, the indigineous resistant wild mustard plants had 4 to 5 leaves. Natural infestations of Canadian thistle were controlled with Lontrel 360 Herbicide applied at a rate of 300 g ai ha^{-1} of clopyralid. The herbicide was applied when the Canada thistle seedlings were in the rosette to pre-bud stage of growth.

Wild mustard densities were determined in each plot on July 17, 2002 (20 days after herbicide application). A 0.25 m^2 quadrat was used to assess the number of wild mustard plants that survived herbicide treatment. The quadrat was tossed in two random directions in each plot. Wild mustard plants that had survived herbicide treatment were either just beginning to bolt or they were well into the inflorescence stage of growth. Visual ratings for wild mustard control were taken at the same time as plant counts for wild mustard densities. Control of wild mustard was subjectively measured on a quantitative scale ranging from 0% to 100%, where 0% represented no herbicidal control and 100% represented complete herbicidal control. In late August, the entire experimental area was mowed prior to seed maturation of both the canola crop and the

wild mustard population. The experimental area was mowed to prevent or minimize resistant wild mustard seed return.

Statistical Analyses. Statistical analysis of the dose response curves from the growth experiments closely followed the procedure outlined by Seefeldt et al. (1995). Initially a log-logistic model was fitted to the data.

$$y = C + (D\text{-}C)/(1 + \exp(b(\ln(x) - \ln(GR_{50})))), \qquad \text{(Eq. 1)}$$

where y = shoot dry matter (percentage of untreated control), x = herbicide dosage (g ai ha^{-1}; a small positive value of 1.0 was assigned to 0 g ai ha^{-1} dosage to allow for calculation of natural logarithms), C = lower limit (asymptote) of the response curve, D = upper limit, b = slope, and GR_{50} = dosage (g ai ha^{-1} of herbicide that reduced shoot dry matter by 50% relative to the untreated control). The exp refers to e (the base of the natural logarithm). Individual curves were statistically tested systematically for common C and D, common b, and common GR_{50} using the lack-of-fit F test at the 0.05 level of significance, as outlined by Seefeldt et al. (1995).

One of the advantages of using the curve described by (Eq. 1) is that the parameters are biologically meaningfull. The upper limit D corresponds to the mean response of the control and the lower limit C is the mean response at very high doses (note that the lower limit is not necessarily zero). The parameter b described the slope of the curve around the GR_{50}. The greater the value of b, the steeper the slope of the curve (Seefeldt et al.1995).

Subsequently, to estimate the magnitude of resistance levels, the GR_{50} term in the above model was replaced by the resistance factor (RF) multiplied by the derived value of GR_{50UM00} (i.e. RF x GR_{50UM00}, where GR_{50UM00} is the dosage (g ai ha^{-1}) that reduced

shoot dry matter for a given population by 50% relative to untreated plants for UMWM00-00, the susceptible biotype). Models were fitted to the data using a derivative-free nonlinear regression procedure (PROC NLIN) using SAS. Coefficients of determination (r^2) were calculated as described by Kvalseth (1985) using the residual sum of squares value from the SAS output. As outlined by Seefeldt et al. (1995), SAS provides only one residual sum of squares value for the model as a whole, even though parameters for several functions (i.e., S and R response curves) are estimated simultaneously (Friesen et al. 2000). Standard errors are presented (Table 2). The standard error of the parameter estimate is a measure of confidence, and if the standard error is large, the parameter is poorly estimated. Parameter estimates can be considered statistically significant at the 0.05 level if the standard error is less than half the numerical value of the estimate (Koutsoyiannis 1977).

Data from field trials were subjected to analysis of variance (ANOVA) and means were separated using Fisher's Protected LSD test at the 0.05 level of significance (Gomez and Gomez 1984). Standard errors are included to provide additional statistical information, as suggested by Morse and Thompson (1981).

CHAPTER 3

RESULTS AND DISCUSSION

Growth Room Experiments

1) Whole plant dose response to spray applications of ethametsulfuron herbicide.

Results of the growth room experiments provided clear evidence that the four wild mustard populations suspected to be resistant to ALS inhibitor herbicides were in fact, resistant to ethametsulfuron. The response of pot cultured plants treated by spray applications of ethametsulfuron is shown in Figure 1, Table 2.

▽ UMWM02-17	△ UMWM02-08
◇ UMWM02-01[a]	○ UMWM00-00
□ UMWM02-05[a]	

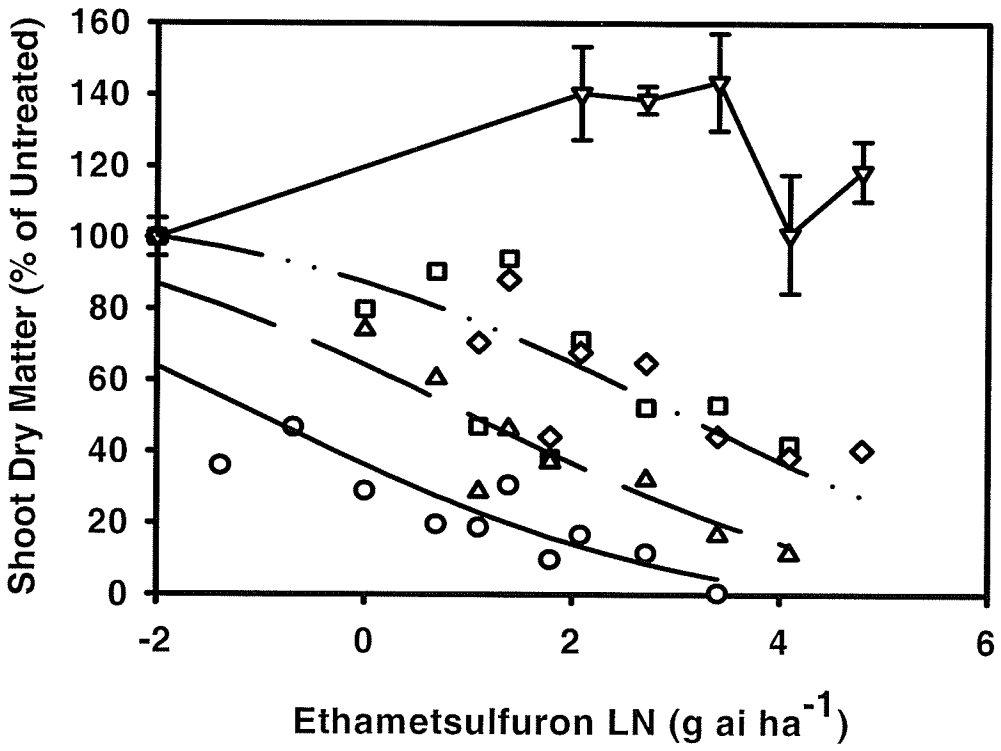

Figure 1. The effect of ethametsulfuron herbicide on shoot dry biomass expressed as a percent of the untreated control on 1 susceptible (UMWM00-00) and 4 ALS inhibitor herbicide resistant wild mustard biotypes. Bars around mean points represent +/- standard error. Common models in this figure are indicated by [a].

Table 2. Parameter estimates for models of the relationship between herbicide dose and wild mustard biomass as affected by 1) ethametsulfuron, 2) ethametsulfuron tank mixed with 1500 g ai ha^{-1} malathion, 3) imazethapyr 4) thifensulfuron or 5) 2,4-D. Values in parentheses are standard errors. *C*, *D*, *b*, and GR_{50} are model parameters. See Materials and Methods for the models.

	Parameter Estimates					
Ethametsulfuron Biotype	*C*	*D*	*b*	GR_{50} (g ai ha^{-1})	[d]R/S Ratio	Overall model r^2
UMWM00-00	-8.39 (25.98)	110.50 (19.82)	0.47 (0.24)	0.34 (0.33)		0.81
UMWM02-05	-8.39 (25.98)	110.50 (19.82)	0.47 (0.24)	20.07 (16.87)	58.60 (16.11)	
UMWM02-08	-8.39 (25.98)	110.50 (19.82)	0.47 (0.24)	2.60 (2.25)	10.56 (3.87)	
UMWM02-01	-8.39 (25.98)	110.50 (19.82)	0.47 (0.24)	20.07 (16.87)	58.60 (16.11)	
[a]UMWM02-17	----------	----------	----------	----------	----------	
Ethametsulfuron with [b]malathion Biotype	*C*	*D*	*b*	GR_{50} (g ai ha^{-1})	[d]R/S Ratio	Overall model r^2
UMWM00-00	1.51 (6.46)	105.00 (7.27)	0.57 (0.12)	0.07 (0.03)		0.91
UMWM02-05	1.51 (6.46)	105.00 (7.27)	0.57 (0.12)	1.89 (0.81)	15.53 (5.15)	
UMWM02-08	1.51 (6.46)	105.00 (7.27)	0.57 (0.12)	0.24 (0.12)	3.52 (1.45)	
UMWM02-01	1.51 (6.46)	105.00 (7.27)	0.57 (0.12)	10.12 (4.10)	149.2 (47.44)	
UMWM02-17	1.51 (6.46)	105.00 (7.27)	0.57 (0.12)	29.29 (11.88)	432.1 (136.70)	
Imazethapyr Biotype	*C*	*D*	*b*	GR_{50} (g ai ha^{-1})	[d]R/S Ratio	Overall model r^2
UMWM00-00	9.89 (4.95)	111.7 (5.03)	2.66 (1.03)	0.35 (0.07)		0.93
UMWM02-05	9.89 (4.95)	111.7 (5.03)	0.88 (0.16)	0.35 (0.07)	0.64 (0.02)	
UMWM02-08	9.89 (4.95)	111.7 (5.03)	0.88 (0.16)	0.35 (0.07)	0.64 (0.02)	
UMWM02-01	9.89 (4.95)	111.7 (5.03)	0.88 (0.16)	0.35 (0.07)	0.64 (0.02)	
UMWM02-17	9.89 (4.95)	111.7 (5.03)	2.66 (1.03)	1.08 (0.16)	3.08 (0.31)	
Thifensulfuron Biotype	*C*	*D*	*b*	GR_{50} (g ai ha^{-1})	[d]R/S Ratio	Overall model r^2
UMWM00-00	10.58 (2.43)	120.00 (c)	1.59 (0.26)	0.04 (0.004)		0.93
UMWM02-05	10.58 (2.43)	120.00 (c)	0.56 (0.06)	0.04 (0.004)	1.03 (0.25)	
UMWM02-08	10.58 (2.43)	120.00 (c)	1.59 (0.26)	0.04 (0.004)	1.03 (0.25)	
UMWM02-01	10.58 (2.43)	120.00 (c)	0.56 (0.06)	0.29 (0.09)	7.25 (3.50)	
UMWM02-17	10.58 (2.43)	120.00 (c)	0.56 (0.06)	0.29 (0.09)	7.25 (3.50)	
2,4-D Biotype	*C*	*D*	*b*	GR_{50} (g ai ha^{-1})	[e]R/S Ratio	Overall model r^2
UMWM00-00	8.99 (1.83)	120.00 (c)	2.09 (0.24)	1.92 (0.14)		0.94
UMWM02-05	8.99 (1.83)	120.00 (c)	2.09 (0.24)	1.92 (0.14)	----------	
UMWM02-08	8.99 (1.83)	120.00 (c)	2.09 (0.24)	1.92 (0.14)	----------	
UMWM02-01	8.99 (1.83)	120.00 (c)	2.09 (0.24)	1.92 (0.14)	----------	
[f]UMWM02-17	8.99 (1.83)	120.00 (c)	2.09 (0.24)	1.92 (0.14)	----------	
UMWM04-04	8.99 (1.83)	120.00 (c)	0.70 (0.12)	7.08 (1.58)	----------	

[a]UMWM02-17 indicates that no convergence was met for the sigmoidal model.
[b]malathion mixed at the rate of 1500 g ai ha^{-1} with ethametsulfuron herbicide.
[c]*D* had to be bound in the non-linear regression procedure; hence a standard error was not calculated.
[d]R/S ratio was calculated by the non-linear regression procedure in SAS.
[e]R/S ratio was not calculated for this dose response experiment
[f]UMWM02-17 indicates that parameter estimates are based on a single run for the sigmoidal model.

Individual plant responses to herbicide treatment were visually inconsistent within each resistant wild mustard population. Notice for example the size of the standard deviation around the means for biotype UMWM02-17. This inconsistency is not surprising because each resistant wild mustard population was not true breeding with respect to the resistance trait (i.e. a small proportion of individuals within each resistant population may have been susceptible to the herbicide treatment). In addition, wild mustard readily outcrosses. According to Jasieniuk et al. (1996), a newly arisen resistance mutation will initially be present in the heterozygous state. In a highly cross-pollinated weed species such as wild mustard, most of the progeny from resistant heterozygote plants are most likely susceptible to herbicide treatment because of the initial lack or extreme rarity of plants carrying the resistance allele in the population (Jasieniuk et al. 1996).

The log-logistic model described the response of four out of five wild mustard biotypes very well as indicated by the high r^2 value (0.81) (Table 2). For one biotype (UMWM02-17), a log-logistic model could not be fitted to the data and only the treatment means and standard errors are presented (Figure 1). Biotypes UMWM00-00, UMWM02-05, UMWM02-08 and UMWM02-01 all had a common *C* (lower limit of the dose response curve), *D* (upper limit of the dose response curve) and *b* (slope) (Table 2), thus the curves are parallel. It has been suggested that parallel dose response curves indicate that the herbicide was acting at the same site of action in each biotype but that the affinity of the herbicide at its site of action is reduced for the resistant biotypes (Streibig et al. 1993). In most cases, ALS herbicide resistance is conferred by a single semidominant nuclear gene and is endowed by modification of the target site by multiple

point mutations (Beckie et al. 2001). Varying levels of resistance have been associated with specific regions or domains and different amino acid substitutions result in quite predictable patterns of cross-resistance. For example, proline changes in Domain A of the ALS gene are likely responsible for the high level of insensitivity to sulfonylurea herbicides and moderate to low levels of resistance to the imidazolinone herbicides (Devine et al. 1991; Guttieri et al. 1995).

Overall, the response to increasing dosages of ethametsulfuron was similar (not statistically different) only for biotypes UMWM02-05 and UMWM02-01 (Figure 1). Furthermore, biotypes UMWM02-05 and UMWM02-01 had a common GR_{50} (g ai ha^{-1} that reduced above ground shoot biomass by 50% relative to the untreated control) of 20.07 g ai ha^{-1}. This common GR_{50} was relatively high in comparison to biotype UMWM02-08 (2.60 g ai ha^{-1}) and to susceptible biotype UMWM00-00 (0.34 g ai ha^{-1}) (Table 2). A previous study conducted by Veldhuis et al. (2000) on ethametsulfuron-resistant wild mustard from Vegreville, Alberta indicated that under controlled environmental conditions, herbicide doses of ethametsulfuron that inhibited growth by 50% (ED_{50}) were >100 and <1 g ai ha^{-1} for ethametsulfuron-resistant and susceptible wild mustard, respectively.

Levels of resistance were detectable both visually and quantitatively and were statistically significant (Figure 1, Table 2). The R/S ratios derived from the herbicide dosages required to inhibit shoot dry matter accumulation by 50% (GR_{50}) calculated from the log-logistic model, indicate that biotypes UMWM02-05 and UMWM02-01 were highly resistant to ethametsulfuron (R/S ratio of 58.60). In contrast, UMWM02-08 demonstrated a much lower level of resistance to ethametsulfuron (R/S ratio of 10.56)

(Figure 1, Table 2). A previous study conducted by Jeffers et al. (1995) on ethametsulfuron-resistant wild mustard biotypes in Alberta, Canada indicated that the resistant population was considerably more resistant to ethametsulfuron than the susceptible population, with ED_{50} values indicating a 48 fold difference in susceptibility (Jeffers et al. 1995).

2) Whole plant dose response to spray applications of ethametsulfuron herbicide tank mixed with 1500 g ai ha^{-1} of malathion.

The response of pot cultured plants treated by spray applications of ethametsulfuron in the presence of malathion are shown in Figure 2.

▽ UMWM02-17	△ UMWM02-08
◇ UMWM02-01	○ UMWM00-00
□ UMWM02-05	

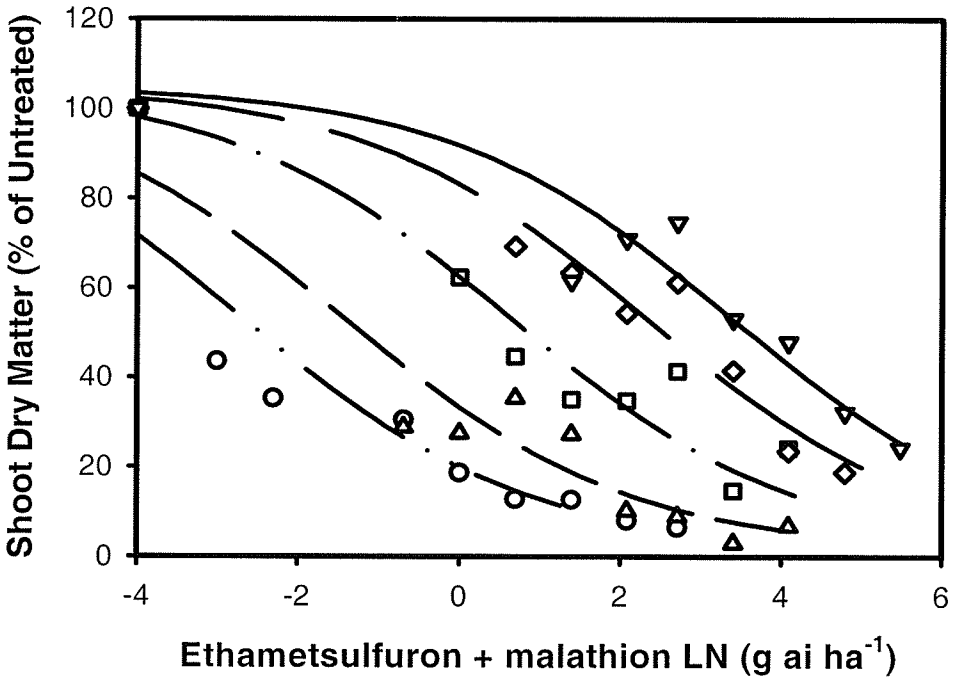

Figure 2. The effect of ethametsulfuron herbicide tank mixed with 1500 g ai ha^{-1} of malathion on shoot dry biomass expressed as a percent of the untreated control on 1 susceptible (UMWM00-00) and 4 ALS inhibitor herbicide resistant wild mustard biotypes.

A log-logistic model best described the relationship between shoot growth and herbicide dosage for all wild mustard biotypes as indicated by the high r^2 value (0.91) (Table 2). As shown in Table 2, the susceptible and resistant dose-response curves all had the same lower (*C*) and upper (*D*) limits and the same slope (*b*) producing statistically distinct but parallel dose response curves. Although the GR_{50} values differed among wild mustard biotypes, the GR_{50} for ethametsulfuron was reduced for all wild mustard biotypes when the herbicide was tank-mixed with 1500 g ai ha^{-1} of malathion (Table 2). This result indicates that malathion may interact with ethametsulfuron in all four ALS-resistant wild mustard biotypes and in the susceptible biotype to reduce the amount of ethametsulfuron required to reduce above ground shoot biomass by 50% relative to the untreated control. As shown by Table 2, the GR_{50} for biotypes UMWM02-05 and UMWM02-08 were substantially reduced by the addition of 1500 g ai ha^{-1} malathion to ethametsulfuron (from 20.07 and 2.60 to 1.89 and 0.24 g ai ha^{-1} respectively). In comparison, the GR_{50} for biotypes UMWM02-01 and UMWM02-17 did not approach the GR_{50} of the susceptible biotype UMWM00-00 but were still reduced in comparison to the GR_{50} values when ethametsulfuron was applied alone (Table 2).

The R/S ratios derived from the herbicide dosages required to inhibit shoot dry matter accumulation by 50% (GR_{50}) calculated from the log-logistic model, indicated that biotypes UMWM02-01 and UMWM02-17 continued to demonstrate high levels of resistance to ethametsulfuron even in the presence of malathion (Table 2). However, the addition of 1500 g ai ha^{-1} malathion to ethametsulfuron had a relatively greater effect on biotype UMWM02-17 than on biotype UMWM02-01 as its above ground shoot biomass continued to be reduced as the herbicide dosage of ethametsulfuron was increased (Figure

2, Table 2). Furthermore, the strong interactive effect of malathion with all ethametsulfuron herbicide treatments allowed for the dose response results for biotype UMWM02-17 to be fitted to a log-logistic model (Figure 1-2). As shown in Table 2, there was no GR_{50} for UMWM02-17 in the absence of 1500 g ai ha^{-1} malathion while the GR_{50} for UMWM02-01 was halved when malathion was added to all ethametsulfuron herbicide treatments. These results strongly indicate that the addition of 1500 g ai ha^{-1} of malathion to ethametsulfuron acted as a partial pesticide restorer agent in biotypes UMWM02-01 and UMWM02-17. It may be likely therefore, that biotypes UMWM02-01 and UMWM02-17 possess more than one mechanism of resistance to ALS inhibitor herbicides.

In comparison to biotypes UMWM02-01 and UMWM02-17, biotypes UMWM02-05 and UMWM02-08 demonstrated a much lower level of resistance to ethametsulfuron in the presence of malathion than in its absence (Table 2). However, in comparison to biotype UMWM02-08, biotype UMWM02-05 demonstrated a much higher level of resistance to ethametsulfuron in the presence of malathion (R/S ratio of 3.52 and 15.53, respectively). On the basis of these results it is likely that malathion acted as a partial pesticide restorer agent in UMWM02-05 and as a relatively complete pesticide restorer agent in UMWM02-08. Therefore, these results suggest that ethametsulfuron resistance in biotype UMWM 02-05 may be partially conferred by enhanced metabolism. In contrast, it is likely that biotype UMWM02-08 has an herbicide sensitive ALS and is largely dependent upon Cytochrome P450-mediated enzymes for the rapid metabolism of ethametsulfuron into polar, herbicidally inactive products.

Malathion has previously been shown to be an excellent pesticide restorer agent to increase the phytotoxicity of some sulfonylurea herbicides such as primsulfuron and chlorsulfuron in *Z. mays* and *L. rigidium*, respectively. For example, seedlings of a chlorsulfuron-resistant *L. rigidium* biotype that was treated with chlorsufuron in combination with 1000 g ai ha^{-1} malathion exhibited greater levels of mortality and reduced dry biomass in comparison to seedlings of *L. rigidium* that were treated with chlorsulfuron alone (Christopher et al. 1994). In our experiment, we observed a similar effect in UMWM02-08 as the ethametsulfuron GR_{50} decreased from 2.60 g ai ha^{-1} in the absence of malathion to 0.24 g ai ha^{-1} in the presence of 1500 g ai ha^{-1} malathion (Figures 1-2, Table 2). However, it is not clear whether ethametsulfuron metabolism in UMWM02-08 is due to an innate cytochrome P450 (cyt P450) system that is also found in UMWM00-00 and is simply enhanced in the resistant biotype, or whether this P450 system is specific to only the resistant biotype. Our results are supported by Veldhuis et al. (2000) who demonstrated that PBO (a cyt P450 inhibitor) inhibited the metabolism of ethametsulfuron in a biotype of *S. arvensis* from Alberta, Canada. Our results are also supported by Preston et al. (1996) who demonstrated that malathion inhibited sulfonylurea metabolism in *L. rigidium* and resulted in greater levels of weed control. However, these latter authors concluded that the resistance of their *L. rigidium* biotype was not limited to Cytochrome P450-mediated enzyme detoxification because multiple resistance (two or more genetic-based types of resistance) has been commonly exhibited in *L. rigidium*, even to several herbicides families with entirely different modes of action (Preston et al. 1996).

3) Whole plant dose response to spray applications of imazethapyr.

Phytotoxicity for pot cultured plants treated with spray applications of imazethapyr is shown in Figure 3. Wild mustard biomass as a function of herbicide dose was modeled using log-logistic models where possible.

▽ UMWM02-17	△ UMWM02-08[a]
◇ UMWM02-01[a]	○ UMWM00-00
□ UMWM02-05[a]	

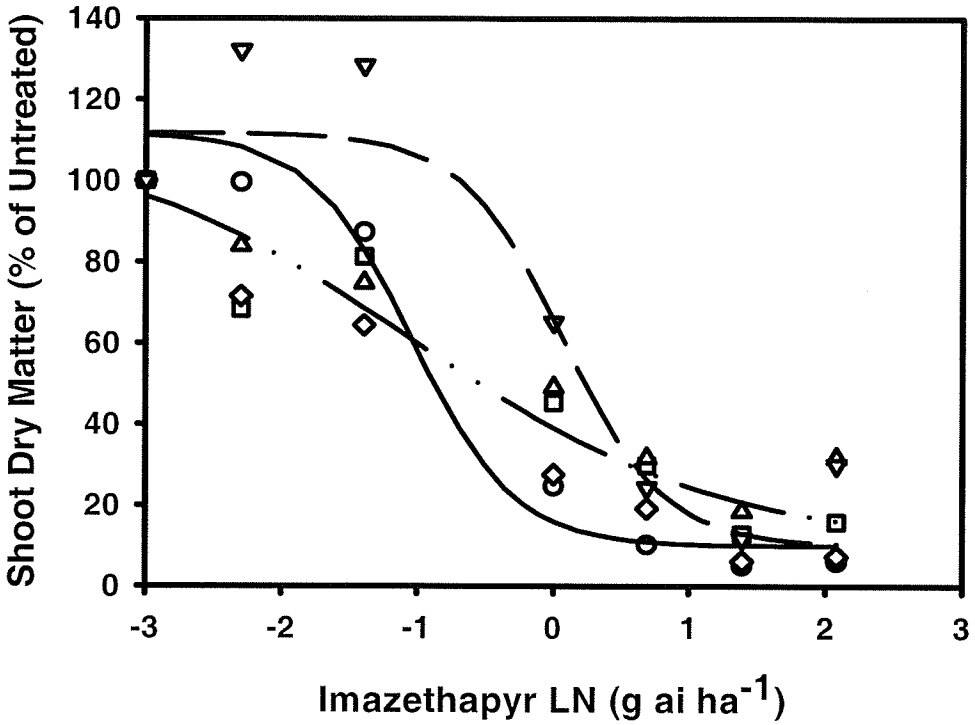

Figure 3. The effect of imazethapyr herbicide on shoot biomass expressed as a percent of the untreated control on 1 susceptible (UMWM00-00) and 4 ALS inhibitor herbicide resistant wild mustard biotypes. Common models in this figure are indicated by [a].

As shown in Table 2, the log-logistic model described the response of all wild mustard biotypes very well, as indicated by the high r^2 value (0.93). The observed mean dry biomass values (Figure 3) indicate that both the susceptible and the resistant dose

response curves for both susceptible and resistant biotypes had the same upper (*D*) and lower (*C*) limits, but did not share a common slope (*b*). Furthermore, biotypes UMWM00-00 and UMWM02-17 appeared to have parallel dose response curves (Figure 3) which upon further analysis proved to be the case (Table 2). This possibly suggests that that the herbicide was acting at the same site of action in each of these two biotypes (Seefeldt et al. 1995).

Results of growth room experiments provided clear evidence that three of the wild mustard biotypes resistant to ethametsulfuron (UMWM02-08, UMWM02-05 and UMWM02-01) with ALS inhibitor resistance were not cross resistant to imazethapyr as the GR_{50} values for these biotypes did not differ from that of the susceptible biotype (Table 2). In contrast, biotype UMWM02-17 exhibited a low level of cross-resistance to imazethapyr (GR_{50} of 1.08 g ai ha^{-1}). This wild mustard biotype demonstrated 3-fold resistance to imazethapyr compared to the susceptible biotype UMWM00-00, while UMWM02-05, UMWM02-08 and UMWM02-01 demonstrated no resistance to imazethapyr at the whole plant level (Figure 3). These results thus suggest that biotype UMWM02-17 probably has a different point mutation or a combination of point mutations on the ALS gene that are distinct from the point mutations on the ALS gene in the other 3 ethametsulfuron-resistant biotypes.

Cross-resistance between ALS inhibitor herbicide families has been previously reported to result from a single point mutation or a combination of 2 point mutations on the ALS gene, where each separate mutation results in resistance to a different family of ALS inhibitors (Lee et al. 2000). Guttieri et al (1992) reported that sulfonylurea resistant *X. strumarium L.* biotypes with little or no cross-resistance to other ALS inhibitor

herbicides such as imazethapyr, exhibit a proline to threonine, glycine, arginine, leucine or serine point mutation at amino acid residue 173 of the ALS gene.

Thus far, ALS inhibitor resistance has been explained with single gene systems that operate in a dominant or semi-dominant manner (Mallory-Smith et al. 1990). Murray et al. (1995) and Seefeldt et al. (1998) found that gene segregation for a single nuclear gene resulting in herbicide resistance could be described by a modified Punnet square (Punnet 1907). They used a discriminatory herbicide dose to differentiate between dominant, semi dominant and recessive heritability for herbicide resistance. At a low herbicide dosage, the resistant allele will appear dominant. If herbicide dose can be used to clearly discriminate between resistant, intermediate and susceptible biotypes, the phenotype, based on a Punnett square model, will segregate as a semi-dominant trait (Seefeldt et al. 1998).

Previous research on ALS-resistant biotypes has not shown a clear cross-resistance pattern that is consistent for all populations of a certain species (Devine et al. 1991). Biotype UMWM02-17 had only a low level of resistance to imazethapyr. In a field situation, UMWM02-17 would probably be controlled with a normal rate of imazethapyr in the field, thus demonstrating resistance to a sulfonylurea, but not an imidazolinone herbicide. Therefore, imazethapyr would not act as a selective agent on this particular biotype in the field, unless it was used at very low rates (1/10 to 1/1000 of recommended field rate). Imazethapyr could still be used as a weed control option for the wild mustard biotypes tested here (Lovell et al. 1996).

4) Whole plant dose response to spray applications of thifensulfuron.

The response of pot cultured plants treated with spray applications of thifensulfuron is shown in Figure 4.

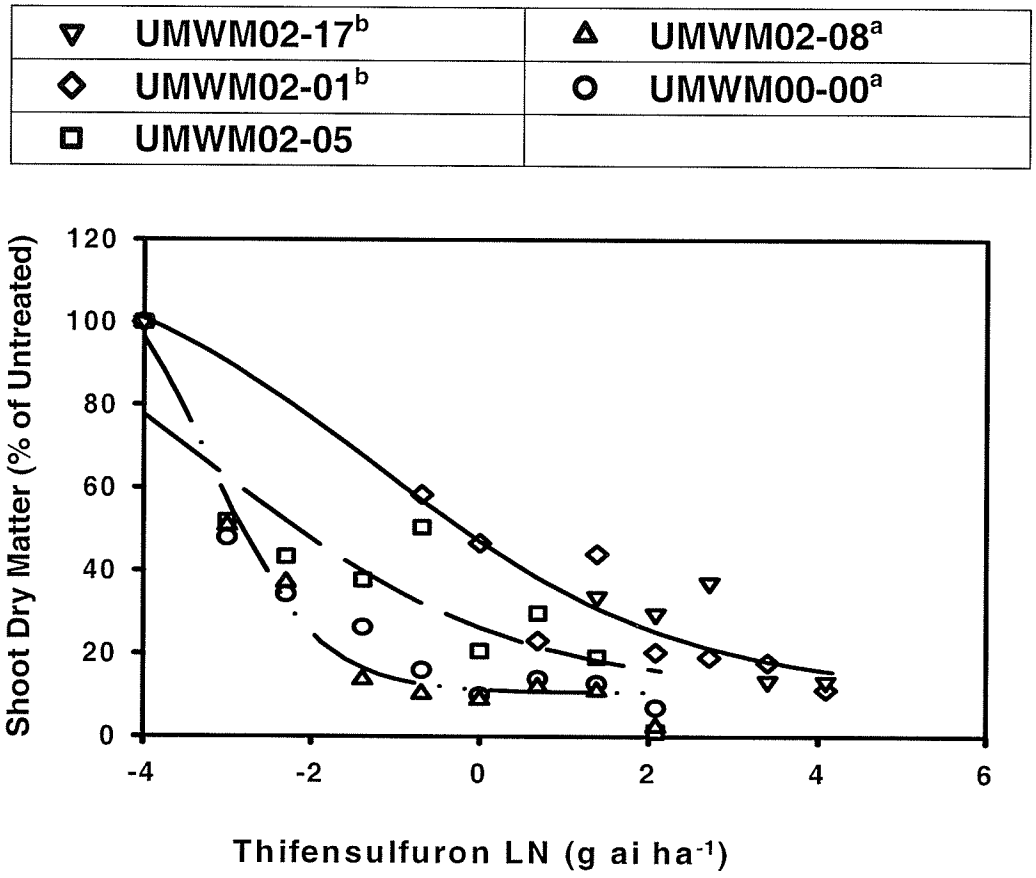

Figure 4. The effect of thifensulfuron herbicide on shoot dry biomass expressed as a percent of the untreated control on 1 susceptible (UMWM00-00) and 4 ALS inhibitor herbicide resistant wild mustard biotypes. Common models in this figure are indicated by [a] or [b].

A log-logistic model best described the relationship between shoot growth and herbicide dosage for all wild mustard biotypes as indicated by the high r^2 value (0.93). As shown in Table 2, the susceptible and resistant dose response curves for the susceptible and resistant biotypes all had a common upper (D) and lower (C) limits, but

different slopes (*b*). Table 2 also indicates that biotypes UMWM00-00 and UMWM02-08 had a steeper slope than biotypes UMWM02-05, UMWM02-01 and UMWM02-17 (Figure 4).

Overall, the response to thifensulfuron was similar (models were not statistically different) for biotypes UMWM00-00 and UMWM02-08 and for biotypes UMWM02-01 and UMWM02-17 (Figure 4). Results of the growth room experiments provided clear evidence that biotypes UMWM02-05 and UMWM02-08 were not cross-resistant to thifensulfuron as the GR_{50} value did not differ from that of the susceptible biotype (Table 2). In contrast, biotypes UMWM02-01 and UMWM02-17 exhibited a low level of resistance to thifensulfuron (0.29 g ai ha^{-1}). These respective wild mustard biotypes demonstrated 7.25-fold resistance to thifensulfuron compared to the susceptible biotype (UMWM00-00), while UMWM02-05 and UMWM02-08 demonstrated no resistance to thifensulfuron at the whole plant level (Table 2). These results indicate that thifensulfuron could still be used as a weed control option for biotypes UMWM02-05 and UMWM02-08 but perhaps not for biotypes UMWM02-01 and UMWM02-17.

Cross-resistance to imazethapyr and thifensulfuron has been previously reported in Powell amaranth (*Amaranthus powellii* S. Wats), redroot pigweed (*Amaranthus retroflexus)* and Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Beckie et al 2000). Ferguson et al. (2001) and Sprague et al. (1997) noted high levels of cross-resistance to thifensulfuron in each of these species, with resistance factors ranging from 270 to >3700-fold. Cross-resistance to thifensulfuron has also been reported in an imazethapyr and triazine-resistant biotype of common waterhemp (*Amaranthus rudis* Sauer) from Bound County, Illinois (Foes et al. 1998). Based on *in vivo* enzyme assays, Foes et al.

(1998) reported that the ALS in the resistant biotype was 8-fold less sensitive to thifensulfuron than the ALS in the susceptible biotype. Whole plant efficacy trials also indicated that the resistant biotype required more than >270 g ha^{-1} to inhibit growth by 50% (GR_{50}) (Foes et al. 1998).

Cross-resistance to thifensulfuron has also been reported in a biotype of chlorsulfuron-resistant kochia from Winnipeg, Manitoba (Friesen et al. 1993). However, the response of this chlorsulfuron-resistant biotype to thifensulfuron was highly variable at both the whole plant and enzyme levels. For example, Friesen et al. (1993) reported that the resistant kochia was extremely resistant to thifensulfuron at the whole plant level (180-fold), but only moderately resistant at the enzyme level (4-fold). Saari et al. (1993) postulated that whole plant injury caused by ALS inhibiting herbicides is time and environment-dependent and the degree of plant injury observed is therefore influenced by when and under what conditions that evaluation is made. They also speculated that barriers to herbicide retention, uptake and translocation (leaf turgor and orientation, composition and thickness of leaf waxes and physiological state of the plant) may reduce whole plant susceptibility even though site of action susceptibility remains unchanged (Saari et al. 1993).

5) Whole plant dose response to spray applications of 2,4-D.

Figures 5 describes the response of pot cultured plants treated with 2,4-D. A log-logistic model best described the relationship between shoot growth and herbicide dosage for all wild mustard biotypes as indicated by the high r^2 value (0.94) (Table 2). For biotype UMWM02-17, the log-logistic model was fitted to a single experimental run as the first experimental run over estimated plant shoot biomass at the whole plant level. As

shown in Table 2, the susceptible and resistant dose response curves for the susceptible and resistant biotypes all had a common upper (*D*) and lower (*C*) limit. Table 2 also indicates that the slope (*b*) for biotype UMWM04-04 differed from the other 5 wild mustard biotypes (Figure 5).

○ UMWM04-04	□ UMWM02-05[a]
▽ UMWM02-17[a]	△ UMWM02-08[a]
◇ UMWM02-01[a]	○ UMWM00-00[a]

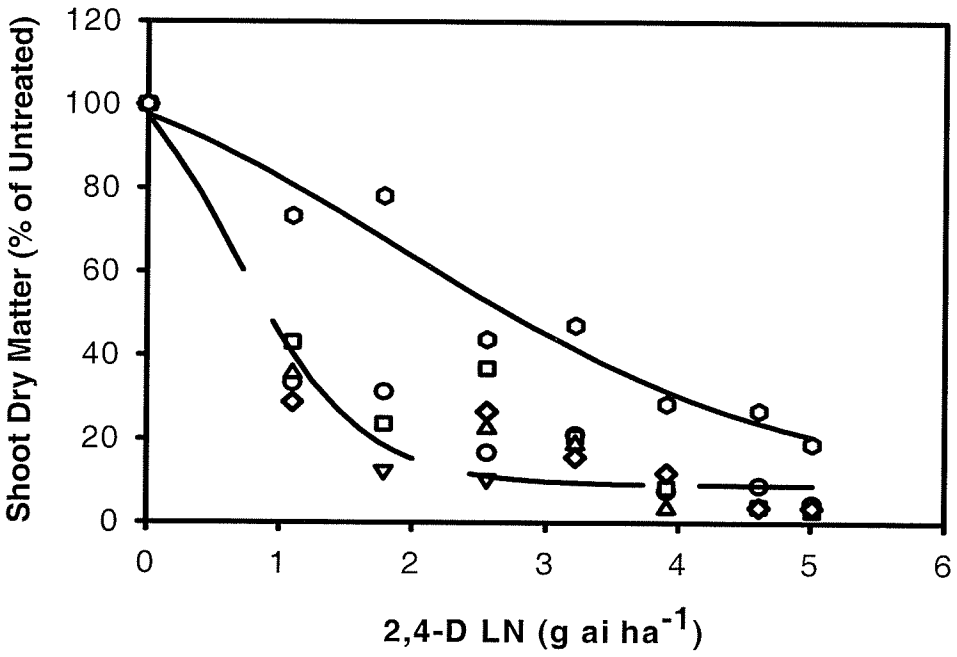

Figure 5. The effect of 2,4-D herbicide on shoot dry biomass as expressed as a percent of the untreated control on 1 susceptible (UMWM00-00) and 4 ALS inhibitor herbicide resistant wild mustard biotypes and on 1 group 4 resistant wild mustard population (UMWM04-04). Common models in this figure are indicated by [a].

Overall, the response to increasing dosages of 2,4-D was similar (models were not statistically different) for the susceptible biotype UMWM00-00 and for the ALS resistant biotypes UMWM02-05, UMWM02-08, UMWM02-01 and UMWM02-17 (Figure 5). Furthermore, the ALS resistant biotypes were not cross-resistant to 2,4-D at the whole

plant level as the GR_{50} values did not differ from that of the susceptible biotype UMWM00-00 (Table 3). In contrast, biotype UMWM04-04 (the group 4 resistant check) exhibited a high level of resistance to 2,4-D (7.08 g ai ha^{-1}) in comparison to the other ALS resistant and ALS susceptible wild mustard biotypes. These results strongly indicate that 2,4-D could still be used as a weed control option for the susceptible biotype UMWM00-00 and for biotypes UMWM02-05, UMWM02-08, UMWM02-01 and UMWM02-17.

Field Experiment

In 2002, one ALS inhibitor resistant wild mustard (UMWM02-05) population was evaluated in a separate field experiment to determine the efficacy of group 2 herbicides in competition with a competitive canola crop. Visually, the Roundup Ready canola crop in the 2002 field experiment was vigorous and competitive throughout the growing season. Since this experiment was conducted in only one farmer's field, *S. arvensis*, UMWM02-05 was the only biotypethat was naturally present. As shown in Table 3, the number of wild mustard plants per square meter decreased as ethametsulfuron herbicide dosage increased. However, the recommended herbicide dosage of ethametsulfuron (7.5 g ai ha^{-1}) did not provide a satisfactory level of wild mustard control, that is, UMWM02-05 proved to be resistant to the recommended dosage of ethametsulfuron under field conditions.

Table 3. Response of UMWM02-05 (wild mustard biotype population) to treatments of 1) ethametsulfuron, 2) ethametsulfuron tank mixed with 1500 g ai ha^{-1} malathion, 3) imazethapyr or 4) thifensulfuron in competition with Roundup Ready© canola. Values in parentheses are standard errors.

Herbicide Treatment	Herbicide Rate (g ai ha^{-1})	Visual Rating (% of control)	Wild Mustard Density (m^{-2})
Untreated control	0	0	264.00 (48.22)
Ethametsulfuron	7.5	13.33	253.33 (56.44)
Ethametsulfuron	15	23.33	194.67 (70.09)
Ethametsulfuron	30	40	157.33 (74.24)
Ethametsulfuron	60	53.33	173.33 (21.83)
Ethametsulfuron with [1]malathion	7.5	10	269.33 (26.68)
Ethametsulfuron with [1]malathion	15	16.66	152.00 (46.88)
Ethametsulfuron with [1]malathion	30	20	229.33 (70.70)
Ethametsulfuron with [1]malathion	60	56.66	176.00 (36.62)
Imazethapyr	15	40	296.00 (76.04)
Thifensulfuron	15	94.33	26.67 (22.78)
LSD (0.05)			158.09

[1]malathion mixed at the rate of 1500 g ai ha^{-1} with ethametsulfuron herbicide.

In Canada, a herbicide must provide at least an 80% reduction in wild mustard plant density or shoot dry biomass for the term "control" to be listed on the commercial herbicide label, as opposed to the term suppression (Friesen et al. 1993). As shown in Table 3, even the highest dose of ethametsulfuron (60 g ai ha^{-1}) did not result in wild mustard "control".

Spray applications of ethametsulfuron in combination with 1500 g ai ha^{-1} malathion did not increase the mortality or control of UMWM02-05. Herbicide treatments with ethametsulfuron in the presence of malathion resulted in visual control ratings and wild mustard densities that were similar to herbicide treatments with ethametsulfuron alone (Table 3). For example, at a dosage of 15 g ai ha^{-1}, spray applications of ethametsulfuron in the presence and absence of 1500 g ai ha^{-1} malathion resulted in visual control ratings of 16.66% and 23.33% respectively. Results of this field experiment are in agreement with the results of the growth room experiments; that is, UMWM02-05 continued to exhibit a high level of resistance to ethametsulfuron even

with the addition of 1500 g ai ha^{-1} malathion. The relatively poor activity of ethametsulfuron on UMWM02-05 in the presence of malathion indicates strongly that malathion did not act as an effective pesticide restorer agent on this particular biotype.

Thifensulfuron was the only herbicide that provided effective control of this particular ethametsulfuron-resistant wild mustard biotype (Table 3) showing that UMWM02-05 is sensitive to other sulfonylurea herbicides. In contrast, UMWM02-05 exhibited a high level of cross-resistance to imazethapyr as the recommended herbicide dosage failed to reduce wild mustard densities below that of the untreated control (Table 3). The latter result is not in agreement with the results of the growth room experiment in which UMWM02-05 was found not to be cross-resistant to imazethapyr at the whole plant level (Figure 3, Table 2).

These results indicate that biotype UMWM02-05 varies widely in its response to these three herbicides, from little apparent herbicidal effect to almost complete growth inhibition (mortality). Since UMWM02-05 continued to demonstrate a high level of resistance to ethametsulfuron in the presence of 1500 g ai ha^{-1} malathion in the field, the results of the field experiment do not fully support the results of the growth room experiments. Furthermore, these results suggests that UMWM02-05 possess a single resistance mechanism to ALS inhibitor herbicides and is resistant to ethametsulfuron due an herbicide insensitive ALS. If resistance is site-of-action- mediated, it is likely that UMWM02-05 is cross-resistant to imazethapyr due to a point mutation on the ALS gene (Lee et al. 2000, Foes et al. 1998).

Differences in results between the field experiment and the growth room experiments stresses the importance of assessing the response of putative resistant wild

mustard populations in the field in a competitive crop, particularly if resistance levels are low, because growing conditions for plants in pots in the growth room are quite different from those in the field. Furthermore, many herbicides, particularly ALS inhibitors, tend to be more active in the growth room than in the field (Friesen et al. 2000).

Although it is reasonable to expect that the majority of wild mustard populations in Manitoba are susceptible to ethametsulfuron, imazethapyr and thifensulfuron the results of our field experiment suggest that it is impossible to accurately predict the field response of any given population without testing it. It is apparent that patterns of cross-resistance in ALS-inhibitor resistant weed biotypes cannot be predicted based on field histories but instead must be assessed for each weed population (Friesen et al. 2000).

CHAPTER 5

GENERAL DISCUSSION

Thifensulfuron, ethametsulfuron and imazethapyr were registered for commercial use in western Canada in 1989, 1990 and 1991 respectively. The wild mustard populations used in this study are evidence that selection by ALS inhibitors for resistant biotypes can occur rapidly (for example, within 10 years of the introduction of imazethapyr). ALS herbicide resistant biotypes of wild mustard have been previously selected for in other regions of western Canada (Jeffers et al. 1995, Veldhuis et al. 2000).

The results presented in this thesis confirm that UMWM02-05, UMWM02-08, UMWM02-01 and UMWM02-17 were resistant to a broad range of ALS-inhibiting herbicides, but were not resistant to 2,4-D. For example, in initial growth room experiments, dosages of 2,4-D as low as 3 g ai ha^{-1} substantially reduced plant shoot dry matter below 45% for all ALS resistant and susceptible wild mustard biotypes (Figure 5). While the precise mechanism of resistance was not determined for any of the ALS resistant wild mustard populations used in our experiments; the results indicate that biotype UMWM02-17 possesses an altered target site and is also resistant to ALS inihibitor herbicides due to enhanced metabolism. The results of the growth room experiments along with the field experiment, also indicate that biotypes UMWM02-05 and UMWM02-01 are resistant to ALS inihibitor herbicides due to an altered target site; whereas biotype UMWM02-08 is resistant to ethametsulfuron via enhanced metabolism.

In initial growth room experiments, population UMWM02-17 exhibited a high level of resistance to ethametsulfuron. Even 120 g ai ha^{-1} of ethametsulfuron, which is more than 10 times the dosage recommended for control under field conditions, did not

reduce average plant shoot dry biomass below 100% in comparison to the untreated control (Figure 1). In contrast, the susceptible population UMWM00-00 was fully controlled at the recommended dosage (7.5 g ai ha^{-1}) (Figure 1). Furthermore, UMWM02-17 did not show any symptoms of visual injury such as leaf necrosis and chlorosis until the plants were sprayed with more than 30 g ai ha^{-1} of ethametsulfuron.

The addition of 1500 g ai ha^{-1} malathion to all ethametsulfuron herbicide treatments increased the phytotoxicity of ethametsulfuron in biotype UMWM02-17. For example; at a herbicide dosage of 30 g ai ha^{-1}, average plant shoot dry matter was reduced below 40% (Figure 2, Table 2). The interactive effect of malathion with ethametsulfuron on biotype UMWM02-17 indicates that malathion can interact with ethametsulfuron at the whole plant level (R/S ratio of 432). These findings are supported by Preston et al. (1996) and Christopher et al. (1994) who noted that 1000 g ai ha^{-1} malathion had an interactive effect with chlorsulfuron in a biotype of *L. rigidium* (Preston et al. 1996, Christopher et al 1994). For example, the addition of 1000 g ai ha^{-1} malathion to all chlorsulfuron herbicide treatments reduced the LD_{50} of chlorsulfuron resistant *L. rigidium* from 0.470 to 0.105 (Preston et al. 1996). Christopher et al. (1994) noted that the chlorsulfuron LD_{50} for the *L. rigidium* resistant biotype decreased from 293.5 g ai ha^{-1} in the absence of 1000 g ai ha^{-1} malathion to 84.6 g ai ha^{-1} in the presence of malathion.

In whole plant dose response experiments, UMWM02-17 demonstrated a higher level of resistance to the sulfonylurea herbicides than to imazethapyr. For example, the R/S ratios derived from the herbicide dosages required to inhibit shoot dry matter accumulation by 50% (GR_{50}) indicate that UMWM02-17 exhibited moderate and low

levels of resistance to thifensulfuron and imazethapyr, respectively (Table 2). A similar pattern of resistance and cross resistance has been reported for a chickweed biotype from Alberta, Canada (Devine et al. 1991) and in biotypes of kochia and Russian thistle from the western US (Saari et al. 1992; Devine and Eberlyn 1997). According to Sprague et al. 1997, there are different possible mutations within the ALS system that confer a spectrum of resistance to these herbicides. Although some mutations confer broad-spectrum resistance to all ALS inhibitors, other mutations provide resistance to a single class of ALS inhibitor. This renders the prediction of resistance patterns difficult (Sprague et al. 1997).

Our results provide strong evidence that UMWM02-17 is likely resistant to ethametsulfuron, thifensulfuron and imazethapyr due to an altered target site that confers resistance to a broad spectrum of ALS inhibitor herbicides. However, the interactive effect of malathion with ethametsulfuron in the resistant population suggests that this biotype may also have more than one resistance mechanism to ALS inhibiting herbicides. It is possible that in addition to possessing an altered target site, resistance may also be conferred in part, by enhanced metabolism.

Unlike *Lolium* spp., wild mustard biotype populations do not seem prone to developing herbicide resistance (Christopher et al. 1994). Therefore, the cross-resistance patterns exhibited by wild mustard population UMWM02-17 to ALS inhibitor herbicides is relatively uncommon (Christopher et al. 1994). The resultant cross–resistant pattern between the imidazolinone and sulfonylurea herbicides demonstrated by UMWM02-17 suggests that cross-resistance is likely a function of the location of the mutation and the way in which it affects the binding sites of different herbicides (Hall and Devine 1990).

On the other hand, the development of multiple herbicide resistance mechanisms is not rare within a weed species. Preston et al (1996) reported a biotype of *Lolium rigidium* Gaudin (VLR 69) that showed multiple resistances to at least nine dissimilar herbicide chemistries. This biotype was shown to have enhanced metabolism to herbicides that inhibit Photosystem II, ALS and Acetyl-coenzyme A carboxylase. In addition to enhanced metabolism, this biotype was also shown to have a resistant form of acetyl coenzyme A that showed 31, 4 and 20-fold resistance to diclofop acid, fluazifop acid and haloryfop acid, respectively (Preston et al. 1996). Similarly, Friesen et al (2000) reported that it is unlikely that resistance to imazethabenz, flamprop and fenoxaprop-P is conferred by a single resistance mechanism such as multiple altered target sites in 3 populations of *Avena fatua* (wild oat) from Manitoba, Canada. Furthermore, population genetics theory indicates that it would be very rare for 3 different target site mutations to evolve in a plant or plant population over a short period of time (Jasienuik et al. 1996)

Biotypes UMWM02-05 and UMWM02-08 displayed a similar pattern of resistance to ALS inhibitors that were tested in the growth room. However, the level of ethametsulfuron resistance that these two wild mustard biotypes demonstrated was markedly different. For example, at an herbicide dosage of 30 g ai ha^{-1} the average plant shoot dry biomass for biotypes UMWM02-08 and UMWM02-05 was reduced to approximately 53% and 16% respectively (Figure 1). Even at 60 g ai ha^{-1}, which is more than 8 times the recommended dosage rate in the field, the average plant shoot dry biomass for biotype UMWM02-05 was 30% greater than biotype UMWM02-08 (Figure 1). These results thus indicate that biotypes UMWM02-05 and UMWM02-08 possess different mechanisms of herbicide resistance to ethametsulfuron at the whole plant level.

The addition of 1500 g ai ha^{-1} malathion to all ethametsulfuron herbicide treatments increased the phytotoxicity of ethametsulfuron in both UMWM02-05 and UMWM02-08 (Figure 1-2). For example, the level of ethametsulfuron resistance for UMWM02-05 was reduced from 58.60 fold to 15.53 fold in the absence and presence of malathion (Table 2). Similarly, the level of ethametsulfuron resistance for UMWM02-08 was reduced from 10.56 fold to 3.52 fold when 1500 g ai ha^{-1} malathion was added to all ethametsulfuron herbicide treatments (Table 2). The results of the growth room experiments thus indicate that malathion can increase herbicide susceptibility in biotype UMWM02-08 but not in biotype UMWM02-05, suggesting that biotype UMWM02-08 possesses an enhanced metabolism and biotype UMWM02-05 possess an altered target site.

Although UMWM02-05 and UMWM02-08 displayed the same level of herbicide resistance to thifensulfuron and imazethapyr in the growth room, results of the field experiment indicate that UMWM02-05 is cross-resistant to imazethapyr. For example, when imazethapyr was sprayed at the recommended field rate, the average number of wild mustard plants per square meter were not reduced below that of the untreated control (Table 3). Boutalis and Powles (1995) suggest that within species differences in sensitivity to imidazolinone herbicides may be due to different mutations in the herbicide binding domains of ALS. That is, if a single amino acid change in the ALS enzyme is responsible for the increased insensitivity to inhibition, then one binding domain might be affected differently than another domain (Saari 1993). The different patterns of cross-resistance displayed by these two wild mustard biotypes suggest that the sites overlap but are not identical (Ferguson et al. 2001). The observations made in this study agree with

other research showing weed populations developing resistance to ALS inhibitors to which they were not previously exposed (Mallory-Smith et al. 1990, Saari et al. 1994, Sprague et al. 1997 and Ferguson et al. 2001).

Like biotype UMWM02-05, biotype UMWM02-01 demonstrated an extremely high level of resistance to ethametsulfuron (R/S ratio of 58.60) (Table 2). Even 120 g ai ha^{-1} did not reduce average plant shoot dry biomass below 40% (Figure 1). Furthermore, the addition of 1500 g ai ha^{-1} malathion did not increase the phytotoxicity of ethametsulfuron in biotype UMWM02-01. For example, the GR_{50} for UMWM02-01 was not reduced when ethametsulfuron was tank mixed with malthion (Table 2). Although UMWM02-01 did not prove to be resistant to imazethapyr at the whole plant level, this biotype was moderately resistant to thifensulfuron (R/S ratio of 7.25) (Table 2). Even though herbicide binding studies were not conducted in our study, it appears that the alteration of the resistant ALS enzyme greatly reduced the binding affinity of ethametsulfuron and thifensulfuron. This alteration did not affect the binding affinity of imazethapyr. Therefore, the degree of cross resistance expressed by this particular biotype to the sulfonylurea and imidazolinone herbicides is likely a function of the location of the mutation and the way in which it affects the binding sites of the different herbicides (Saari et al. 1993).

It is not unexpected that populations of wild mustard could be selected for resistance to ALS inhibitors. Wild mustard can occur at high densities in southern Manitoba (Van Acker and Oree 1999) and, prior to the introduction of triazine herbicide-tolerant canola cultivars, there were no effective in-crop herbicides for control of wild mustard in this crop (Blackshaw et al. 1989a). In the past 25 years, as canola acreage has

expanded, wild mustard has increased in frequency and abundance throughout the region (Manitoba Weed Survey, 2002). Acetolactate synthase inhibitors have been the products of choice for wild mustard control in Brassicaceae crops since the introduction of ethametsulfuron in 1990, and some of these herbicides, including ethametsulfuron, have a long soil residual activity, exerting selection pressure for several years (Beckie and McKercher 1989). Since the introduction of imidozolinone-resistant canola in 1996 (Hall et al. 1998) and the registration of imazethapyr and thifensulfuron, ALS inhibitors can now be used in most crops grown in western Canada. It can be predicted that ALS-resistant wild mustard will continue to be selected for as the use of these herbicides expands (Hall et al. 1998).

Until the commercialization of sulfonylurea herbicides in the 1980's, farmers in western Canada had depended on phenoxy herbicides for control of wild mustard in cereals for more than 3 decades. Even though auxin herbicides have been used extensively for approximately 40 years, the incidence of resistance to these herbicides has been relatively low (Debreuil 1996). Despite this long term use of phenoxy herbicides, resistance occurrences in Manitoba have only occurred in fields where 2,4-D or other phenoxy herbicides were intensively and extensively used and where farmers had shared equipment (Debreuil et al. 1996). Furthermore, resistance to phenoxy herbicides has only been selected for in wild mustard after 20 to 30 years of selection pressure. Therefore, it is probable that a stable, heritable mutation conferring resistance to auxin-type herbicides in broadleaf weeds in general and in wild mustard in particular, is quite rare (Debreuil et al. 1996). However, it is possible that subsequent mutation and/or pollen movement followed by selection pressure could give rise to 'double' resistant wild mustard

populations (plants resistant both to auxin-type herbicides and ALS inhibitors). This would lead to the resurgence of wild mustard as a serious weed problem across the Canadian Prairies (Debreuil et al. 1996).

In canola, incremental weed management gains have paralleled the development of new herbicide technology. For example, the introduction of ethametsulfuron provided an opportunity for growers to control Cruciferae weeds that are closely related to canola (Blackshaw 1989a, Swanton and Chandler 1989) without the yield penalty associated with the triazine resistant varieties (Harket et al. 2000). Another example of the incremental weed management gains is the recent introduction of HTC varieties (excluding triazine tolerance) (Harker et al. 2000). However, extensive use of HTC will most probably influence the development of herbicide-resistant weeds. Growing glufosinate and glyphosate tolerant canola may help delay the threat of herbicide-resistant weeds by encouraging the use of novel in-crop herbicide groups. HTC's are already providing flexibility on some western Canadian farms with wild oat populations resistant to more than three mode of action groups. On the other hand, the popularly of HTC may encourage the overuse of some herbicides and the increased selection intensity may increase the number of resistant weed individuals in a once susceptible population. Glyphosate is a major component of zero tillage and direct seeding systems, and is also economically important for pre-harvest control of difficult perennial weeds such as Canadian thistle, quackgrass and perennial sowthistle. Selection of weeds resistant to glyphosate herbicide, however "unlikely" (Waters, 1991) has already occurred (Powles et al. 1998) and can threaten the use of gylphosate in agricultural ecosystems. Similarly, growing imazethapyr/ imazamox resistant canola encourages more in-crop applications

of imidazolinone herbicides, which may contribute to the already significant problem of weed resistance to ALS inhibitors (Saari et al. 1994). In terms of weed resistance management, glufosinate may be the least risky of the HTC herbicides commercially available, given its relatively limited use in Canadian prairie cropping systems (Harker et al. 2000).

CHAPTER 6

CONCLUSIONS

The differences in the patterns of cross-resistance, coupled with the fact that the four ALS resistant wild mustard populations originated from locations many kilometers apart, provides strong evidence that resistance evolved independently in the populations that we studied. It then follows that the resistant populations may not have identical mechanisms of resistance and characterization of resistance in one population cannot be extrapolated to others. This has both agronomic and physiological implications. From an agronomic point of view, it would be impractical to attempt to eradicate and/or isolate outbreaks of resistance within a defined geographic area, since it is probable that resistance occurs in most populations at varying frequencies depending on their exposure to ALS inhibitors. From a physiological point of view, it would seem likely that more than one mechanism of resistance can confer varying degrees of resistance to ALS inhibitor herbicides in wild mustard (Heap et al. 1993).

The difference in patterns of cross-resistance exhibited by these populations complicates the practical advice being given to farmers. The general advice offered to farmers is to use an Integrated Weed Management (IWM) strategy for the control of wild mustard and to use herbicides with different modes of action (Heap et al. 1993). Although this advice is appropriate, it is difficult to implement given the scale of farms today and the common practice across the Canadian Prairies of continuous cropping of annual spring crops under reduced tillage regimes and the resultant reliance on herbicides for weed control. This farming practice has evolved because of cash flow requirements,

lifestyle choice and economics and therefore, will be difficult to change voluntarily (Friesen et al. 2000).

It is not hard to learn from pesticide use history how to delay the evolution of herbicide resistance. Resistance has predominantly evolved where a single herbicide chemical class or group was used annually with high selection pressure or residual activity or where short-lived herbicides were repeatedly used. Resistances have not evolved where herbicide rotations or mixtures have been used, despite multiple treatments (Shaner et al. 1991). Time and time again, it has been shown that crop rotations, especially among crops with different growth morphologies and phenologies can reduce weed densities and increase weed diversity. By voluntarily adopting an IWM approach, herbicides and other cultural methods of weed control, the potential for resistance evolution is reduced because there is less selection pressure being applied to the weeds (Shaner 1991; Saari et al. 1993; Friesen et al. 2001 and Nazarko 2002). Cultural weed control practices that encourage biological diversity such as the use of crop rotations, cover crops and intercrops may increase and encourage nutrient cycling interactions with marginal and off-farm organisms and the self regulation of pest populations (Barberi 2002). If voluntary measures are not taken to conserve and protect our herbicides resources, it is possible that many more producers will have to make the mandatory decision to adapt an alternative means of weed control. IWM is the safest way to avoid herbicide resistance and to ensure that herbicides will continue to provide an efficient and cost effective means of weed control (Nazarko 2002).

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