Inheritance of Multiple Herbicide Resistance in Wild Oat (*Avena fatua* L.).

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Karlowsky, Jocelyn. M.Sc., The University of Manitoba, March, 2004. Inheritance of multiple herbicide resistance in wild oat (*Avena fatua* L.). Major Professor: Anita L. Brûlé-Babel.

The inheritance of multiple herbicide resistance was studied in two wild oat (*Avena fatua* L.) populations, UMWO12-01 and UMWO12-03. These populations have developed resistance to three distinct herbicides, imazamethabenz, flamprop and fenoxaprop. Crosses were made between each resistant population and a susceptible population (UM5) and between the two resistant populations. Inheritance was studied using classical plant genetic techniques. Parental and F_2 plants, and F_2 -derived F_3 ($F_{2:3}$) families were treated with the three herbicides, and classified as resistant (R) or susceptible (S). F_2 plants and $F_{2:3}$ families produced from crosses between the resistant populations and a susceptible population segregated in 3R:1S and 1 homozygous R:2 segregating:1 homozygous S ratios, respectively. These ratios indicate a single dominant nuclear gene controls resistance to each of these herbicides. F_2 plants and $F_{2:3}$ families produced from crosses between the resistant populations segregated for resistance/susceptibility, which indicated that the genes that confer resistance to imazamethabenz and flamprop are different in each population. Individual $F_{2:3}$ families responses to each herbicide demonstrated that resistance to each herbicide was controlled by a separate dominant nuclear gene and these genes are not independent of each other, indicating possible linkage between resistance to each herbicide. This provides insight as to how these populations developed multiple resistance in the absence of selection with two of the herbicides (imazamethabenz and flamprop).

1.0 INTRODUCTION

Herbicide resistance has become a problem in many agricultural regions worldwide. Resistance has evolved in several weed species in 59 countries, including Canada (Heap, 2003 www.weedscience.com). Herbicide resistance has developed to most modes of action. On the Canadian prairies herbicide resistance was first reported in the late 1980s, with the identification of trifluralin resistant green foxtail (*Setaria viridis* (L.) P. Beauv.), chlorosulfuron resistant chickweed (*Stellaria media* (L.) Vill.) and kochia (*Kochia scoparia* (L.) Schrad.) (Morrison and Devine, 1994). Resistance in wild oat was first confirmed in Manitoba in 1990 (Heap et al.1993). This population had developed resistance to acetyl-coA carboxylase (ACCase) inhibitors. Recent surveys have found that 2.4 million hectares and 1.2 million hectares in Saskatchewan and Manitoba, respectively are infested with ACCase inhibitor resistant wild oat (Beckie et al. 1999). It has been estimated that over $4 million is spent by producers annually in Saskatchewan and Manitoba to control herbicide resistant wild oat through alternative herbicide use (Beckie et al. 1999). Resistance has typically evolved following repeated exposure to herbicides with the same mode of action. However resistance has developed to herbicides in populations that have not been repeatedly exposed to the specific mode of action (Friesen et al. 2000, Mansooji et al, 1992). Multiple herbicide resistance has developed in populations of wild oat, as well as other species (Friesen et al. 2000). These populations are resistant to more than one herbicide mode of action.

The inheritance of herbicide resistance greatly influences the development and spread of resistant populations (Jasieniuk et al. 1996). Single herbicide resistance has

been studied in several different species. The majority of these studies have shown that resistance is due to one or two partially dominant nuclear genes. The development of multiple herbicide resistance is not as well understood. Multiple herbicide resistance may be the result of the accumulation of multiple resistance genes following repeated exposure to different modes of action or may be due to a single resistance gene, which confers resistance to more than one mode of action. Studying the inheritance of multiple herbicide resistance will help in determining how these populations developed. This information is also needed for the development of effective strategies to delay the development of resistance and to control existing resistant populations.

The objectives of this thesis were to: 1) determine the mode of inheritance of resistance to imazamethabenz, flamprop and fenoxaprop-P in two multiple herbicide resistant wild oat populations and 2) determine the relationship between the different resistances and between the populations. These objectives were met using classical plant genetic techniques.

2.0 LITERATURE REVIEW

2.1 Wild Oat

2.1.1 Occurrence

Wild oat (*Avena fatua* L.) is one of the most serious problem weeds found in western Canada and causes economic losses through decreased crop yield and crop quality, and increased dockage and seed cleaning costs (Sharma and Vanden Born, 1978). Wild oat ranks second in relative abundance among weeds found in the Great Plains of Canada. Wild oat typically occurs in patches throughout fields. The natural spread of wild oat patches is limited, as seed is typically shed on the ground when mature. However, the spread of wild oat may occur through transportation of seed, seed cleaning equipment and other farm implements. It has been demonstrated that harvesting equipment can spread wild oat seed over 100 m (Shirtliffe, 1999).

2.1.2 Biology and Population Structure

Wild oat is a predominantly self-pollinating hexaploid species ($2n = 6x = 42$). Populations of wild oat have been shown to be highly autogamous, and even within small areas of fields, unrelated patches can be identified (Andrews et al. 1998). The level of outcrossing has been reported to range between less than one and 12 % (Imam and Allard, 1965; Murray, 1996). Low levels of outcrossing between different genotypes within populations allow for genetic recombination creating a range of genotypes, maintaining genetic variability of the population, while high levels of inbreeding create populations comprised primarily of coexisting homozygous individuals (Imam and Allard, 1965).

2.2 Herbicide Resistance

2.2.1 Definition of Herbicide Resistance

Herbicide resistance has been defined in a number of ways. For the purpose of this thesis, herbicide resistance will be defined as "the naturally occurring heritable ability of some weed biotypes within a population to survive a herbicide treatment that would, under normal conditions of use, effectively control that weed population" (Heap, 1997). A population of weeds is considered resistant when 30% of the individuals are resistant (Gressel and Segel, 1978).

2.2.2 Occurrence of Herbicide Resistance

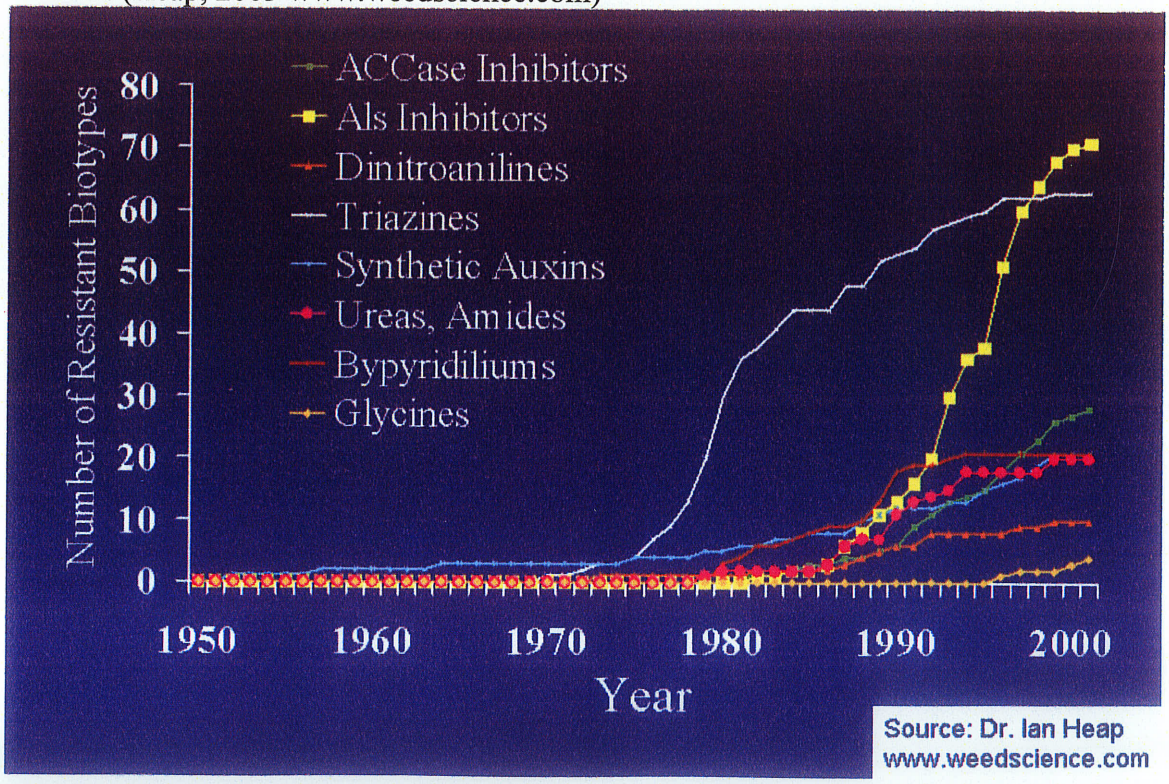
Since the initial reports of herbicide resistance, the number of new reports of resistance has been on the rise and the area of land infested with resistant weeds is increasing rapidly (LeBaron, 1991). Herbicide resistant weeds have been identified in 59 countries (Heap, 2003 www.weedscience.com). In Canada, 29 different biotypes exhibiting resistance to one or more of 7 different herbicide groups have been identified (Heap, 2003 www.weedscience.com). Resistance has developed to almost all herbicide classes used to manage weeds in cereal and small grain production (Mathews, 1994). It has been estimated that 210,000 fields worldwide (Heap, 2003 www.weedscience.com) are infested with resistant populations.

2.2.3 History of Resistance Development

Harper (1956) discussed the development of herbicide resistance in 1956. However, it was not until 1968 that Ryan (1970) first confirmed herbicide resistance in a weed. Ryan (1970) reported that a biotype of common groundsel (*Senecio vulgaris* L.) was resistant to the recommended rate of simazine. From 1970 until 1977, there was an

average of one new herbicide resistant biotype identified each year (Heap, 1997). After 1977, the number of new cases of resistance reported increased to an average of nine cases each year (Heap, 1997). In the 1980s, not only was there a rapid increase in the number of resistant biotypes, but resistance had developed to a wide range of herbicides (Shaner, 1995). The proportions of biotypes resistant to the different herbicide groups changed as weeds evolved resistance to herbicides with new modes of action that were introduced through the late 1970s and early 1980s (Heap, 1997), resulting in a decline in the proportion of biotypes resistant to triazines (Figure 2.1). Today resistance has developed to all herbicide modes of action.

Figure 2.1. Occurrence of herbicide resistance to eight herbicide groups from 1950-2000. (Heap, 2003 www.weedscience.com)



Herbicide resistance was first recognized on the Canadian prairies in the late 1980s (Morrison and Devine, 1994). During this period, trifluralin-resistant green foxtail

was reported in Manitoba (Morrison et al. 1989); chlorosulfuron-resistant chickweed was reported in Alberta and chlorosulfuron-resistant kochia in Saskatchewan (Morrison and Devine, 1994). Cases of resistance have increased, and in 1999, approximately one in four fields studied in Manitoba contained wild oat resistant to more than one group of herbicides (Beckie et al. 1999).

2.2.4 Development of Resistance

To date, recorded cases have shown the shift in weed populations from susceptible to resistant may be gradual or rapid, from as little as four years up to at least two decades (Cousens and Mortimer, 1995). Since repeated use of herbicides with similar modes of action seems to be a precursor in the selection of resistance (Jasieniuk et al.1996) the herbicide grouping system was created. Herbicides are divided into groups based on their modes of action (Table 2.1). This system allows the producer to make herbicide choices that would help to prevent continued use of similar modes of action. However, there have been several documented cases of weeds being resistant to a herbicide that they have not been exposed to (Friesen et al. 2000; Mansooji et al. 1992; Somody et al. 1984; Kibite and Harker, 1992).

Table 2.1. Herbicide groups classified by mode of action based (adapted from Mallory-Smith and Retzinger, 2003)

Group	Site of Action	Chemical Family
1	Inhibition of ACCase	Aryloxyphenoxypropionate
		Cyclohexanedione
2	Inhibition of ALS	Imidazolinone
		Pyrimidinylthio-benzoate
		Sulfonylamino-carbonyltriazolinone
		Sulfonylurea
		Triazolopyrimidine

Group	Site of Action	Chemical Family
3	Microtubule assembly inhibitors	Dintroaniline
		Pyridazine
		None
4	Synthetic auxins	Phenoxy
		Benzoic acid
		Carboxylic acid
		Quinoline carboxylic acid
5	Inhibition of photosynthesis at PS II site A	Phenyl-carbamate
		Pyridazinone
		Triazine
		Triazinone
		Triazolinone
		Uracil
6	Inhibition of photosynthesis at PS II site B than Group 5)	Benzothidiazole
		Nitrile
		Phenyl-pyridazine
7	Inhibition of photosynthesis at PS II site A (different binding behavior than Group 5)	Amide
		Urea
8	Inhibition of lipid synthesis	Thiocarbamate
		None
9	Inhibition of EPSPS	None
10	Inhibition of glutamine synthetase	None
11	Inhibition of carotenoid synthesis at unknown site	Triazole
12	Inhibition of PDS	Pyridazinone
		Pyridinecarboxamide
		Other
13	Inhibition of DOXP synthase	Isoxazolidinone
14	Inhibition of PPO	Diphenylether
		N-phenylphthalamide
		Oxadiazole
		Phenylpyrazole
		Pyrimidindione
		Thiadiazole
		Triazinone
		Triazolone
		Other

Group	Site of Action	Chemical Family
15	Inhibitors of synthesis of very long-chain fatty acids	Acetamide
		Chloroacetamide
		Oxyacetamide
		Tetrazolinone
		Other
16	Unknown	Benzofuran
17	Unknown	Organoarsenical
18	Inhibition of DHP	Carbamate
19	Inhibition of indoleacetic acid transport	Pthalamate
		Semicarbazone
20	Inhibition of cellulose synthesis site A	Nitrile
21	Inhibition of cell wall synthesis site B	Benzamide
22	Photosystem I electron diverters	Bipyridylium
23	Inhibition of mitosis	Carbanilate
24	Membrane disrupters (uncouplers)	Dinitrophenol
25	Unknown	Arylaminopropionic acid
26	Unknown	Various
27	Inhibition of 4-HPPD	Isoxazole
		Pyrazole
		Triketone

2.2.5 Mechanisms of Resistance

Plants can exhibit resistance in a number of ways. These include metabolic detoxification before the herbicide reaches the target site; overproduction of the natural, endogenous substrate with which the phytotoxic compound competes in metabolic function; modified substrate binding on the site of the protein; and altered uptake, translocation or compartmentation (Duesing, 1983). Of the mechanisms of resistance reported, altered target site, enhanced detoxification and sequestration of the herbicide away from its target site are the most common (Rubin, 1991). The majority of resistant weeds worldwide exhibit target site resistance to a wide range of herbicide mechanisms

of action (Powles and Holtum, 1994; Heap, 2003 www.weedscience.com), leaving them resistant to other herbicides with the same mechanism of action and not resistant to other herbicide mechanisms of action. The second most common mechanism of herbicide resistance worldwide is enhanced herbicide metabolism (Hall et al. 1999). When resistance is due to enhanced metabolism, multiple resistance to other classes of herbicides is a concern, including herbicides that may have never been used on that biotype before (Devine, 1997).

2.2.6 Multiple Resistance

Multiple herbicide resistance has been defined as the capacity of weeds to survive a range of herbicides from different chemical classes (Hall et al. 1994). This type of resistance can occur when a population of weeds develops a mechanism of resistance that confers resistance to more than one class of herbicide or that have accumulated more than one mechanism of resistance (Hall et al. 1999).

The most notable cases of multiple herbicide resistance that have developed are resistant populations of rigid ryegrass (*Lolium rigidum* Gaud.) in Australia, blackgrass (*Alopecurus myosuroides* Huds.) in England and wild oat in North America (Powles and Howat, 1990; Moss, 1990; Hall et al. 1994; Beckie et al. 2001), making these weeds very difficult to control.

The first report of a weed species with resistance to chemically dissimilar herbicides and herbicides with different modes of action was in 1982, when a resistant population of rigid ryegrass was identified in Australia (Heap and Knight, 1982; 1986). Since this first case of multiple resistance, populations of multiple-group resistant rigid ryegrass can now be found in all mainland states of southern Australia (Heap, 1991).

One extremely resistant population has been identified with resistance to nine classes of herbicides, accounting for six herbicide modes of action (Burnet et al. 1994). Multiple herbicide resistance has also been identified in populations of green foxtail in Manitoba and Saskatchewan (Morrison and Devine, 1994; Beckie et al. 1999), false cleavers (*Galium spurium* L.) (Hall et al. 1998) and populations of pigweed (*Amaranthus* spp). (Ferguson et al. 2000).

Multiple group herbicide resistance in wild oat has developed throughout the Canadian Prairies, however, more cases of multiple resistance have been reported in Manitoba than in Alberta or Saskatchewan (Hall et al. 1999; Beckie et al. 1999). In 1997, 27% of cereal fields surveyed in Manitoba had populations of wild oat that exhibited resistance to more than one chemical group. (Beckie et al. 1999). Four of these populations have developed resistance to Group 1, 2, 8, and 25 herbicides. This leaves no herbicides registered for use in Manitoba to control these quadruple resistant wild oat populations in wheat, leaving producers with fewer cropping/weed control options. It has been hypothesized that multiple-group herbicide resistance in populations of wild oat is due to either a single, non-target site resistance mechanism, such as enhanced metabolism of the herbicides, or the sequential accumulation of different resistant mechanisms as the result of repeated selection by different herbicide modes of action (Beckie et al, 2001). It has been suggested that multiple resistance due to enhanced metabolism is more likely, as many populations of wild oat have developed resistance to chemical modes of action they were not repeatedly exposed to (Friesen et al. 2000; Beckie et al. 1999, Somody et al. 1984).

2.3 Acetyl-Coenzyme A Carboxylase (ACCase) Inhibitor Resistance

2.3.1 History of ACCase Inhibitor Use

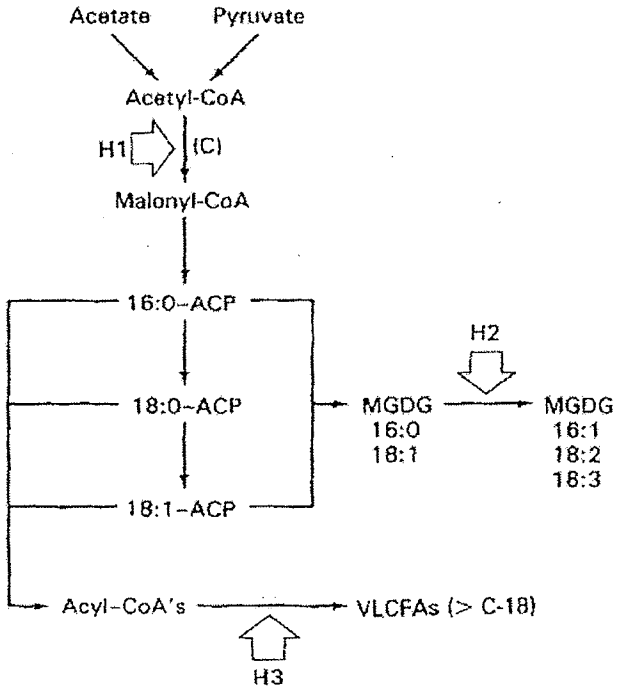
ACCase inhibitors include two chemical families, the aryloxyphenoxypropionates (APPs) and cyclohexanediones (CHDs). These herbicides were first introduced in the late 1970s and early 1980s. Since their introduction, these herbicides have been used widely to control annual and perennial grassy weeds. In Manitoba, from the 1980s and early 1990s ACCase inhibitors were used more widely than all other wild oat herbicides combined (Bourgeois and Morrison, 1997). The frequency of ACCase inhibitor or Group 1 (Table 2.1) herbicide use in the prairies increased throughout the 1990s. In Saskatchewan 56% of cropped fields were treated with Group 1 herbicides in 1996. In 1997 in Alberta and Manitoba, 49 and 69% of cropped fields were treated with Group 1 herbicides, respectively (Beckie et al. 1999). ACCase inhibitors have gained widespread acceptance and as of 1997 accounted for more than 5% of global herbicide sales (Heap, 1997).

2.3.2 Mode of Action of ACCase Inhibitor Herbicides

Both classes of Group 1 herbicides, the APPs and CHDs inhibit ACCase, an essential enzyme for fatty acid biosynthesis (Devine and Shimaburkuro, 1994). These herbicides bind to ACCase in sensitive grass species, slowing down and stopping fatty acid synthesis, which in turn causes cell growth and division to slow down and stop (Hall et al. 1999). This typically results in wilting and chlorosis of the leaves, followed by drying and necrosis. ACCase inhibitor herbicides are thought to be active in the chloroplasts or plastids (Hall et al. 1999; Devine et al. 1993b; Owen, 1991). It is here where pyruvate and acetate are converted to acetyl coenzyme A by the chloroplastidic

pyruvate dehydrogenase complex and the stromal acetyl coenzyme A (CoA) synthase (Figure 2.2). The acetyl Co A is then carboxylated by ACCase to form malonyl CoA, which is then catalyzed to form fatty acids (Owen, 1991). ACCase inhibitor herbicides provide control of grassy weeds in dicotyledonous crops, as broadleaf species are insensitive to the ACCase inhibitor herbicides (Hall et al. 1999). Selectivity between cereal crops and grassy weeds is due to rapid metabolism of ACCase inhibitors by the crop and slow metabolism in the weed species (Hall et al. 1999).

Figure 2.2. Site of action of ACCase inhibitors in major synthesis routes of saturated, unsaturated and very long chain fatty acids (VLCFAs) in leaves. H1, H2 and H3 denote herbicidal inhibition sites. C = acetyl-CoA carboxylase; ACP = acyl carrier protein; MGDG = monogalactosyldiaglyceride (Devine et al. 1993b)



H1: aryl-propanoic acids and similar structures; cyclohexanediones

H2: pyridazinones

H3: thiocarbamates, halogenated acids (?)

2.3.3 Cases of ACCase Inhibitor Resistance

2.3.3.1 ACCase Inhibitor Resistance Worldwide

The first case of ACCase inhibitor resistance was reported in 1982 when a population of rigid ryegrass in South Australia was found to be resistant to diclofop (Heap and Knight, 1982). Since this initial report many populations with resistance to both APP and CHD herbicides have been identified. As of 1997 it was estimated that there were more than 3000 sites with ACCase inhibitor resistant rigid ryegrass in Australia (Heap, 1997). In addition to Australia, ACCase inhibitor resistant *Lolium* spp. have been reported in Chile, France, South Africa, Spain, the United Kingdom and the United States (Heap, 1997).

Resistance to ACCase inhibitors has developed in several grassy species throughout many agricultural regions. Thirty-two species that have developed resistance have been identified in 27 countries (Heap, 2003 www.weedscience.com). These resistant species are a major economic concern due the large acreage of land infested and the limited number of alternative herbicides that can be used for control (Heap, 1997). Species that have developed resistance include green foxtail, large crabgrass (*Digitaria sanguinalis* L.), goosegrass (*Eleusine indica* (L.) Gaertn), giant foxtail (*Setaria faberi* Herrm.), johnsongrass (*Sorghum halepense* (L.) Pers.), blackgrass, rigid ryegrass, Italian ryegrass (*Lolium multiflorum* Lam.), and *Avena* spp. (Heap, 2003 www.weedscience.com). Cross-resistance to the chemically unrelated APP and CHD herbicides has also developed in blackgrass, wild oat, winter wild oat (*Avena sterilis* ssp. *ludoviciana*), goosegrass, rigid ryegrass and green foxtail (Mortimer, 1993 www.plantprotection.org). Cross-resistance occurs when a weed biotype is resistant to

two or more herbicides within the same mode of action group due to the presence of a single resistance mechanism (Heap, 1997).

ACCase inhibitor resistance in *Avena* spp. has developed in Australia, Chile, France, South Africa, Spain, the United Kingdom, the United States and Canada (Heap, 1997). The first confirmed cases of wild oat resistant to Group 1 herbicides in the United States was found in the Willamete Valley, Oregon in 1990 (Seefeldt et al. 1994). Diverse patterns of cross-resistance were observed in wild oat populations from the U.S. (Seefeldt et al. 1994), and wild oat and winter wild oat populations from Australia (Mansooji et al. 1992).

2.3.3.2 ACCase Inhibitor Resistance in Canada

One of the first cases of ACCase inhibitor resistance in Canada was identified in 1990 when a producer from south-central Manitoba reported a lack of control of green foxtail by fenoxaprop-p-ethyl and sethoxydim (Heap and Morrison, 1996). A field study conducted in Saskatchewan in 1996 found that one in every 20 fields surveyed contained green foxtail with Group 1 herbicide resistance. Green foxtail populations have also developed cross-resistance to APP and CHD herbicides (Heap and Morrison, 1996). It has been speculated that Group 1 herbicide resistant green foxtail is a more abundant and widespread problem in Manitoba than it is in Saskatchewan, as there is a greater use of Group 1 herbicides and a greater number of green foxtail populations in Manitoba (Beckie et al. 2001).

The first cases of herbicide resistant wild oat in Canada were identified in 1990 (Heap et al. 1993). Three wild oat populations from Manitoba and one population from Saskatchewan were found to be resistant to ACCase inhibitors. By 1993 the number of

ACCase inhibitor resistant wild oat populations had risen to more than one hundred (Morrison and Devine, 1994). A recent study showed that Group 1 herbicide resistant wild oat occurred in 53% of fields surveyed in Manitoba (Beckie et al. 1999). The cost of controlling wild oat populations resistant to Group 1 herbicides in Manitoba by using alternative herbicides is estimated to be $1.3 million annually (Beckie et al 1999). Cases of resistance have also increased in Alberta and Saskatchewan. Fifty-seven percent of fields surveyed in Alberta have Group 1 resistant wild oat, and it is estimated to be in one in nine fields where annual crops are grown in Saskatchewan (Beckie et al. 1999). The frequency of use of ACCase inhibitors is directly related to the occurrence of Group 1 resistance in wild oat (Bourgeois and Morrison, 1997), with resistance typically developing after six to ten applications.

Wild oat populations in Canada have also developed cross-resistance to APP and CHD herbicides, with the first case being reported in 1990 (Heap et al. 1993). Bourgeois et al. (1997) identified three types of cross-resistance patterns in wild oat using a petri-dish bioassay. Type A included populations that were resistant to APP herbicides with little or no resistance to CHD herbicides; type B had low to moderate levels of resistance to all Group 1 herbicides; and type C had high levels of resistance to all Group 1 herbicides.

2.3.4 Mechanisms of Resistance to ACCase Inhibitors

In the majority of weeds exhibiting ACCase inhibitor resistance the mechanism of resistance has been an alteration in the herbicide target site of the plant, reducing sensitivity to APP and CHD herbicides (Devine and Shimaburkuro, 1994). Alterations in the target site have been identified as the mechanism of resistance in biotypes of *Avena*

spp. (Cocker et al. 2000; Seefeldt et al. 1994), rigid ryegrass, Italian ryegrass (Betts et al. 1992), blackgrass, johnsongrass and green foxtail. Seefeldt et al. (1996) studied the mechanisms of resistance in wild oat populations with cross-resistance to APP and CHD herbicides and found that resistance in populations with different patterns of cross-resistance were due to different mutations leading to less sensitive ACCase. The ACCase is less sensitive, as the target site for the specific herbicide is altered. In addition to target site resistance, enhanced metabolism of ACCase inhibitors has been identified as the source of resistance in *Avena* spp. (Cocker et al. 2000), rigid ryegrass and johnsongrass. In the *Avena* spp. investigated by Cocker et al. (2000) enhanced metabolism was observed as the breakdown of the herbicide into non-toxic metabolites. In rigid ryegrass populations it has been hypothesized that enhanced metabolism may be due to elevated activity of cytochrome P450 monoxygenases which detoxifies the herbicide (Preston et al. 1996). This would give these populations the ability to metabolize several different herbicides. Bradley et al. (2001) found that overproduction of ACCase was correlated with ACCase inhibitor resistance in johnsongrass. The effects of ACCase inhibitors on electrogenic potentials have also been investigated. Devine et al. (1993a) concluded "that the effects of diclofop and related herbicides on membrane proton influx are an important component of the mechanism of action in some species." However, the importance of membrane potentials as a resistance mechanism is questionable (Devine, 1997).

2.4 Acetolactate Synthase (ALS) Inhibitor Resistance

2.4.1 History of ALS Inhibitor Use

Acetolactate synthase inhibitor, or Group 2 (Table 2.1), herbicides first became commercially available in 1982. The ALS inhibitor herbicides include five chemical

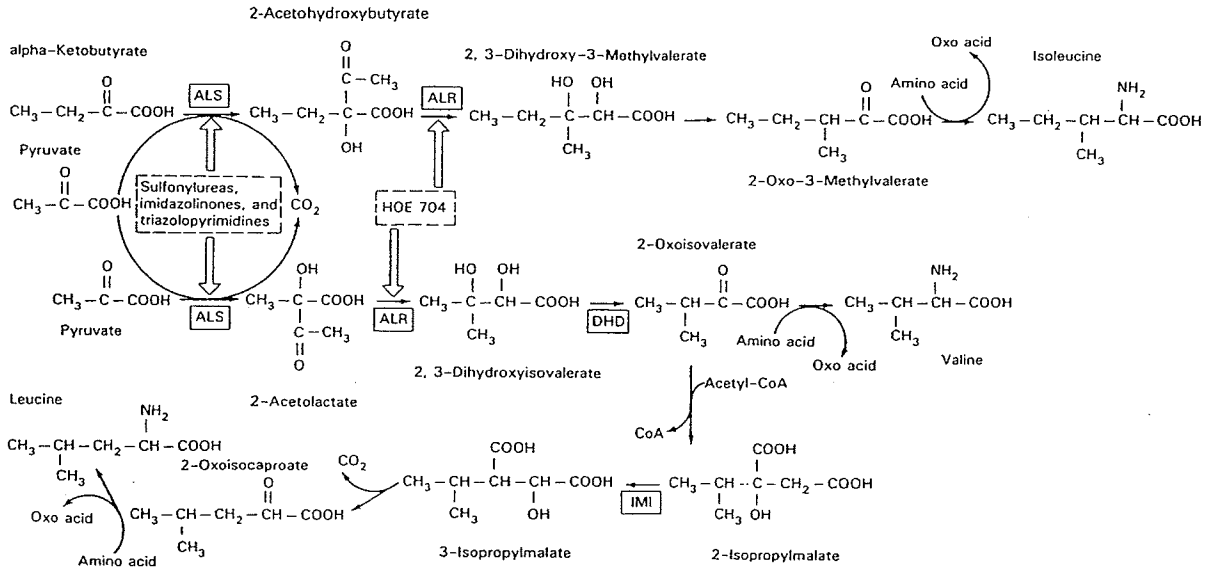
families, the imidazolinones, pyrimidinylthio-benzoates, sulfonylamino-carbonyltriazolinone sulfonylureas, and triazolopyrimidines (Mallory-Smith and Retzinger, 2003). Over the past 20 years these herbicides have become widely used worldwide for selective weed control in numerous crops. The acceptance of ALS inhibitor use is thought to be due to their low effective dosage rates, high efficacy, low mammalian toxicity, low environmental risk, broad spectrum of control and in crop selectivity (LeBaron and McFarland, 1990a, Saari et al. 1994). In 1994, ALS inhibitors accounted for more than 17% of global herbicide sales, a larger proportion of sales than any other single herbicide group (Heap, 1997). Group 2 herbicides are also used frequently by Canadian farmers. In 1995, 45% of cereal crops and 8% of canola crops in Alberta were sprayed with Group 2 herbicides (Beckie et al. 2001). Group 2 herbicides are used across the prairies, but are applied more often in Alberta and Manitoba than in Saskatchewan (Beckie et al. 2001). ALS inhibitors are also popular in Ontario, with 75% of the soybean crop and 30% of the corn crop being treated with at least one ALS inhibitor (Beckie et al. 2001).

2.4.2 Mode of Action of ALS Inhibitor Herbicides

Group 2 herbicides inhibit ALS, one of three regulation sites in the branched chain amino acid pathway, and the ALS enzyme is feedback regulated by valine, leucine and isoleusine (Figure 2.3) (Shaner, 1991; Saari et al. 1994). ALS is located in the chloroplasts of green plant tissue and plastids of non-green tissue (Hall et al. 1999). ALS condenses 2 molecules of pyruvate to form acetolacate, a precursor of leucine and valine, and CO_2. ALS also synthesizes the condensation of pyruvate and ketobutyrate to form CO_2 and acetohydroxybutyrate, a precursor of isoleucine (Devine et al., 1993b). In

sensitive species, synthesis of valine, leucine and isoleucine is decreased, which leads to amino acid deficiencies in the plant. The lack of valine, leucine and isoleucine is thought to be the primary cause of plant death (Shaner, 1991). However, other secondary effects of ALS inhibitors have been implicated in causing plant death, including inhibition of cell division, a build up of toxic intermediary compounds, and a decrease in phloem transport (Hall et al. 1999; Shaner, 1991). The initial symptom of response to ALS inhibitor herbicides in sensitive species is the inhibition of plant growth, followed by chlorosis, necrosis and terminal bud death (Hall et al. 1999). ALS inhibitors affect both broadleaf and grass species. Selectivity results from the ability of the crop to metabolize the herbicide to non-toxic compounds (Hall et al. 1999; Hartnett et al. 1990).

Figure 2.3. Branched-chain amino acid biosynthesis pathway. ALS, acetolactate synthase; ALR acetolactate reductoisomerase; DHD,2,3-dihydroxyacid dehydratase; IMI, isopropylmalate isomerase (Devine et al. 1993b)



2.4.3 Cases of ALS Inhibitor Resistance

One of the first cases of ALS inhibitor resistance was reported in 1987, when a population of prickly lettuce (*Lactuca serriola* L.) from a continuous no-till winter wheat field in Idaho was not controlled by sulfonylureas (Mallory-Smith et al. 1990). Resistance in this population developed after only five applications of sulfonylurea herbicides. This population was also found to be cross-resistant to some of the imidazolinone herbicides. Herbicide resistant kochia biotypes were first reported in the Canadian prairies around this time as well (Morrison and Devine, 1994). Populations of Group 2 resistant kochia are now widespread throughout the western United States and Canada (Guttieri et al. 1995). Resistant populations of chickweed in western Canada and resistant species of pigweed in Ontario have also become an "abundant and widespread" problem (Beckie et al, 2001).

Cases of herbicide resistance to ALS inhibitors have increased at a greater annual rate than the development of resistance to any other herbicide group since the late 1980s and early 1990s (Heap, 1997; Heap 2003 www.weedscience.com). Resistant biotypes were observed within five years of their commercial introduction (Hall et al. 1999). The rapid increase in cases of resistance has been speculated to be due to an initial high frequency of resistant individuals in weed populations before herbicide use, the persistence of Group 2 herbicides and the large acreage treated with these herbicides (Heap, 1997). There are currently more weed species resistant to ALS inhibitors than to any other herbicide group, representing at least 80 dicot and monocot species including wild oat (Heap, 2003 www.weedscience.com). As with ACCase inhibitor resistance,

cross-resistance to ALS inhibitors has been identified in a number of species (Volenberg, et al, 2001, Mallory-Smith et al, 1990, Heap and Knight, 1986; Moss and Cussans 1991).

2.4.3.1 ALS Inhibitor Resistance in Wild Oat

Group 2-herbicide resistance in wild oat has been found throughout the Canadian prairies. One of the first cases of documented group 2 resistance in wild oat occurred in the Swan River region of northwestern Manitoba. Three producers reported unsatisfactory control of wild oat infestations sprayed with imazamethabenz (Friesen et al. 2000). In all three cases this was the first time imazamethabenz was used on these populations. Producer records suggested that these populations were isolated and evolved resistance independently of one another. These populations were also resistant to fenoxaprop-P (Group 1) and flamprop (Group 25). Since this initial report, cases of reported ALS inhibitor herbicide resistance in wild oat have increased. In 1997, 23% of grain elevator screenings in Saskatchewan contained Group 2 resistant wild oat (Beckie et al, 1999). In the same year, 21% of fields surveyed in Manitoba also contained ALS inhibitor resistant wild oat (Beckie et al. 1999).

2.4.4 Mechanisms of Resistance to ALS Inhibitors

In the majority of Group 2 resistant weed species, resistance it due to an alteration in the gene coding for ALS, resulting in an insensitive form of the ALS enzyme (Saari et al, 1994; Shaner, 1991). Studies have shown that several different single amino acid substitutions have resulted in resistant ALS (Tranel and Wright, 2002). Different levels of resistance and patterns of cross-resistance have been shown to be associated with these specific amino acid substitutions (Foes et al. 1999). While most cases of resistance are due to the presence of a mutated ALS gene, non-target site resistance has been observed

in populations of rigid ryegrass (Christopher et al. 1992) and downy brome (*Bromus tectorum* L.) (Mallory-Smith et al. 1999). Resistance in the downy brome population is thought to be a result of metabolization of the herbicide (Mallory-Smith et al. 1999). Resistance in the rigid ryegrass biotype investigated by Christopher et al, (1992) was thought to be due to enhanced metabolism, in addition to target site resistance. In a separate population of rigid ryegrass exhibiting resistance to a number of different herbicides, including ALS inhibitors, enhanced metabolism was believed to be the source of resistance (Burnet et al, 1994, Preston et al. 1996). The mechanism of imazamethabenz resistance has been studied in a biotype of wild oat from North Dakota (Nandula and Messersmith, 2000). The primary mechanism of resistance in this population was reduced metabolization of imazamethabenz-methyl to the biologically active imazamethabenz acid.

2.5 Flamprop Resistance

Flamprop is a member of the aminopropionate family (Group 25) (Table 2.1) that was registered for use as a post emergent wild oat herbicide in 1978 (Morrison et al, 1992). Flamprop is no longer registered for use in Canada. Flamprop is a polycyclic alkonic acid (PCA) (Duke and Kenyon, 1975). When PCAs are applied to sensitive species, growth is arrested, followed by chlorosis and eventually meristematic necrosis (Duke and Kenyon, 1975). Low concentrations of this type of herbicide causes membrane disruption, growth inhibition of apical, internodal and root meristems, disruption of auxin action, and the inhibition of fatty acid biosynthesis (Duke and Kenyon, 1975). However, the mode of action of PCAs has yet to be determined (Duke and Kenyon, 1975).

The first case of *Avena* spp. resistant to flamprop was reported in 1984, when Somody et al. (1984) screened populations of *Avena fatua* and *Avena sterilis* with a number of dissimilar herbicides. This research found populations with resistance from locations that had not been treated previously with the herbicides under investigation, including flamprop. Prior to 1992 there were no reported cases of flamprop resistant wild oat in Canada (Morrison et al. 1992). However, Kibite and Harker (1992) screened six different *Avena* spp. and found flamprop-methyl resistance in 38 accessions from four species including *A. abyssinica*, *A. brevis*, *A. sativa*, and *A. strigosa*. Flamprop resistant wild oat was identified in northwestern Manitoba in 1994, with the discovery of three populations with multiple resistance to flamprop, fenoxaprop-P and imazamethabenz (Friesen et al. 2000). These populations developed resistance in the absence of selection with either flamprop or imazamethabenz. Since the confirmation of flamprop resistance in Canada in 1994 many other resistant populations have been identified, including four populations with multiple resistance to flamprop, imazamethabenz, fenoxaprop-P and triallate/difenzoquat (Beckie et al. 1999). Surveys conducted in 1997 identified Group 25 resistant wild oat, with either single or multiple-group resistance in 28% of all fields studied in Manitoba, and 12% of fields with cross-resistant wild oat in Saskatchewan (Beckie et al. 1999).

2.6 Genetics of Herbicide Resistance

2.6.1 Inheritance Studies

Many factors have been identified that affect the development of herbicide resistance in weed species. These factors include the number of alleles involved in the expression of functional resistance, the frequency of resistance alleles in unselected

populations, the reproduction and breeding characteristics of the species, the longevity of seed in the soil, the intensity of selection that differentiates resistant individuals from susceptible, the relative fitness of resistant and susceptible genotypes, and the mode of inheritance of the resistance allele(s) (Cousens and Mortimer, 1995). Of these factors, the inheritance of resistance alleles is the focus of this thesis. The inheritance of resistance influences the likelihood and rate of resistance development, and spread of resistance in a population (Maxwell and Mortimer, 1994; Duesing, 1983; Jasieniuk et al. 1996). Genetic components of resistance remain important after resistance has developed, as they predict the likelihood of the population reverting back to susceptibility (Christoffers, 1999). Knowledge of the inheritance of herbicide resistance is also needed to create a strategy for long term control and prevention of herbicide resistant weeds (Cousens and Mortimer, 1995; Christoffers, 1999).

2.6.1.1 Nuclear versus Cytoplasmic Inheritance

Reviews of literature regarding herbicide resistance have shown that in most cases of herbicide resistance, the resistance allele is nuclear encoded (Jasieniuk et al. 1996). The only known exception is triazine resistance, where the resistance gene is cytoplasmicaly inherited through plastid genes (Souza Machado et al. 1978; Hirschberg and McIntosh 1983). However, Anderson and Gronwald (1987) found a biotype of velvetleaf (*Abutilon theophrasti* Medik.) where resistance to atrazine is under nuclear control. Cytoplasmicaly inherited traits can spread quite rapidly throughout populations, as all seed borne on resistant plants will also be resistant and there is no genetic recombination of the resistance genes (Jasieniuk et al. 1996). Cytoplasmicaly inherited traits spread only slightly faster than dominant nuclear traits. Differences in the rate of

spread are most important in the early stages of selection, when frequencies of cytoplasmic and dominant genes increase much faster than recessive genes (Mortimer, 1993 www.plantprotection.org). Studies of inheritance of ACCase inhibitor resistance in many species, including wild oat have shown resistance to be under the control of nuclear genes (Murray et al. 1995; 1996). ALS inhibitor resistance has also shown to be nuclear encoded and to follow qualitative inheritance (Tranel and Wright, 2002).

2.6.1.2 Dominant versus Recessive Expression

The majority of cases of herbicide resistance are controlled by a dominant or partially dominant gene(s). The only cases reported where resistance was controlled by a recessive gene were for trifluralin resistant green foxtail (Jasieniuk et al. 1994) and dinitroaniline resistant goosegrass (Zeng and Baird, 1997). When subjected to selection, the frequency of dominant resistant alleles increase at a greater rate than recessive alleles in highly outcrossing species. However, in populations that are mainly self-pollinating, recessive and dominant alleles increase in frequency at a similar rate (Jasieniuk et al. 1996). Partial dominance of a resistance allele causes heterozygous individuals to be less resistant than resistant homozygous individuals, but still more resistant than susceptible individuals. The partially resistant phenotype may be the result of a single partially dominant gene or a dominant gene modified by pleiotropy or other factors (Cousens and Mortimer, 1995). It has been speculated that cases of resistance controlled by dominant or partially dominant genes are more common as "a recessive resistance mutation has a poor chance of establishment in a weed population because heterozygotes are susceptible and will be eliminated when treated with the herbicide" (Jasieniuk et al. 1995). Murray et al. (1995; 1996) found that a single partially dominant nuclear gene governed ACCase

inhibitor resistance in populations of wild oat. Other research has shown that ACCase inhibitor resistance in *Avena* spp. populations was due to a dominant nuclear gene (Kibite et al. 1995; Barr et al. 1992). ALS inhibitor resistance in wild oat and other species has also been shown to be controlled by dominant or partially dominant genes in most cases (Tranel and Wright, 2002).

An important point was made by Seefeldt et al (1998) in relation to characterizing the expression of a resistance allele when expression is partially dominant. They noted that the herbicide rate used to screen populations may affect the interpretation of the inheritance of resistance. For example, if a cross is made between a homozygous resistant (RR) and homozygous susceptible (rr) plant and the resulting offspring (Rr) are then self pollinated, the F_2 generation will segregate genotypically in a 1RR:2Rr:1rr ratio. If a relatively high herbicide rate is used to screen the F_2 generation the heterozygous individuals (Rr) may be classified as susceptible, and the F_2 generation would segregate, phenotypically in a 1 resistant: 3 susceptible ratio. This would lead to the conclusion that a single recessive gene confers resistance. If a low herbicide rate is used then the heterozygous individuals may be classified as resistant and the F_2 generation would segregate in a 3 resistant:1 susceptible ratio. This would lead to the conclusion that a single dominant gene confers resistance.

2.6.1.3 Number of Genes

Herbicide resistance is controlled by one or two nuclear dominant genes for the majority of resistant weed populations (Jasieniuk et al. 1996). However, notable exceptions include a population of chlorotoluron resistant blackgrass (2 genes) (reviewed in Jasieniuk et al.1996), siduron resistance in foxtail barley (*Hordeum jubatum* L.) (3

genes) (Schooler et al. 1972) and ALS resistance in perennial ryegrass (*Lolium perenne* L.) (Mackenzie et al. 1997). If many genes in a population control resistance, then there should be a progressive response to herbicide selection if the genes act in an additive fashion, each contributing a small increase in resistance (Mortimer, 1993 www.plantprotection.org). It follows that when subjected to selection by the herbicide, fitness may increase in genotypes as a result of genetic recombination over time. Resistance conferred by a polygenic system will result in a slower evolution of resistant populations than with a single gene system (Mortimer, 1993 www.plantprotection.org). Single gene resistance is thought to be more likely to occur because most herbicides are target site specific and exert strong selection pressure, both conditions that favor monogenic resistance inheritance (Jasieniuk et al. 1996).

Resistance to ACCase inhibitors has been shown to be controlled by single genes in many weed species, including wild oat (Murray et al. 1995; 1996; Kibite et al. 1995). In most cases ALS-inhibitor resistance has also been shown to be controlled by single genes, the exception being perennial ryegrass as noted above (Tranel and Wright, 2002).

2.6.2 Evolution of Resistance

The development of herbicide resistance in weed populations is an evolutionary process. For evolution to occur there must be genetic variation in a weed population. This variation may be due to mutation or migration events. When variable populations are exposed to selection pressure imposed by agricultural practices, including the application of herbicides for weed control, the populations adapt. Several factors have been identified that influence the evolution of resistance. In addition to the mechanisms of inheritance, these factors include gene mutation, initial frequency of resistance alleles,

relative fitness of resistant and susceptible genotypes, mating system and gene flow (Cousens and Mortimer, 1995; Jasieniuk et al. 1996; Maxwell and Mortimer, 1994).

2.6.2.1 Mutation

Genetic variation in a population is a function of mutation and migration events either in the present or the past. These mutation and migration events occur independently of herbicide application. Mutation rates are typically quite low. In eukaryotes the rate of spontaneous mutation has been estimated to be about 5×10^{-6} per locus per cell division (Raven et al. 1992). However, even with mutation rates this low, it is possible to select for resistance if a large number of individuals are treated with herbicides (Jasieniuk et al. 1996). Natural variability for response to a specific herbicide may pre-exist in a population without the occurrence of new mutations. Herbicide resistant genotypes have been found in weed populations, including populations of wild oat, that have not been treated with herbicide (Somody et al, 1984, Kibite et al. 1995). Selection through repeated application of a herbicide on such heterogeneous populations would then increase the frequency of resistant individuals in the population. The initial frequency of resistance, whether initially present or due to subsequent mutation or migration, affects the number of generations needed for resistance to develop to a detectable level in the population (Jasieniuk et al. 1996).

2.6.2.2 Migration

The movement of resistance genes between populations and individuals greatly affects the evolution of resistance. In plants, gene flow may occur through either pollen flow or seed dispersal. Gene flow introduces a source of resistance to a previously susceptible population (Jasieniuk et al. 1996). The rate of gene flow will be a factor in

determining the time it takes for resistance to develop. One study has shown that outcrossing in wild oat grown in a wheat crop was between 0.08% and 0.05% at low and high densities, respectively (Murray et al. 2002). The same study found that in flax the rate of outcrossing in wild oat was higher, between 0.07% and 0.16% at low and high densities, respectively. While pollen spread is typically assumed to be the major method of gene flow between populations, the role of seed movement may be just as, or more important (Jasieniuk et al. 1996). It has been speculated that the long distance spread of resistance may be more dependant on factors such as the movement of farm machinery and the use of seed lots contaminated with resistant weed seed (Seefeldt et al. 1998, Friesen et al. 2003). Andrews et al. (1998) confirmed the importance of seed movement in the spread of ACCase inhibitor resistant wild oat. In this study they found several separate patches with a common genotype at distances up to 500 m apart. Along with known levels of outcrossing in wild oat (Imam and Allard, 1965; Murray, 1996), Andrews et al. (1998) concluded that the spread of resistant wild oat is probably not primarily due to pollen flow but is more likely due to seed spread. The relative importance of seed movement compared to pollen flow in the spread of resistant wild oat is underastandable, as wild oat is a highly self-pollinating species.

2.6.2.3 Selectivity of Herbicides

The selection pressure created by the recurrent use of the same herbicide mode of action is one of the most important factors determining the rate of resistance development within a weed population (Jasieniuk et al. 1996; Mortimer et al. 1993 www.plantprotection.org). Unfortunately the qualities that make a herbicide successful are also the qualities that lead to the development of resistance. Most of the herbicides

currently used are applied at rates that result in control of 90% or more of the susceptible individuals (Morrison et al. 1992). With such high selection intensity any resistant individuals quickly become the dominant phenotype in the population (Jasieniuk et al. 1996). Other characteristics of herbicides that contribute to the evolution of resistance are a single target site, highly specific modes of action, long term soil residual activity and frequent application (LeBaron and McFarland, 1990b).

2.6.2.4 Fitness in the Absence of Herbicides

The relative fitness of resistant and susceptible individuals in the absence of selection affects the rate at which populations become predominantly resistant. It has been shown through population genetics models that relative fitness can affect changes in the proportion of resistant to susceptible individuals in years when the herbicide is not applied (Jasieniuk et al. 1996). If the biotypes are equally fit, then the frequency of resistant biotypes will remain constant in seasons when the herbicide is not applied. However, if the resistant biotype is less fit than the susceptible biotype, then the frequency of resistant plants will decline each season the herbicide in question is not applied, slowing the overall development of resistance.

Quantifying the differences in fitness between biotypes is quite difficult as there are difficulties associated with the choice of material for comparison, the conditions under which fitness is compared and the choice of characteristics used to measure fitness (Jasieniuk et al. 1996). While differences in growth characteristics may exist between resistant and susceptible individuals, differences in the ability to reproduce are of more importance in regards to selection. Consequently, few studies have been conducted in this area. A reduction in fitness in triazine resistant biotypes has been reported in most

species in absence of the herbicide (reviewed in Gressel and Segel, 1990). Conversely, Holt and Thill (1994) concluded that ALS inhibitor resistance conferring mutations have a subtle effect on growth, but do not consistently reduce fitness.

2.6.2.5 Multiple Herbicide Resistance

The evolution of multiple herbicide resistance has become a concern, particularly with the high occurrence of multiple herbicide resistant wild oat on the Canadian Prairies. Multiple herbicide resistance can occur by either enhanced metabolism of more than one herbicide mode of action or by the accumulation of multiple mechanisms of resistance. Accumulation of resistance mechanisms was determined to be responsible for the development of populations of green foxtail resistant to Group 1 and Group 3 herbicides (Heap and Morrison, 1996). These resistant biotypes were initially selected with Group 3 herbicides, and after resistance had developed treatment with Group 1 herbicides selected for a second mechanism of resistance. A similar situation has developed with multiple resistance in an amaranth population (Ferguson et al. 2000).

Evolution of multiple resistance in wild oat has not been previously investigated, however, it has been observed that frequent use of Group 1 herbicides appears to precede the development of multiple resistance in wild oat (Beckie et al. 1999). It has been speculated though that "the evolution of individuals with multiple mechanisms of resistance is slowed, particularly since wild oat is primarily self pollinating" compared to multiple resistance development in cross-pollinating species (Beckie et al. 2001). Therefore, in wild oat it would be unlikely that resistance would develop through pollen flow from a population with one mechanism of resistance to a population with an entirely different mechanism of resistance. This type of evolution of multiple resistance is more

probable in a highly outcrossing species. In addition, multiple resistance has developed in populations in the absence of selection (Friesen et al. 2000; Beckie et al. 1999, Somody et al. 1984). The probability of multiple mechanisms of resistance in an unselected population is the product of the probabilities of natural resistance to each herbicide and therefore should be rare in the absence of selection (Wrubel and Gressel, 1994).

2.6.2.6 Strategies for Delaying Resistance

Based on an understanding of the evolution of herbicide resistance, strategies to delay and control resistance can be developed. Several strategies have been proposed including changes in herbicide use, non-chemical control measures, and the conservation of susceptible weeds (Shaner 1995). Selection pressure created by herbicides has the greatest impact on resistance development and can readily be controlled by the producer. Rotating herbicide groups (Table 2.1) or modes of action has been shown to be effective in delaying the development of target-site resistance (Bourgeois and Morrison, 1997). The degree of success of herbicide rotation depends on the relative fitness of the resistant and susceptible biotypes in the absence of selection (Jasieniuk et al. 1996). However, it had been noted that the development of metabolism-based multiple herbicide resistance will limit the effectiveness of herbicide group rotation, as resistance may evolve to multiple herbicide groups at once. (Beckie et al. 2001).

The use of herbicide mixtures to delay the development of resistance has also been investigated. For a mixture to effectively delay resistance the less-resistance prone components should control the same weeds, have the same persistence, have a different target site, be degraded in a different manner and preferably exert negative cross-

resistance to the vulnerable herbicide (Wrubel and Gressel, 1994). Negative cross-resistance refers to the situation where individuals resistant to the vulnerable herbicide are more susceptible than the wild type to the mixing partner. However for this system to work it must be assumed that the mixture will not select for a single mechanism of action that confers resistance to all components of the mixture. In addition, the lower rates of herbicides that can be used in mixtures may decrease the rate of target site resistance evolution and enhance the development of metabolism based resistance (Wrubel and Gressel, 1994).

Many non-chemical control methods have been suggested for weed resistance management, including delayed seeding, the use of rotational crops, tillage, summer fallow and hand weeding (Nalewaja, 1999). These methods may be particularly important for the control of wild oat along with herbicide rotation since wild oat is largely self-pollinating and the seed is not naturally spread great distances (Seefeldt et al. 1998). Maintaining susceptible weed populations has been identified as a potential method of delaying the spread of herbicide resistance (Maxwell, 1992). By maintaining susceptible individuals the overall selection intensity is reduced and is believed to help slow the evolutionary process. However, this method is unlikely to be effective (Jasieniuk et al. 1996), especially in self-pollinating populations unless the susceptible population is quite large and close to the resistant population (Roush et al. 1990).

3.0 MATERIALS AND METHODS

3.1 Material

The two multiple herbicide resistant wild oat populations under investigation in this study originated in the Swan River region of Manitoba, Canada (Figure 3.1) and were first described by Friesen et al. (2000). Populations UMWO12-01 and UMWO12-03 were found in fields located 5 km apart. These populations were first identified when farmers reported unsatisfactory control of wild oat in spring wheat crops when sprayed with imazamethabenz. Imazamethabenz had never been used on these populations before. Seed from populations UMWO12-01 and UMWO12-03 was collected by the producers from obvious wild oat plants/patches in the field. The farmers' records indicated that the farmers had not shared equipment, and based on field histories, it is considered unlikely that these populations developed from a single mutation event followed by subsequent spread between the fields (Table 3.1) (Friesen et al. 2000).

Figure 3.1. Manitoba, Canada – Swan River and Winnipeg (http://travel.yahoo.com/p-travelguide-577744-map_of_manitoba-I)

Table 3.1. Cropping histories and graminicide herbicide usage on fields where multiple herbicide resistance in wild oat populations UMWO12-01 and UMWO12-03 were identified (modified from Friesen et al. 2000).

	UMWO12-01		UMWO12-03[a]	
Year	**Crop**	**Herbicide (Group)**	**Crop**	**Herbicide (Group)**
1983	*Triticum aestivum*	Diclofop (1)		
1984	*Brassica napus*	Dinitramine (3)+ triallate (8) PPI[b], glyphosate (9) preplant, sethoxydim (1)		
1985	*Hordeum vulgare*	Difenzoquat (8)		
1986	*H. vulgare*	-		
1987	*H. vulgare*	-		
1988	*B. napus*	Fenoxaprop (1)		
1989	*T. aestivum*	Diclofop (1)		
1990	*T. aestivum*	Flamprop (25)		
1991	*H. vulgare*	-	*T. aestivum*	Fenoxaprop[c,d] (1)
1992	*B. napus*	Ethafluralin (3) PPI[b], sethoxydim (1)	*T. aestivum*	Triallate (8) PPI[b], difenzoquat (8)
1993	*T. aestivum*	-	*T. aestivum*	Difenzoquat (8)
1994	*T. aestivum*	Imazamethabenz[c] (2)	*B. napus*	Ethafluralin (3) PPI[b]
1995	*H. vulgare*	Triallate (8) PPI[b]	*T. aestivum*	Imazamethabenz[c] (2)

[a] prior to 1991 diclofop was primarily used for wild oat control.
[b] PPI = preplant incorporated.
[c] instances when farmers observed poor control.
[d] ACCase inhibitor resistance was confirmed for this population in 1991.

Wild oat seedlings grown from seed received from the producers were treated with 100 g a.i./ha of fenoxaprop-P. For each population, seed was harvested from 20 surviving plants and pooled to form a base population. The populations were classified by Friesen et al. (2000) as having multiple-herbicide resistance to imazamethabenz, flamprop and fenoxaprop-P (Table 3.2). Both populations had similar resistance factors for imazamethabenz and flamprop, 7.2 and 8.7 times more resistant than the susceptible biotype, respectively. Resistance factors were slightly different for fenoxaprop-P (2.0 for UMWO12-01 and 2.9 for UMWO12-03). The susceptible biotype used in this study was

UM5. It was collected from Portage La Prairie Manitoba, and has been used and characterized in numerous studies (Heap et al. 1993; Murray et al. 1995; 1996; Devine et al. 1993a).

Table 3.2. Response of susceptible and resistant wild oat populations to imazamethabenz, flamprop and fenoxaprop-P under growth room conditions. (adapted from Friesen et al. 2000)

Herbicide/Population	GR_{50} (g/ha)[a]	RF[b]
Imazamethabenz		
UM5	54.8	
UMWO12-01	393.0	7.2
UMWO12-03	393.0	7.2
Flamprop		
UM5	19.7	
UMWO12-01	173.0	8.6
UMWO12-03	173.0	8.6
Fenoxaprop-P		
UM5	16.9	
UMWO12-01	33.8	2.0
UMWO12-03	49.3	2.9

[a]GR_{50} = dosage in g/ha of herbicide that reduced shoot dry matter by 50% relative to the untreated control.
[b]RF = resistance factor, R/S GR_{50}

3.2 Growing Conditions

All wild oat seed used in this study was dehulled in order to enhance germination. Germination procedures used were similar to those used in other studies (Heap et al. 1993; Murray et al. 1995). Seed was germinated in 9 cm diameter plastic petri plates lined with Whatman No. 1 filter paper. Plates were moistened with a 0.1% KNO_3 solution and were placed in the dark for 48 hours in a refrigerator set at 4 C. Prior to refrigeration, plates were wrapped with parafilm to ensure seeds would not dry out. Petri plates were then moved to room temperature (20-25 C) in the dark for a further 48 hours. This procedure was slightly modified for the F_2 derived F_3 generation ($F_{2:3}$). To ensure

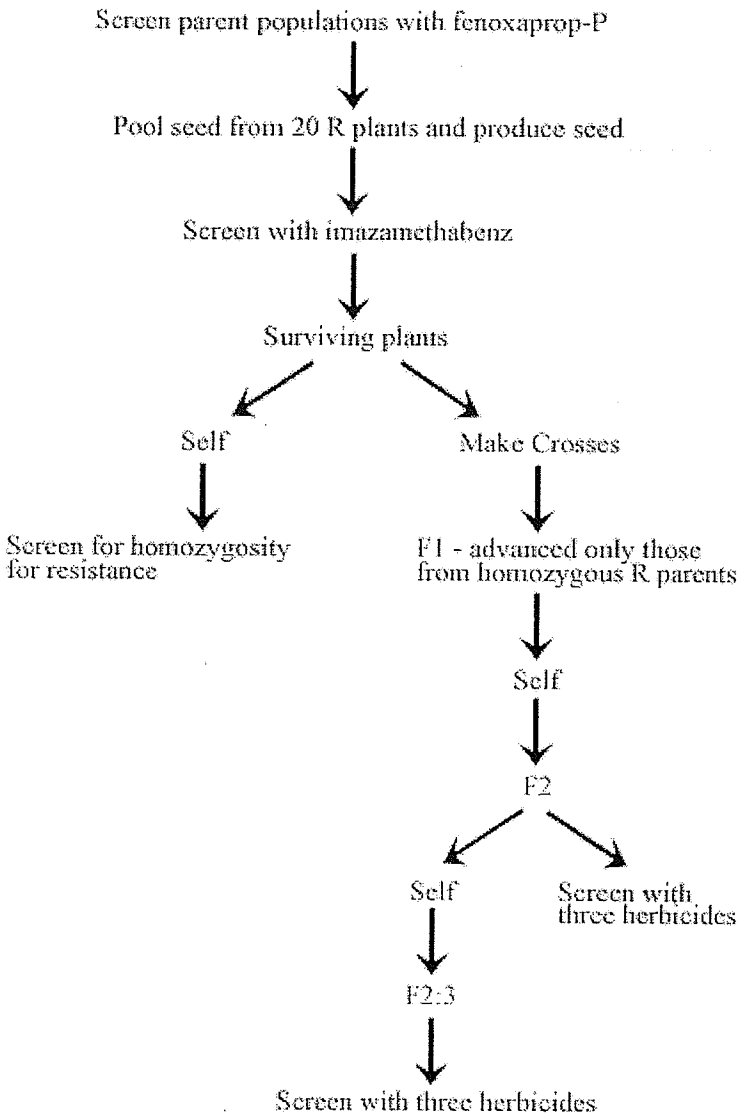
more even germination, $F_{2:3}$ seeds were pierced with a dissecting needle after seed had been at 4 C in the dark for a 24 hour period. Seeds were pierced on the dorsal side next to the embryo and kept at 4 C in the dark for an additional 48 hours, followed by 48 hours at room temperature in the dark. In this case parafilm was not used on the petri plates until after the seeds had been pierced.

Germinated seeds were planted in a potting mixture of clay loam/sand/peat moss in a 2:1:1 ratio by volume. Plants used to make initial crosses and produce subsequent generations were grown in 15 cm clay/plastic pots. Plants that were screened with the different herbicides were grown in flats containing the same soil mixture. Plastic flats that were used measured 53 cm x 26 cm and contained 48 individual cells in each flat. One seed was planted per cell. The soil mixture was changed for the $F_{2:3}$ generation, due to the suspected presence of herbicide residues in the clay loam component. A 1:1:1 volumetric ratio of clay loam/sand/peat moss was used to address this concern. At this time, two seeds were planted per cell for the resistant/susceptible checks to ensure enough checks were present despite possible herbicide residues in the soil. All plants were grown in growth rooms/cabinets with a 16 hour photoperiod and approximate irradiance of 200 $\mu E\ m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD) for population development and 600 $\mu E\ m^{-2}s^{-1}$ PPFD for screening populations. The temperature regime was 21/15 C day/night and relative humidity was not controlled. Plants were watered as needed and fertilized weekly with a dilute solution of water soluble fertilizer (20-20-20) at a rate of approximately 2.4 g/L Peters Professional Water Soluble Fertilizer (W.R. Grace & Co., Fogelsville PA) (equivalent to 40 g/ha N).

3.3 Population Development

Population development is outlined in Figure 3.2. Prior to F_1 production, UMWO12-01 and UMWO12-03 parental populations were screened with imazamethabenz at 400 g a.i./ha to ensure that each parent was resistant. In order to determine if each parent was homozygous for resistance, panicles from each plant were self-pollinated by enclosing individual panicles in glycine crossing bags. This was done at the same time as different panicles on the same plants were used to make crosses. Progeny produced by selfing resistant parents were tested for segregation of resistance/susceptibility to imazamethabenz, flamprop and fenoxaprop-P. Only seed from those plants found to be homozygous for resistance were carried on to produce the next generation. A total of 2,149 seedlings from selfed parental plants were screened with the three herbicides

Figure 3.2. Flow diagram of population development



To maximize seed production two crossing methods were used. The first method involved hand pollination of emasculated panicles (Brown, 1980). Emasculation was done manually by removing immature anthers with forceps. Hands and all tools were sterilized with 95% ethanol to prevent accidental pollination. Immature and over mature florets were removed prior to emasculation and only the primary florets were pollinated.

In this method anthers from paternal plants were directly transferred to emasculated maternal florets. Wild oat has been shown to dehisce in the afternoon (Raju et al. 1985) and therefore growth room lights and day-time temperatures were timed to come on at 2:00 a.m. for morning pollination. Anthers began to dehisce approximately 6 hours after the lights in the growth room came on and continued to dehisce for about 3 hours. Pollination of each floret was conducted for two days in a row to ensure successful fertilization. Paternal anthers were left in the florets to maximize the amount of pollen in each floret. Crossing was conducted for approximately a 7 day period for each panicle, as this was the duration of time needed for all stigmas on emasculated panicles to mature. In order to identify how many times each floret had been pollinated a system of marking each individual floret was devised. Awns were clipped from florets to designate emasculation. One glume was clipped on the first day of pollination and the other glume was clipped on the second day of pollination.

The second method of pollination used was the approach method described by McDaniel et al. (1967). For this method, female parents were emasculated and placed in glycine bags with paternal panicles. The paternal panicles were still attached to their respective plants to ensure pollen viability. Bags were shaken daily to maximize pollen movement within the crossing bag. After approximately one week, male panicles were removed so seed from the paternal parent was not mixed with seed from the maternal parent.

Forty-two crosses were made using the hand pollination method and 19 crosses were made using the approach method. A total of 369 and 58 seeds were produced using each method, respectively. The average number of seeds produced per panicle using the

hand pollination technique was 8.8, while an average of only 3.1 seeds were produced per cross using the approach method.

During all stages of seed production, panicles of interest were covered with glassine/perforated plastic bags to prevent contamination with unwanted pollen. All bags were closed at the base of the panicles to ensure all shed seed would be collected. Crosses were made between each of the resistant biotypes and the susceptible parent (UMWO12-01/UM5, UM5/UMWO12-01, UMWO12-03/UM5 and UM5/UMWO12-03) as well as between the resistant biotypes (UMWO12-01/UMWO12-03 and UMWO12-03/UMWO12-01). A minimum of 10 crosses were made for each of the combinations of crosses listed above.

Four F_1 hybrids from each reciprocal cross were self-pollinated to produce the F_2 generation. Only 3 F_2 families were screened with the herbicides due to time and space limitations. These families were chosen based upon results obtained from screening selfed parental seed. Due to the same limitations, only one F_1 family per each reciprocal cross was advanced to the $F_{2:3}$ generation, chosen again on the basis of results from the screening of parental seed. Approximately 80 F_2 plants per F_1 cross were grown out and self pollinated to produce the $F_{2:3}$ generation. Seed from each plant was harvested and stored separately at each stage of population development to maintain distinct pedigrees for each plant. Family sizes were determined based on the following formula, which calculates the minimum family size required to have at least one of each genotype present:

$P = 1 - (1 - p)^N$ where: P = probability of at least one individual of the lowest frequency class being present

p = frequency of the genotype with the lowest frequency

N = number of individuals

Assuming that resistance is controlled by one dominant nuclear gene (p = 0.25), as is the case most often (Jasieniuk et al. 1996), if a family size of 80 individuals is chosen the probability of having at least one of the lowest frequency class present is approximately 1.000. If resistance is controlled by 2 genes (p = 0.0625), then the probability of having at least one of the lowest frequency class present for a family size of 80 becomes 0.994. Probability values this high indicate that with a family size of 80 all genotypes should be present in detectable amounts.

3.3.1 Cross Identification System

All crosses are identified using a system of letters and numbers. The female parent used in the original cross is the first individual listed in the cross identification, to the left of the slash symbol, the male parent is listed to the right. Each parent is identified with a number and letter(s). The number represents the biotype of the parent (1 = UMWO12-01, 3 = UMWO12-03 and 5 = UM5) and the letters represent the specific parental plant. For example, F_1 seed identified as 1A/5F was produced by a cross between plant A from the resistant biotype UMWO12-01 as the female parent and plant F from susceptible biotype UM5 as the male parent. F_1 seedlings planted from each cross are identified with a number following the description of the original cross (i.e. 1A/5F 1, 1A/5F 2, etc.). This same "name" is used to identify the family of F_2 seedlings from each

specific F_1 plant. Approximately 80 F_2 seedlings were grown out to produce $F_{2:3}$ families. Each specific F_2 seedling and associated $F_{2:3}$ family was identified by a letter following the name of the specific F_1 plant of family origin. For example 1A/5F 1-A is an $F_{2:3}$ family A derived from an F_2 plant that came from F_1 plant number 1 of the cross between plant A from UMWO12-01 population and plant F from UM5 population.

3.4 Screening Techniques

Plants in all generations were divided into treatment groups, each sprayed with a separate herbicide. Plants were sprayed at the 2-3 leaf stage, approximately 14 days after planting, for imazamethabenz (Group 2) and fenoxaprop-P (Group 1). Plants were sprayed with flamprop (Group 25) at the 3-4 leaf stage, approximately 21 days after planting. These wild oat growth stages correspond to herbicide label recommendations. Commercially available herbicide formulations were used throughout the study. Herbicide rates used for this project were based on previous studies of the same populations (Friesen et al. 2000). These rates provided a clear distinction between resistant and susceptible checks, with approximately 5% of the checks displaying the alternate reaction. Rates of imazamethabenz, flamprop and fenoxaprop-P used were 350 g a.i./ha plus 0.25% v/v Agral 90, 300 g a.i./ha and 90 g a.i./ha, respectively. Resistant checks from each population in question and susceptible checks were planted in each flat of material being tested to ensure validity of results. A cabinet sprayer equipped with a flat-fan nozzle set to deliver 117 L/ha of spray solution at 310 kPa in a single pass was used to apply the herbicides.

A limited amount of F_1 seed was produced and therefore resulting F_1 plants were not sprayed to determine resistance levels. Forty F_2 seedlings from each reciprocal cross

were treated with each of the herbicides and segregation patterns were determined. This family size was determined based on the formula given in section 3.3. A family size of 40 was chosen based on the capacity of the flats used. This kept individual families in separate flats. With an assumption of a single dominant nuclear gene for resistance, the probability of the least frequent genotype being present in a family size of 40 is approximately 1.000. If two genes are needed for resistance then the probability of the least frequent genotype being present is 0.924. Both probabilities support the sufficiency of a family size of 40. A total of 5,655 F_2 seedlings were screened. Twenty $F_{2:3}$ seedlings from each of the reciprocal crosses were screened with each of the herbicides and segregation patterns were determined. This family size was also determined using the formula given in section 3.3. In this case F_2 results indicated that one locus was involved per herbicide so a family size of 20 was justified. The probability of the least frequent genotype being present is 0.997 for a family size of 20 in this case. The number of $F_{2:3}$ families screened for each cross ranged from 17 to 73. These tests were conducted to confirm the validity of the F_2 results and to determine if resistances in the different populations were due to the same mutation. A total of 686 $F_{2:3}$ families of consisting of 15 to 20 individuals each were screened.

Plants were rated as resistant (R) or susceptible (S) after herbicide application based on a visual rating. Plants showing signs of recovery and new growth after treatment were considered R, while plants exhibiting no new growth and tissue death were considered S. Typical response types for each herbicide are illustrated in Figures 3.3-3.5. Plants were rated 2, 3 and 4 weeks after treatment to ensure that plants had fully

responded to herbicide treatments. Data reported in tables is from ratings taken four weeks after herbicide treatment.

Figure 3.3. Response types four weeks after treatment with 350 g a.i./ha imazamethabenz plus 0.25% v/v Agral 90. Susceptible phenotype on the left, resistant phenotype on the right.

Figure 3.4. Response types four weeks after treatment with 300 g a.i./ha flamprop. Susceptible phenotype on the left, resistant phenotype on the right.

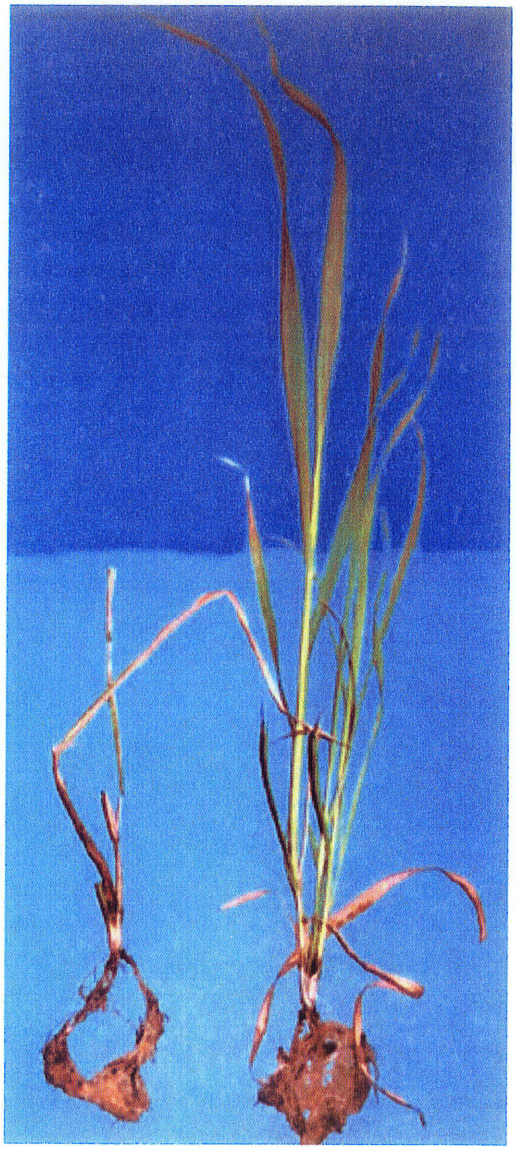


Figure 3.5. Response types four weeks after treatment with 90 g a.i./ha fenoxaprop-P. Susceptible phenotype on the left, resistant phenotype on the right.

$F_{2:3}$ families were classified as homozygous resistant when 1 or fewer individuals did not survive and homozygous susceptible when 2 or fewer individuals survived. All other families were considered to be segregating for the resistance trait. These criteria were set based on the following formula:

$n!/x!y!\ p^x q^y$ where: n = family size

x = number of homozygous dominant individuals

y = number of homozygous recessive individuals

p = frequency of dominant gene

q = frequency of recessive gene

Using this formula, a family size of 20 and assuming resistance is controlled by one dominant gene, the probabilities of misclassifying a resistant population as segregating and a susceptible population as segregating are 0.021 and 1.5×10^{-9}, respectively. These probabilities of error were chosen to correspond with the 0.05 error rate observed in the resistant and susceptible checks planted in each flat based on the herbicide rates chosen for use in this project. Twenty $F_{2:3}$ seeds were planted for each family, however in some cases not all seedlings emerged. Only those families where at least 15 individuals were screened were used for determining segregation ratios. The criteria for classifying families were used for all three herbicides.

3.5 Statistical Analysis

Chi-square tests were used to determine the goodness of fit of the segregation ratios to known genetic models (Strickberger, 1985). Homogeneity chi-square tests were used to determine if data could be pooled for the reciprocal crosses and for the different

experiments (Strickberger, 1985). Linkage between resistances to the different herbicides was determined based on methods as described by Strickberger (1985).

4.0 RESULTS AND DISCUSSION

4.1 Parental Screening

Initially 42 different parental combinations of crosses were made between the resistant and susceptible populations. To ensure homozygosity for resistance, seed produced from self-pollinating each resistant parental plant was screened with the three herbicides. The number of seedlings screened with each herbicide was determined based on the amount of selfed seed produced by each parent, with a maximum of 20 seedlings being screened per herbicide. Based on the results of the parental screening, 14 crosses were eliminated due to a lack of homozygosity for flamprop resistance (Table 4.1). All parents were found to be homozygous for imazamethabenz resistance. The results of the parental seed screening with imazamethabenz were not surprising, as the parents used to make the crosses had all survived treatment with imazamethabenz. Results from the parental seed screening with fenoxaprop-P showed that none of the UMWO12-01 parents were homozygous for resistance. As a result no crosses involving UMWO12-01 were further investigated with regards to fenoxaprop-P resistance. The fact that crosses were eliminated due to results obtained by this step demonstrates the importance of testing parental plants for homozygosity. Due to space and time limitations, 3 crosses from each combination of reciprocal crosses were advanced to the F_2 generation and screened (these appear in boldface in Table 4.1). Only one of each of these reciprocal crosses was screened in the $F_{2:3}$ generation (1A/5F +1A/5AA, 5W/1I, 3AX/5BC, 5AT/3AO, 1Y/3N and 3AK/1AL).

Table 4.1. Number of resistant parental plants over total tested screened with the three herbicides to ensure parental homozygosity for resistance.

	Number of Resistant Plants (Total Tested)					
	Maternal Parent			Paternal Parent		
Cross[a]	Imaz.[b]	Flamp.[c]	Fenox.[d]	Imaz.[b]	Flamp.[c]	Fenox.[d]
UMOW12-01/UM5 (R/S)						
1A/5F	**20(20)**	**18(19)**	**0(15)**			
1A/5AA	**20(20)**	**18(19)**	**0(15)**			
1B/5G	**20(20)**	**20(20)**	**11(17)**			
1B/5P	**20(20)**	**20(20)**	**11(17)**			
1C/5H	**20(20)**	**19(20)**	**0(14)**			
1D/5J	20(20)	17(20)	0(20)			
1D/5AD	20(20)	17(20)	0(20)			
1E/5R	20(20)	17(20)	1(20)			
1F/5Y	20(20)	15(19)	0(16)			
1K/5AG	20(20)	13(20)	0(19)			
1M/5AI	20(20)	12(18)	0(19)			
UM5/UMWO12-01 (S/R)						
5V/1H				20(20)	16(20)	2(14)
5W/1I				**20(20)**	**20(20)**	**0(18)**
5X/1J				**20(20)**	**20(20)**	**10(17)**
5AE/1N				**20(20)**	**20(20)**	**0(18)**
5AF/1O				20(20)	16(20)	0(16)
5AP/1W				20(20)	16(19)	0(18)
UMWO12-03/UM5 (R/S)						
3AS/5AX	10(10)	8(8)	9(9)			
3AU/5AY	**15(15)**	**14(14)**	**13(13)**			
3AV/5AZ	**18(18)**	**14(14)**	**14(14)**			
3AX5BC	**11(11)**	**11(11)**	**8(8)**			
3AZ/5BE	2(2)	2(2)	2(2)			
UM5/UMWO12-03 (S/R)						
5AS/3AN				**13(13)**	**10(10)**	**12(12)**
5AT/3AO				**15(15)**	**11(11)**	**14(14)**
5AU/3AP				11(11)	11(11)	9(9)
5AV/3AQ				11(11)	10(10)	10(10)
5AW/3AR				**18(18)**	**15(15)**	**13(13)**
5BD/3BA				11(11)	9(9)	10(10)
5BF/3BC				10(10)	8(8)	8(8)

	Number of Resistant Plants (Total Tested)					
	Maternal Parent			Paternal Parent		
Cross[a]	**Imaz.**[b]	**Flamp.**[c]	**Fenox.**[d]	**Imaz.**[b]	**Flamp.**[c]	**Fenox.**[d]
UMWO12-01/MWO12-03 (R/R)						
1Q/3C	13(13)	6(13)	0(11)	6(6)	5(5)	4(4)
1U/3J	20(20)	8(19)	0(19)	20(20)	19(19)	20(20)
1U/3AH	20(20)	8(19)	0(19)	16(16)	13(13)	15(15)
1V/3K	**20(20)**	**18(20)**	**0(18)**	**20(20)**	**19(19)**	**19(19)**
1V/3AG	20(20)	18(20)	0(18)	8(8)	7(7)	7(7)
1Y/3N	**16(16)**	**15(15)**	**1(15)**	**20(20)**	**19(19)**	**16(16)**
1AH/3AD	1(1)	1(1)	0(1)	20(20)	19(19)	19(19)
1AO/3AT	**10(10)**	**9(10)**	**0(9)**	**20(20)**	**16(17)**	**15(15)**
UMWO12-03/UMWO12-01 (R/R)						
3P/1Z	**6(6)**	**6(6)**	**6(6)**	**20(20)**	**20(20)**	**10(15)**
3Q/1AA	9(9)	9(9)	6(6)	18(18)	14(18)	0(14)
3R/1AC	20(20)	19(19)	13(13)	4(4)	1(4)	0(3)
3X/1AJ	**6(6)**	**5(5)**	**4(5)**	**15(15)**	**11(12)**	**0(12)**
3AK/1AL	**20(20)**	**19(19)**	**16(17)**	**11(11)**	**9(10)**	**0(10)**

[a] crosses that appear in boldface font were advanced to the F_2 generation.
[b] imazamethabenz
[c] flamprop
[d] fenoxaprop-P

4.2 Imazamethabenz (Group 2) Results

4.2.1 Resistant/Susceptible and Susceptible/Resistance Results

4.2.1.1 F_2 Results

Results from screening the F_2 families from the UMWO12-01/UM5, UM5/UMWO12-01, UMWO12-03/UM5 and UM5/UMWO12-03 crosses with imazamethabenz are reported in Table 4.2. Data has been pooled within families where acceptable, based on chi-square homogeneity values and the Yates correction factor has been used where appropriate for all analyses (Strickberger, 1985). When using chi-square analysis a probability of 0.05 or greater indicated that the data did not significantly differ from the ratio tested.

Of the UMWO12-01/UM5 crosses the F_2 families from 1A/5F were homogeneous enough to be pooled and segregated in a 3R:1S ratio. The 4 F_2 families from cross 1A/5AA were not homogeneous, however 3 of these families also segregated in a 3R:1S ratio. F_2 families from the 1C/5H cross were also homogeneous but did not fit a 3R:1S ratio, however 3 of these 4 families fit a 3R:1S ratio when tested individually.

For the reciprocal crosses only 5AE/1N were homogeneous and fit a 3R:1S ratio. F_2 families from 5W/1I and 5X/1J were not homogeneous, but 5 of these 8 families did fit a 3R:1S ratio. For 2 of the 3 families that did not fit the 3R:1S ratio, probabilities were close to the acceptable 0.05 level. A 3R:1S segregation pattern is expected when one dominant nuclear gene is responsible for resistance in each resistant population. The resistance gene is believed to be of nuclear and not cytoplasmic origin as results from reciprocal crosses were not statistically different.

In some cases the segregation ratio observed was reversed, i.e 1R:3S. Crosses involving 1B as the female parent (1B/5G and 1B/5P) were homogeneous and segregated in this ratio along with one F_2 family from the 5X/1J cross. F_2 family 1A/5AA 2 did not closely fit a 1R:3S ratio, but more susceptible than resistant individuals were observed. This reversal of dominance is believed to be the result of the herbicide rate used to screen the populations (Seefeldt et al. 1998). The rate used may have been at a threshold level causing heterozygous individuals to display the resistant phenotype a majority of the time, while only occasionally displaying the susceptible phenotype. It may also be possible that the resistance trait does not exhibit complete dominance, as has been shown in other cases of ALS inhibitor resistance in other weed species (reviewed in Tranel and Wright, 2002). Differences in the phenotypic responses of the heterozygotes may also

involve an interaction of genotype, herbicide rate and growth cabinet environments. While all growth cabinets were set to the same specifications, some variation between cabinets is expected. All F_2 families involving 1B (1B/5G and 1B/5P) as the female parent were screened in the same growth cabinet in the same run and 6 of these 7 families segregated in a 1R:3S ratio. The particular environmental conditions during this specific run may account for the reversal of dominance.

Results from crosses involving UMWO12-03 also segregated in a 3R:1S ratio for the majority of crosses. All F_2 families for crosses 3AX/5BC and 5AW/3AR were homogeneous and fit a 3R:1S ratio. F_2 families from crosses 3AU/5AY, 3AV/5AZ and 5AT/3AO were also homogeneous, but did not fit a 3R:1S ratio. However, probability values for 3AU/5AY and 3AV/5AZ did not differ greatly from the acceptable 0.05 level. Individually, the F_2 families from 5AT/3AO fit a 3R:1S ratio. F_2 families from 5AS/3AN were not homogeneous, but all 4 families did fit a 3R:1S ratio. The 3R:1S ratio observed indicates that one dominant gene was responsible for resistance to imazamethabenz in population UMWO12-03. Since reciprocal crosses segregated in the same manner the gene appears to be nuclear in origin rather than cytoplasmic.

Table 4.2. Segregation for imazamethabenz resistance in the F_2 generation of resistant/susceptible and susceptible/resistant crosses treated with 350 g a.i./ha imazamethabenz plus 0.25% v/v Agral 90

Cross[a]	Resistant	Susceptible	Ratio	χ^2	Probability[b]
	Number of Plants				
UMWO12-01/UM5 (R/S)					
1A/5F	101	31	3:1	0.162	0.69
1A/5AA 1	24	11	3:1	0.467	0.49
1A/5AA 2	14	17	1:3	5.688	0.02
1A/5AA 3	22	11	3:1	0.818	0.37
1A/5AA 4	33	5	3:1	2.246	0.13
1B/5G + 1B/5P	79	182	1:3	3.863	0.05
1C/5H	83	44	3:1	6.302	0.01
UM5/UMWO12-01 (S/R)					
5W/1I 1	25	12	3:1	0.730	0.39
5W/1I 2	26	9	3:1	0.010	0.92
5W/1I 3	23	15	3:1	3.509	0.06
5W/1I 4	36	4	3:1	4.033	0.04
5X/1J 1	24	13	3:1	1.523	0.22
5X/1J 2	27	10	3:1	0.009	0.92
5X/1J 3	21	15	3:1	4.481	0.03
5X/1J 4	15	23	1:3	3.509	0.06
5AE/1N	75	27	3:1	0.118	0.73
UMWO12-03/UM5 (R/S)					
3AU/5AY	123	26	3:1	4.530	0.03
3AV/5AZ	84	42	3:1	4.667	0.03
3AX/5BC	69	32	3:1	2.405	0.12
UM5/UMWO12-03 (S/R)					
5AS/3AN 1	29	4	3:1	2.273	0.13
5AS/3AN 2	20	13	3:1	2.919	0.09
5AS/3AN 3	19	10	3:1	0.931	0.33
5AS/3AN 4	23	13	3:1	1.815	0.18
5AT/3AO	80	42	3:1	5.781	0.02
5AW/3AR	66	28	3:1	1.149	0.28

[a] crosses not followed by a number designation indicate pooled results from three/four different F_1 plants. Data was pooled based on chi-square homogeneity tests. Crosses where a number designation does follow the cross indicates that results are from an individual F_1 plant.

[b] probabilities of 0.05 or greater indicate that the data does not differ significantly from the tested ratio.

4.2.1.2 $F_{2:3}$ Results

To clarify F_2 results $F_{2:3}$ families were screened with imazamethabenz. Results from screening $F_{2:3}$ families are reported in Table 4.3. $F_{2:3}$ families developed from UMWO12-01/UM5 and UM5/UMWO12-01 crosses segregated in a 1 homozygous resistant : 2 segregating: 1 homozygous susceptible ratio. This ratio was in agreement with the F_2 results, which indicated one dominant nuclear gene was responsible for imazamethabenz resistance in this population. When results from crosses 1A/5F, 1A/5AA and 5W/1I were combined, the data still fit a 1:2:1 ratio. This further confirmed that the resistance gene was nuclear based rather than cytoplasmic. $F_{2:3}$ families from UMWO12-03/UM5 and UM5/UMWO12-03 crosses also segregated in a 1:2:1 ratio. When these reciprocal crosses were pooled the results also fit a 1:2:1 ratio. These results confirmed F_2 results and results from other studies (Tranel and Wright, 2002), indicating that one dominant nuclear gene was encoding for imazamethabenz resistance in populations UMWO12-01 and UMWO12-03.

Table 4.3. Segregation for imazamethabenz resistance in the $F_{2:3}$ generation of resistant/susceptible and susceptible/resistant crosses treated with 350 g a.i./ha imazamethabenz plus 0.25% v/v Agral 90

Cross[a]	Resistant	Segregating	Susceptible	Ratio	χ^2	Probability[b]
	Number of Families					
UMWO12-01/UM5 (R/S)						
1A/5F + 1A/5AA	9	26	11	1:2:1	0.956	0.62
UM5/UMWO12-01 (S/R)						
5W/1I	3	11	3	1:2:1	1.471	0.48
UMWO12-01/UM5 + UM5/UMWO12-01 (R/S + S/R)						
1A/5F + 1A/5AA + 5W/1I	12	37	14	1:2:1	2.047	0.36
UMWO12-03/UM5 (R/S)						
3AX/5BC	4	21	7	1:2:1	3.688	0.16
UM5/UMWO12-03 (S/R)						
5AT/3AO	20	39	10	1:2:1	4.072	0.13
UMWO12-03/UM5 + UM5/UMWO12-03 (R/S + S/R)						
3AX/5BC + 5AT/3AO	24	60	17	1:2:1	4.545	0.10

[a] pooled results from four different F_1 plants. Data was pooled based on chi-square homogeneity tests.

[b] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

4.2.2 Resistant/Resistant Results

4.2.2.1 F_2 Results

Crosses were made between resistant populations to determine if resistance to imazamethabenz was due to the same gene mutation. F_2 seedlings from UMWO12-01/UMWO12-03 and UMWO12-03/UMWO12-01 crosses segregated into resistant and susceptible individuals and results are reported in Table 4.4. If the same gene were responsible for resistance in both populations it would be expected that all F_2 seedlings would be resistant. The fact that there was segregation in the F_2 generation indicated that different genes were involved in the expression o f imazamethabenz resistance in each population. If there were separate dominant resistance genes in each population, the F_2

generation would be expected to segregate in a 15R:1S ratio as was seen in some of the crosses (1AO/3AT 2, 3X/1AJ 2 and 3AK/1AL 2). The 15R:1S scenario can be explained if the presence of at least one dominant allele in an individual confers resistance. However, in the majority of cases the results did not conform to this ratio according to statistical analysis. Some of the F_2 families fit a 9R:7S ratio (1V/3K, 1AO/3AT 1, 3P/1Z 1, 3X/1AJ 1, 3AK/1AL 1 and 4) and others fit a 11R:5S ratio (3P/1Z 2, 3X/1AJ 3 and 4, and 3AK/1AL 3). Both of these ratios can be explained individually using one separate dominant nuclear gene in each population. For the families segregating in a 9R:7S ratio, a single copy of a dominant resistance allele from each population must be present for resistance to be expressed. For the families segregating in a 11R:5S ratio, more than one copy of a resistance allele from either population must be present for resistance to be expressed.

Table 4.4. Segregation for imazamethabenz resistance in the F_2 generation of resistant/resistant crosses treated with 350 g a.i./ha imazamethabenz plus 0.25% v/v Agral 90

Cross[a]	Resistant	Susceptible	Ratio	χ^2	Probability[b]
	Number of Plants				
UMWO12-01/UMWO12-03 (R/R)					
1V/3K	90	57	9:7	1.478	0.22
1Y/3N	107	22	9:7	37.357	9.84×10^{-10}
1AO/3AT 1	23	14	9:7	0.313	0.58
1AO/3AT 2	31	4	15:1	0.840	0.40
UMWO12-03/UMWO12-01 (R/R)					
3P/1Z 1	17	21	9:7	1.606	0.21
3P/1Z 2	27	10	11:5	0.142	0.71
3X/1AJ 1	23	16	9:7	0.033	0.86
3X/1AJ 2	36	3	15:1	0.002	0.97
3X/1AJ 3	27	12	11:5	0.012	0.91
3X/1AJ 4	27	12	11:5	0.012	0.91
3AK/1AL 1	24	15	9:7	0.254	0.61
3AK/1AL 2	32	5	15:1	2.207	0.14
3AK/1AL 3	32	6	11:5	3.539	0.06
3AK/1AL 4	18	17	9:7	0.164	0.69

[a] crosses not followed by a number designation indicate pooled results from two, three or four different F_1 plants. Data was pooled based on chi-square homogeneity tests. Crosses where a number designation does follow the cross indicates that results are from an individual F_1 plant.

[b] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

4.2.2.2 $F_{2:3}$ Results

$F_{2:3}$ data were analyzed to clarify F_2 results. To confirm the 9R:7S observed in the F_2 a 1 homozygous resistant: 8 segregating: 7 homozygous susceptible ratio was tested in the $F_{2:3}$ generation. However the pooled $F_{2:3}$ families did not fit this ratio. A 15R:1S and 11R:5S ratio in the F_2 would result in an expected 7 homozygous resistant: 8 segregating: 1 homozygous susceptible in the $F_{2:3}$, making it impossible to distinguish between the two scenarios. The data observed did not closely fit this 7:8:1 ratio either. This lack of fit may have been due to the family size used in $F_{2:3}$ screening. The

experiment was designed to clarify F_2 results for a single resistance gene for both populations hypothesis, and only 20 individuals are needed to accomplish this. However, in order to get a better understanding of what is truly happening in the $F_{2:3}$, a larger number of individuals would need to be screened for each family. This was not done because of time and space limitations. Since only 20 individuals were screened for each family and based on the criteria set to determine if a family is segregating for resistance or not, it is likely that the number of homozygous resistant families was over estimated and this would lead to a misclassification of the heterozygous families. As an alternative to the 7:8:1 ratio (which did not fit the results), chi-square values were determined for a 15:1 ratio, where homozygous dominant and heterozygous families were classified as the same phenotype (non-susceptible). These results are reported in Table 4.5. When a 15:1 ratio was tested both crosses yield acceptable chi-square values. Based on $F_{2:3}$ results, resistance to imazamethabenz appears to be due to different resistance genes in each population, however the interaction between these genes and the resistant phenotype could not be clearly determined.

Table 4.5. Segregation for imazamethabenz resistance in the $F_{2:3}$ generation of resistant/resistant crosses treated with 350 g a.i./ha plus imazamethabenz 0.25% v/v Agral 90

Cross[a]	Non-susceptible	Susceptible	Ratio	χ^2	Probability[b]
	Number of Families				
UMWO12-01/UMWO12-03 (R/R)					
1Y/3N	38	0	15:1	2.533	0.11
UMWO12-03/UMWO12-01 (R/R)					
3AK/1AL	70	2	15:1	1.481	0.22

[a] pooled results from four different F_1 plants. Data was pooled based on chi-square homogeneity tests.

[b] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

The results obtained for the R/S, S/R, and R/R crosses support the same theory. Crosses involving UM5 indicated that a single dominant nuclear gene confers resistance to imazamethabenz in UMWO12-01 and UMWO12-03. Similar results have been found for other ALS inhibitor resistant weed populations, where resistance is controlled by a single nuclear gene, with varying degrees of dominance depending on the particular population and species (Tranel and Wright, 2002). Results from R/R crosses indicated that different genes were responsible for imazamethabenz resistance in each population based on the production of susceptible individuals in the F_2 and $F_{2:3}$ generations. It is important to note that different parental plants from each population were used to make reciprocal crosses, and this may account for discrepancies found between sets of data.

4.3 Flamprop (Group 25) Results

4.3.1 Resistant/Susceptible and Susceptible/Resistant Results

4.3.1.1 F_2 Results

Results from screening the F_2 families from the UMWO12-01/UM5, UM5/UMWO12-01, UMWO12-03/UM5 and UM5/UMWO12-03 crosses with flamprop are reported in Table 4.6. Data were pooled within families and the Yates correction factor was used where appropriate (Strickberger, 1985).

Of the crosses involving UMWO12-01, the F_2 families from the 1B/5G and 1B/5P were homogeneous enough to be pooled and results fit a 3R:1S ratio. Results from reciprocal crosses also supported a 3R:1S ratio. F_2 families were homogeneous for the 5W/1I cross and the expected ratio. The remaining two reciprocal crosses involving UMWO12-01 as the paternal parent were not homogeneous, however 5 of the 7 F_2 families fit the 3R:1S ratio. As with imazamethabenz, F_2 results for flamprop suggest that one dominant nuclear gene was controlling resistance in population UMWO12-01.

Reciprocal crosses also segregated in a 3R:1S ratio, indicating that the resistance gene was not cytoplasmic.

Three of the families (1A/5F, 1A/5AA and 1C/5H) segregated in the opposite direction. Results for two of these families did not fit a 1R:3S ratio. However, when considered individually, results from 4 of the 8 F_2 families fit a 1R:3S ratio. As with the imazamethabenz results this was believed to be related to the herbicide rate used for screening, causing heterozygotes to display the resistant phenotype for the majority of crosses, and the susceptible phenotype for two of the crosses. Partial dominance of the resistance trait may also to explain a reversal of dominance. Slight environmental differences between growth cabinet conditions may also account for this variation. 1A/5F and 1C/5H families were all screened in the same run in the same growth cabinet. Specific conditions in this run (cabinet) may help explain the reversal of dominance observed.

UMWO12-03/UM5 crosses were all homogeneous and segregated in a 3R:1S ratio. Of the reciprocal crosses only 5AT/3AO was homogeneous, but did not fit a 3R:1S ratio. However 2 of the 4 families from this cross did fit the expected ratio. Of the 7 F_2 families that were not homogeneous, 5 did fit a 3R:1S ratio. These results suggest that one dominant nuclear gene was responsible for flamprop resistance in UMWO12-03.

Table 4.6. Segregation for flamprop resistance in the F_2 generation of resistant/susceptible and susceptible/resistant crosses treated with 300 g a.i./h flamprop.

Cross[a]	Resistant	Susceptible	Ratio	χ^2	Probability[b]
	Number of Plants				
UMWO12-01/UM5 (R/S)					
1A/5F	52	70	1:3	20.208	6.95×10^{-6}
1A/5AA	39	96	1:3	1.089	0.30
1B/5G + 1B/5P	192	67	3:1	0.104	0.75
1C/5H	43	69	1:3	10.715	1.06×10^{-3}
UM5/UMWO12-01 (S/R)					
5W/1I	102	45	3:1	2.469	0.12
5X/1J 1	30	6	3:1	0.926	0.34
5X/1J 2	19	19	3:1	11.368	7.47×10^{-4}
5X/1J 3	22	13	3:1	2.143	0.14
5X/1J 4	28	10	3:1	0	1.00
5AE/1N 1	23	8	3:1	0.011	0.92
5AE/1N 3	25	5	3:1	0.711	0.40
5AE/1N 4	19	15	3:1	5.647	0.02
UMWO12-03/UM5 (R/S)					
3AU/5AY	104	37	3:1	0.116	0.73
3AV/5AZ	98	37	3:1	0.417	0.52
3AX/5BC	78	28	3:1	0.113	0.74
UM5/UMWO12-03 (S/R)					
5AS/3AN 1	29	3	3:1	3.375	0.07
5AS/3AN 2	23	7	3:1	0	1.00
5AS/3AN 3	29	5	3:1	1.412	0.23
5AS/3AN 4	18	15	3:1	6.313	0.01
5AT/3AO	76	59	3:1	25.188	5×10^{-7}
5AW/3AR 1	20	18	3:1	8.982	2.73×10^{-3}
5AW/3AR 2	28	9	3:1	0.009	0.92
5AW/3AR 4	25	8	3:1	0.010	0.92

[a] crosses not followed by a number designation indicate pooled results from three/four different F_1 plants. Data was pooled based on chi-square homogeneity tests. Crosses where a number designation does follow the cross indicates that results are from an individual F_1 plant.

[b] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

4.3.1.2 $F_{2:3}$ Results

$F_{2:3}$ families were screened to verify F_2 results (Table 4.7). Crosses involving UMWO12-01 segregated in a 1 homozygous resistant: 2 segregating:1 homozygous susceptible ratio, which confirmed that a single nuclear gene is responsible for resistance in this population. Reciprocal crosses 1A/5F, 1A/5AA and 5W/1I segregated in the same manner and when these results were pooled also supported a 1:2:1 ratio. Reciprocal crosses segregating in the same fashion indicated that the resistance gene is nuclear in origin and not cytoplasmic.

Results from crosses involving UMWO12-03 were not as clear. Cross 3AX/5BC was homogeneous and fit a 1:2:1 ratio while 5AT/3AO did not. Departure from the 1:2:1 ratio may be due to partial dominance of the resistance trait or due to the herbicide rate used to screen these populations. It is possible that the herbicide rate used was not quite high enough to fully kill the susceptible individuals, which may in turn have resulted in an over-representation of segregating families. The majority of F_2 results involving UMWO12-03 did fit a 3:1 ratio, so it is very likely that one dominant gene was responsible for flamprop resistance in this population.

Table 4.7. Segregation for flamprop resistance in the $F_{2:3}$ generation of resistant/susceptible and susceptible/resistant crosses treated with 300 g a.i./ha flamprop.

Cross[a]	Resistant	Segregating	Susceptible	Ratio	χ^2	Probability[b]
	Number of Families					
UMWO12-01/UM5 (R/S)						
1A/5F + 1A/5AA	10	41	15	1:2:1	4.636	0.10
UM5/UMWO12-01 (S/R)						
5W/1I	9	17	8	1:2:1	0.058	0.97
UMWO12-01/UM5 + UM5/UMWO12-01 (R/S + S/R)						
1A/5F + 1A/5AA + 5W/1I	19	58	23	1:2:1	2.880	0.24
UMWO12-03/UM5 (R/S)						
3AX/5BC	10	22	7	1:2:1	0.113	0.74
UM5/UMWO12-03 (S/R)						
5AT/3AO	21	43	8	1:2:1	7.417	0.02

[a] pooled results from four different F_1 plants. Data was pooled based on chi-square homogeneity tests.

[b] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

4.3.2 Resistant/Resistant Results

4.3.2.1 F_2 Results

Resistant/resistant crosses were made to determine if resistance to flamprop was controlled by the same gene in each population. As with imazamethabenz F_2 results, there was segregation of resistant and susceptible individuals and therefore the same segregation ratios were tested. If the same gene was responsible for resistance in each population then all F_2 plants should be resistant to flamprop. Since segregation was observed it was clear that there were separate resistance genes in each population. 9R:7S, 11R:5S and 15R:1S ratios were tested for the F_2 results, and the ratio with the best fit is reported in Table 4.8. A 15R:1S ratio was observed for all of the F_2 families. Chi-square values did not indicate a close fit to the tested ratio for some of these crosses (1V/3K and

1AO/3AT). This may be due to partial dominance of the resistance trait, the herbicide rate used or environmental influences.

Table 4.8. Segregation for flamprop resistance in the F_2 generation of resistant/resistant crosses treated with 300 g a.i./ha flamprop.

Cross[a]	Resistant	Susceptible	Ratio	χ^2	Probability[b]
	Number of Plants				
UMWO12-01/UMWO12-03 (R/R)					
1V/3K	133	17	15:1	6.615	0.01
1Y/3N	108	8	15:1	0.083	0.77
1AO/3AT	56	9	15:1	6.401	0.01
UMWO12-03/UMWO12-01 (R/R)					
3P/1Z	58	5	15:1	0.306	0.58
3X/1AJ	126	6	15:1	0.655	0.42
3AK/1AL	126	12	15:1	1.409	0.24

[a] pooled results from two, three or four different F_1 plants. Data was pooled based on chi-square homogeneity tests.

[b] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

4.3.2.2 $F_{2:3}$ Results

$F_{2:3}$ results were analyzed to help determine the mode of inheritance of flamprop resistance. The ratio used to test the $F_{2:3}$ was 7 homozygous resistant: 8 segregating: 1 homozygous susceptible as this was the ratio that would be expected based on F_2 results of 15R:1S. A full explanation of these segregation ratios can be found in section 4.2.2. Neither of the crosses tested fit a 7:8:1 ratio. As with imazamethabenz results, the lack of fit of $F_{2:3}$ data may be due to the family size used and the criteria set for classifying families as homozygous resistant, segregating or homozygous susceptible. Based on these facts it is likely that there is an over estimation of homozygous resistant families, which would account for the lack of fit. A 15R:1S ratio was tested for the $F_{2:3}$ data by combing the homozygous resistant and heterozygous families as a "non-susceptible" phenotype. These results are reported in Table 4.9. 3AK/1AL results fit a 15R:1S ratio

and 1Y/3N was very close to fitting the same ratio. $F_{2:3}$ results suggested that resistance was due to different genes in UMWO12-01 and UMWO12-03, however the phenotypic expression of the genes is not clear. If these families were screened again with a larger number of individuals in each family interpretation of results may be more decisive, however, this was not possible based on the time limitations for this project.

Table 4.9. Segregation for flamprop resistance in the $F_{2:3}$ generation of resistant/resistant crosses treated with 300 g a.i./ha flamprop.

Cross[a]	Non-susceptible	Susceptible	Ratio	χ^2	Probability[b]
	Number of Families				
UMWO12-01/UMWO12-03 (R/R)					
1Y/3N	60	0	15:1	4.000	0.05
UMWO12-03/UMWO12-01 (R/R)					
3AK/1AL	71	2	15:1	1.535	0.22

[a] pooled results from four different F_1 plants. Data was pooled based on chi-square homogeneity tests.

[b] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

Results from R/S and S/R crosses support the theory that one dominant nuclear gene controls flamprop resistance in each resistant population. Results from R/R crosses show that the populations carry different resistance genes, the genes are independent and segregate in a 15R:1S ratio. While there are currently no published studies that discuss the inheritance of flamprop resistance, these results are similar to the majority of inheritance studies where one dominant nuclear gene confers resistance (Jasieniuk et al. 1996).

4.4 Fenoxaprop-P (Group 1) Results

4.4.1 F_2 Results

Based on the results of the parental screening, only crosses involving UMWO12-03 could be used to study the inheritance of fenoxaprop-P resistance. Families were

pooled and Yates correction factor was used where appropriate (Strickberger, 1985). F_2 results are reported in Table 4.10. All UMWO12-03/UM5 crosses were homogeneous and fit a 3R:1S ratio. The reciprocal crosses behaved in a similar manner. F_2 families from the 5AT/3AO cross were homogeneous and fit a 3R:1S ratio. F_2 families from the 5AS/3AN cross were also homogeneous but did not fit the tested ratio. However 2 of the 4 families from this cross did fit a 3R:1S ratio. F_2 families from 5AW/3AR were not homogeneous, but 2 of the 3 families did fit a 3R:1S ratio, and the family that did not fit had a probability value close to the acceptable 0.05 level. Since the majority of crosses fit a 3R:1S ratio, it appears that one dominant gene conferred resistance to fenoxaprop-P. Reciprocal crosses segregated in the same ratio indicating the resistance gene is nuclear and not cytoplasmic. Similar results have been reported for Group 1 resistance in other wild oat populations (Murray et al. 1995; 1996, Kibite et al. 1995; Seefeldt et al. 1998). Unlike the results for imazamethabenz and flamprop resistance no reversal of dominance was observed.

Table 4.10. Segregation for fenoxaprop-P resistance in the F_2 generation of susceptible/resistant and resistant/susceptible crosses treated with 90 g a.i./ha fenoxaprop-P.

Cross[a]	Resistant	Susceptible	Ratio	χ^2	Probability[b]
	Number of Plants				
UMWO12-03/UM5 (R/S)					
3AU/5AY	99	21	3:1	3.600	0.06
3AV/5AZ	88	21	3:1	1.911	0.17
3AX/5BC	54	10	3:1	3.000	0.08
UM5/UMWO12-03(S/R)					
5AS/3AN	133	18	3:1	13.777	2.06×10^{-4}
5AT/3AO	119	33	3:1	0.877	0.35
5AW/3AR 1	33	4	3:1	3.252	0.07
5AW/3AR 2	24	16	3:1	4.033	0.04
5AW/3AR 4	35	4	3:1	3.769	0.05

[a] crosses not followed by a number designation indicate pooled results from three/four different F_1 plants. Data was pooled based on chi-square homogeneity tests. Crosses where a number designation does follow the cross indicates that results are from an individual F_1 plant.

[b] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

4.4.2 $F_{2:3}$ Results

$F_{2:3}$ results were analyzed to determine if they supported the theory that a single dominant nuclear gene was responsible for conferring resistance to fenoxaprop-P. The $F_{2:3}$ results did not closely fit the expected 1 homozygous resistant: 2 segregating: 1 homozygous susceptible ratio. The number of segregating families was close to the expected value, but there were too many resistant families and too few susceptible families to fit the expected ratio. The reciprocal crosses were segregating in a similar manner, confirming that the resistance gene was nuclear.

Table 4.11. Segregation for fenoxaprop-P resistance in the $F_{2:3}$ generation of susceptible/resistant and resistant/susceptible crosses treated with 90 g a.i./ha fenoxaprop-P.

Cross[a]	Resistant	Segregating	Susceptible	Ratio	χ^2	Probability[b]
	Number of Families					
UMWO12-03/UM5 (R/S)						
3AX/5BC	15	25	3	1:2:1	7.837	0.02
UM5/UMWO12-03 (S/R)						
5AT/3AO	14	17	0	1:2:1	12.935	3.22×10^{-4}

[a] pooled results from four different F_1 plants. Data was pooled based on chi-square homogeneity tests.

[b] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

While F_2 results supported the theory that a single dominant nuclear gene conferred resistance to fenoxaprop-P, $F_{2:3}$ results did not confirm these results. There appeared to be an over-representation of resistant families in the $F_{2:3}$ generation. Closer examination of the F_2 results show that there seemed to be more resistant individuals than expected. Most of the F_2 families segregate in ratios greater than 3:1 (average segregation ratio was 6.2R:1S). However these ratios did not deviate enough to be classified as other than 3R:1S. This lack of fit may be due to the herbicide rate used to screen the different generations of material. UMWO12-03 has a resistance factor of 2.9 for fenoxaprop-P. This is a relatively low level of resistance and as a result a low herbicide rate had to be used to test the population. A low herbicide rate may have led to a disproportionate number of non-susceptible individuals/families, i.e. susceptible escapes in both the F_2 and $F_{2:3}$ generation.

4.5 Linkage Between Herbicide Resistance Genes

4.5.1 Linkage in UMWO12-01

4.5.1.1 Imazamethabenz/Flamprop Linkage

Data obtained from screening resistant/susceptible and susceptible/resistant $F_{2:3}$ families with the different herbicides was used to derive the genotypes of individual F_2 plants (Table 7.1). If the same gene was responsible for both imazamethabenz and flamprop resistance then all F_2 plants would have the same reaction to each herbicide. However, the individual F_2 plants do not behave in this manner as was evident by the presence of non-parental recombinants. For resistant/susceptible and susceptible/resistant crosses non-parental recombinants are those individuals that are resistant to one herbicide and not the other. All other phenotypes (resistant to both or susceptible to both) are parental types. If the resistance genes are independent then the F_2 individuals should segregate in a 9 resistant to both imazamethabenz and flamprop: 3 resistant to imazamethabenz and susceptible to flamprop: 3 susceptible to imazamethabenz and resistant to flamprop: 1 susceptible to both imazamethabenz and flamprop ratio. The pooled data from crosses 1A/5F, 1A/5AA and 5W/1I did not fit this ratio. There are two possible explanations for this lack of fit: 1) either the gene for imazamethabenz or flamprop resistance does not segregate in the expected 3R:1S ratio; or 2) the two resistance genes are linked (Strickberger, 1985).

To determine if the genes are linked, chi-square linkage tests were calculated as described by Strickberger (1985) and are reported in Table 4.12. Segregation of each gene was then tested separately to determine if data fit a 3R:1S ratio. Imazamethabenz and flamprop resistance genes both fit a 3R:1S ratio. The test for the independent

assortment of the genes failed, indicating that the two genes are linked. By dividing the number of non-parental phenotypes by the number of parental phenotypes a *z* value of was 0.01626 obtained. This values corresponds to 10.0 percent recombination value (Strickberger, 1985) indicating the genes for imazamethabenz and flamprop resistances are located relatively close to one another on the same chromosome.

Table 4.12. Chi-square tests to detect the presence of linkage between imazamethabenz and flamprop resistance in UMWO12-01.

Test (Actual)	χ^2	Probability[a]
9:3:3:1 (41:2:3:9)	24.160	2.31×10^{-5}
3:1 imazamethabenz (43:12)	0.297	0.59
3:1 flamprop (44:11)	0.733	0.39
Independence	24.789	6.40×10^{-7}

[a] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

4.5.2 Linkage in UMWO12-03

4.5.2.1 Imazamethabenz/Flamprop Linkage

When comparing derived F_2 genotypes for imazamethabenz and flamprop resistance, the presence of non-parental recombinants indicates that the gene for resistance was different for each herbicide in UMWO12-03 (Table 7.2). A 9:3:3:1 ratio and tests for linkage were investigated as described in section 4.5.5.1, and results are reported in Table 4.13. The data did not fit a 9:3:3:1 ratio indicating that the genes for resistance may be linked. Tests for adherence to the 3R:1S ratios were acceptable for imazamethabenz resistance but not for flamprop resistance. The test for independence also failed, indicating linkage of the genes. However, since the test for 3R:1S segregation of flamprop resistance failed it was not possible to determine how much of the lack of fit to the 9:3:3:1 ratio was due to linkage and how much was due to the relative scarcity of flamprop-susceptible phenotypes.

Table 4.13. Chi-square tests to detect the presence of linkage between imazamethabenz and flamprop resistance in UMWO12-03.

Test (Actual)	χ^2	Probability[a]
9:3:3:1 (81:0:6:10)	41.722	4.60×10^{-9}
3:1 imazamethabenz (81:16)	3.742	0.05
3:1 flamprop (87:10)	11.165	8.34×10^{-4}
Independence	48.887	1.63×10^{-12}

[a] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

4.5.2.2 Imazamethabenz/Fenoxaprop-P Linkage

The presence of non-parental recombinants in the derived F_2 genotypes indicates that there were separate genes for imazamethabenz and fenoxaprop-P resistance in UMWO12-03. This data did not fit a 9:3:3:1 (Table 7.3), and the data for fenoxaprop-P did not fit a 3R:1S ratio (Table 4.14). The test for independence of linkage produced an acceptable chi-square value indicating that the lack of fit to the 9:3:3:1 ratio was not due to linkage. The lack of fit of the fenoxaprop-P data to the 3R:1S ratio indicated that the lack of fit to the 9:3:3:1 ratio was due to the relative scarcity of fenoxaprop-P-susceptible phenotypes.

Table 4.14. Chi-square tests to detect the presence of linkage between imazamethabenz and fenoxaprop-P resistance in UMWO12-03.

Test (Actual)	χ^2	Probability[a]
9:3:3:1 (51:0:9:1)	22.148	6.08×10^{-5}
3:1 imazamethabenz (51:10)	2.410	0.12
3:1 fenoxaprop-P (60:1)	17.754	2.51×10^{-5}
Independence	0.838	0.36

[a] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

4.5.2.3 Flamprop/Fenoxaprop-P Linkage

Due to the presence of non-parental types in the derived F_2 genotypes, linkage between flamprop and fenoxaprop-P resistance genes was investigated as well (Table

7.4). Families segregated in a ratio significantly different from the expected 9:3:3:1 ratio (Table 4.15). Individual segregation ratios for flamprop and fenoxaprop-P were tested and these tests failed. The test for independence for linkage also failed, indicating that there was linkage between these two genes. However as with the investigation of linkage between imazamethabenz and flamprop in UMWO12-03, the tests for individual herbicide resistance failed, making it impossible to determine how much of the departure from the 9:3:3:1 ratio was due to linkage and how much was due to a lack of susceptible phenotypes.

Table 4.15. Chi-square tests to detect the presence of linkage between flamprop and fenoxaprop-P resistance in UMWO12-03.

Test (Actual)	χ^2	**Probability**[a]
9:3:3:1 (59:0:6:2)	29.186	2.05×10^{-6}
3:1 flamprop (59:8)	6.095	0.01
3:1 fenoxaprop-P (65:2)	17.318	3.16×10^{-5}
Independence	7.797	0.01

[a] probabilities of 0.05 or greater indicate that the data does not differ significantly from the ratio being tested.

Unfortunately the interpretation of linkage calculations were not as clear for population UMWO12-03 as they were for UMWO12-01. This may be related to the family size screened. When family sizes were chosen, determination of linkage between resistance genes was not a goal of the research. There does appear to be some degree of linkage between herbicide resistance genes in UMWO12-03 based on the fact that none of the sets of data even came close to fitting a 9:3:3:1 ratio. In addition, the complete absence of some non-parental recombinant types in the derived F_2 genotypes also supported the presence of linkage. It may be possible that if a larger number of plants were screened that the linkage relationship would be quantifiable. The more tightly that two genes are linked, the larger the population size that must been screened to have all

parental and recombinant types present. In this project time and space limitations meant that it was not possible to screen larger populations.

5.0 GENERAL DISCUSSION AND CONCLUSIONS

Herbicide resistance has become a widespread problem throughout agricultural regions worldwide (Heap, 2003 www.weedscience.com), limiting producers' chemical weed control options. The more recent development of multiple herbicide resistance further limits weed control options. In some instances of multiple herbicide resistant wild oat, producers are left with no chemical means of wild oat control in wheat (Beckie et al. 1999). By studying the inheritance of herbicide resistance, insight can be gained as to how these populations developed, how to prevent new populations from evolving and how to manage resistant populations that already exist.

The rate of spread of herbicide resistance is influenced by the number of genes and the interactions of the genes involved in resistance (Jasieniuk et al. 1996). A separate single dominant nuclear gene was responsible for resistance to imazamethabenz, flamprop and fenoxaprop-P for each of the UMWO12-01 and UMWO12-03 populations. This conclusion was consistent with results reported by other research. ACCase inhibitor resistance has been shown to be controlled by a single dominant nuclear gene in other populations of wild oat (Murray et al. 1995; 1996; Kibite et al. 1995; Seefeldt et al. 1998). Most cases of ALS inhibitor resistance have been shown to be controlled by a single partially dominant nuclear gene (Tranel and Wright, 2002). The fact that resistance genes for the same chemical are different in each of the populations indicated that these populations did develop resistance independently and are not the result of the spread of a single resistant population. This is in agreement with producers' records (Friesen et al. 2000). This project is the first report of the investigation of the inheritance of flamprop resistance in wild oat.

The nature of the resistance genes is unclear, as no work has been done on the physiology of the resistance in these populations. In the majority of cases of ACCase and ALS inhibitor resistance, an altered target site has been implicated as the source of resistance (Devine and Shimaburkuro, 1994; Saari et al. 1994; Shaner, 1991). For altered target site resistance it is believed that a single gene mutation may correspond to altered ACCase and ALS. Although different genes are responsible for resistance to these herbicides in each of the multiple resistant wild oat populations used in this study, altered target site may still be the mechanism for resistance. Wild oat is a hexaploid species and an alteration of the ACCase or ALS gene in different genomes may be possible in each population. A similar situation may exist for flamprop resistance, however whether there is one specific target site as the mode of action for flamprop has yet to be determined.

The resistance levels for these herbicides and these populations were relatively low (Table 3.1; Friesen et al. 2000), which is not common for altered target site resistance. With low resistance levels, enhanced herbicide metabolism may be a more likely mechanism of resistance in these populations. Alterations in cytochrome p450 monoxygenases have been shown to cause resistance to ACCase inhibitors in rigid ryegrass (Preston et al. 1996). A similar system may be involved in the wild oat populations used in this project. Different cytochrome p450 monoxygenases may be altered in each wild oat population, which would still account for different resistance genes being identified in UMWO12-01 and UMWO12-03.

Herbicide resistance typically develops as an evolutionary response to repeated selection with the same herbicide mode of action. How these multiple herbicide resistant wild oat populations developed has been a topic of interest in this research, particularly

since these populations were not repeatedly exposed to two of the three herbicides they have developed resistance to. Results of the linkage calculations may provide some insight into how resistance in these populations developed. Results from crosses involving UMWO12-03 and UM5 implied that there was some degree of linkage between flamprop and fenoxaprop-P resistance genes. This population had been repeatedly exposed to ACCase inhibitors (Friesen et al. 2000). If there is linkage between resistance genes to these two herbicide groups it is quite possible that when this population was repeatedly subjected to Group 1 herbicides, selection for Group 25 resistance was also occurring. This would make sense because UMWO12-03 was not repeatedly exposed to flamprop.

The results from this project did not suggest that there was any linkage between imazamethabenz and fenoxaprop-P resistance in population UMWO12-03, so it is unclear how resistance to imazamethabenz developed in this population when it was not repeatedly exposed to imazamethabenz. The lack of linkage between imazamethabenz and fenoxaprop-P resistance in this population may be the result of the inability to detect linkage due to the small family size screened in the $F_{2:3}$ generation (as discussed in section 4.5.2). The data suggested that there was some linkage between imazamethabenz and flamprop resistance genes. This may indicate that there was inadvertent selection of both imazamethabenz and flamprop resistance while selection for resistance to Group 1 herbicides was happening. Unfortunately, because there seemed to be an under-representation of susceptible families in these data sets, leading to speculation as to how resistance developed in these populations and not clear answers.

Results from crosses involving UMWO12-01 and UM5 clearly suggest that there was linkage between imazamethabenz and flamprop resistance in this population. However, this population did not undergo selection with either of these herbicide modes of action. While the parents used in these crosses were not homozygous for resistance to fenoxaprop-P (Table 4.1) work by Friesen et al., (2000) indicated that this population as a whole, is resistant to fenoxaprop-P. As with UMWO12-03 it is then possible that selection with Group 1 herbicides selected for imazamethabenz and flamprop resistance at the same time.

While there are still uncertainties as to how resistance to imazamethabenz and flamprop developed in these wild oat populations developed, it is clear that resistance developed to Group 2 and Group 25 herbicides in absence of selection specifically for these chemical modes of action. This implies that rotation of herbicide groups alone may not be sufficient in preventing the development of herbicide resistance. A range of weed control measures, including herbicide use will be needed to help control and prevent the evolution of resistance. Further research into the mechanisms of resistance to the different herbicide groups in these populations may provide further insight as to how resistance developed in these populations.

5.1 Conclusions

1. Multiple herbicide resistance to imazamethabenz, flamprop and fenoxaprop-P in wild oat populations UMWO12-01 and UMWO12-03 is controlled by a separate resistance gene for each herbicide.

2. Resistance for each herbicide is controlled by a single dominant nuclear gene.

3. Resistance genes differ between the two wild oat populations, indicating that each population developed resistance independently.

4. Multiple herbicide resistance may have developed when selection with fenoxaprop-P and other Group 1 herbicides inadvertently selected for resistance to flamprop and imazamethabenz at the same time.

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7.0 APPENDIX

Table 7.1. Derived F_2 genotypes based on $F_{2:3}$ data for imazamethabenz/flamprop linkage in UMWO12-01. I, i denote the resistant/susceptible form of the imazamethabenz gene. F, f denote the resistant/susceptible form of the flamprop gene.

F_2 Plant	*Genotype*	*F_2 Plant*	*Genotype*	*F_2 Plant*	*Genotype*
1A/5F1-B	ii ff	1A/5F4-C	Ii Ff	1A/5AA4-D	Ii ff
1A/5F1-C	ii ff	1A/5F4-E	II FF	1A/5AA4-G	II FF
1A/5F1-E	Ii Ff	1A/5AA1-B	II FF	1A/5AA4-I	II Ff
1A/5F1-G	ii Ff	1A/5AA1-G	Ii Ff	5W/1I1-D	II FF
1A/5F1-H	II FF	1A/5AA2-A	Ii Ff	5W/1I1-I	Ii Ff
1A/5F1-J	II FF	1A/5AA2-B	Ii Ff	5W/1I2-B	Ii Ff
1A/5F2-A	Ii Ff	1A/5AA2-D	Ii Ff	5W/1I2-E	ii Ff
1A/5F2-B	Ii Ff	1A/5AA2-E	Ii Ff	5W/1I3-A	II FF
1A/5F2-C	ii Ff	1A/5AA2-G	Ii Ff	5W/1I3-B	Ii Ff
1A/5F2-E	ii ff	1A/5AA2-H	Ii Ff	5W/1I3-C	Ii FF
1A/5F2-F	II FF	1A/5AA3-B	Ii Ff	5W/1I3-E	Ii Ff
1A/5F2-J	Ii Ff	1A/5AA3-C	Ii Ff	5W/1I3-G	ii ff
1A/5F2-K	II FF	1A/5AA3-D	Ii ff	5W/1I3-H	II FF
1A/5F3-A	Ii Ff	1A/5AA3-F	ii ff	5W/1I3-I	ii ff
1A/5F3-B	Ii Ff	1A/5AA3-G	ii ff	5W/1I3-L	Ii FF
1A/5F3-E	Ii Ff	1A/5AA3-H	Ii Ff	5W/1I4-A	Ii Ff
1A/5F3-F	Ii Ff	1A/5AA4-A	II FF	5W/1I4-D	Ii Ff
1A/5F3-H	Ii Ff	1A/5AA4-B	ii ff		
1A/5F4-B	Ii Ff	1A/5AA4-C	ii ff		

Summary of Table 7.1:

41 F_2 resistant to both imazamethabenz and flamprop (I_F_)

2 F_2 resistant to imazamethabenz and susceptible to flamprop (I_ff)

3 F_2 susceptible to imazamethabenz and resistant to flamprop (iiF_)

9 F_2 susceptible to imazamethabenz and flamprop (iiff)

Table 7.2. Derived F_2 genotypes based on $F_{2:3}$ data for imazamethabenz/flamprop linkage in UMWO12-03. I, i denote the resistant/susceptible form of the imazamethabenz gene. F, f denote the resistant/susceptible form of the flamprop gene.

F_2 Plant	*Genotype*	*F_2 Plant*	*Genotype*	*F_2 Plant*	*Genotype*
3AX/5BC1-A	Ii Ff	5AT/3AO1-G	Ii Ff	5AT/3AO3-F	Ii Ff
3AX/5BC1-D	Ii FF	5AT/3AO1-H	Ii Ff	5AT/3AO3-G	Ii Ff
3AX/5BC1-E	Ii Ff	5AT/3AO1-I	Ii Ff	5AT/3AO3-H	Ii Ff
3AX/5BC1-F	Ii Ff	5AT/3AO1-J	Ii Ff	5AT/3AO3-I	II FF
3AX/5BC1-I	Ii Ff	5AT/3AO1-K	II FF	5AT/3AO3-J	Ii Ff
3AX/5BC1-J	ii ff	5AT/3AO1-L	Ii Ff	5AT/3AO3-K	II FF
3AX/5BC1-K	Ii FF	5AT/3AO1-M	ii Ff	5AT/3AO3-L	Ii Ff
3AX/5BC1-M	Ii Ff	5AT/3AO1-N	ii ff	5AT/3AO3-M	Ii Ff
3AX/5BC1-N	ii Ff	5AT/3AO1-O	II FF	5AT/3AO3-N	Ii Ff
3AX/5BC1-O	Ii Ff	5AT/3AO1-P	Ii Ff	5AT/3AO3-O	Ii Ff
3AX/5BC1-P	ii ff	5AT/3AO1-Q	Ii Ff	5AT/3AO3-P	ii Ff
3AX/5BC1-Q	II FF	5AT/3AO1-R	ii ff	5AT/3AO3-Q	Ii Ff
3AX/5BC2-D	II FF	5AT/3AO1-S	Ii Ff	5AT/3AO3-R	Ii Ff
3AX/5BC2-E	Ii Ff	5AT/3AO1-T	Ii Ff	5AT/3AO4-A	Ii Ff
3AX/5BC2-F	Ii FF	5AT/3AO2-A	Ii Ff	5AT/3AO4-B	II FF
3AX/5BC2-I	Ii Ff	5AT/3AO2-B	Ii Ff	5AT/3AO4-C	Ii Ff
3AX/5BC2-K	ii Ff	5AT/3AO2-C	Ii Ff	5AT/3AO4-D	ii ff
3AX/5BC2-L	Ii Ff	5AT/3AO2-D	II FF	5AT/3AO4-E	II FF
3AX/5BC2-M	Ii Ff	5AT/3AO2-F	Ii Ff	5AT/3AO4-F	Ii Ff
3AX/5BC2-O	Ii Ff	5AT/3AO2-G	II Ff	5AT/3AO4-G	II FF
3AX/5BC2-P	Ii Ff	5AT/3AO2-H	Ii Ff	5AT/3AO4-H	II FF
3AX/5BC2-Q	Ii Ff	5AT/3AO2-I	II FF	5AT/3AO4-I	Ii Ff
3AX/5BC2-T	Ii Ff	5AT/3AO2-J	II FF	5AT/3AO4-J	Ii FF
3AX/5BC2-U	Ii Ff	5AT/3AO2-K	ii ff	5AT/3AO4-K	Ii Ff
3AX/5BC3-A	ii ff	5AT/3AO2-L	ii ff	5AT/3AO4-L	Ii Ff
3AX/5BC3-B	Ii FF	5AT/3AO2-M	ii ff	5AT/3AO4-M	ii Ff
3AX/5BC3-C	Ii Ff	5AT/3AO2-N	Ii Ff	5AT/3AO4-N	Ii FF
3AX/5BC3-D	ii Ff	5AT/3AO2-O	II FF	5AT/3AO4-O	II FF
5AT/3AO1-A	Ii FF	5AT/3AO2-P	II FF	5AT/3AO4-P	Ii Ff
5AT/3AO1-B	Ii Ff	5AT/3AO3-A	Ii Ff	5AT/3AO4-Q	II FF
5AT/3AO1-C	Ii Ff	5AT/3AO3-B	ii ff	5AT/3AO4-R	II FF
5AT/3AO1-E	II FF	5AT/3AO3-C	II FF		
5AT/3AO1-F	Ii Ff	5AT/3AO3-E	II Ff		

Summary of Table 7.2:

81 F_2 resistant to both imazamethabenz and flamprop (I_F_)

0 F_2 resistant to imazamethabenz and susceptible to flamprop (I_ff)

6 F_2 susceptible to imazamethabenz and resistant to flamprop (iiF_)

10 F_2 susceptible to imazamethabenz and flamprop (iiff)

Table 7.3. Derived F_2 genotypes based on $F_{2:3}$ data for imazamethabenz/fenoxaprop-P linkage in UMWO12-03. I, i denote the resistant/susceptible form of the imazamethabenz gene. P, p denote the resistant/susceptible form of the fenoxaprop-P gene.

F_2 Plant	*Genotype*	*F_2 Plant*	*Genotype*	*F_2 Plant*	*Genotype*
3AX/5BC1-A	Ii Pp	3AX/5BC2-O	Ii Pp	5AT/3AO3-C	II Pp
3AX/5BC1-D	Ii PP	3AX/5BC2-P	Ii Pp	5AT/3AO4-A	Ii Pp
3AX/5BC1-E	Ii Pp	3AX/5BC2-Q	Ii PP	5AT/3AO4-B	II PP
3AX/5BC1-F	Ii Pp	3AX/5BC2-R	Ii Pp	5AT/3AO4-C	Ii PP
3AX/5BC1-G	ii pp	3AX/5BC2-T	Ii Pp	5AT/3AO4-D	ii Pp
3AX/5BC1-I	Ii Pp	3AX/5BC2-U	Ii Pp	5AT/3AO4-E	II PP
3AX/5BC1-J	ii Pp	3AX/5BC3-A	ii Pp	5AT/3AO4-F	Ii PP
3AX/5BC1-K	Ii PP	3AX/5BC3-B	Ii PP	5AT/3AO4-G	II PP
3AX/5BC1-L	II PP	3AX/5BC3-C	Ii Pp	5AT/3AO4-H	II PP
3AX/5BC1-M	Ii Pp	3AX/5BC3-D	ii Pp	5AT/3AO4-I	Ii Pp
3AX/5BC1-N	ii PP	5AT/3AO1-A	Ii PP	5AT/3AO4-J	Ii Pp
3AX/5BC1-O	Ii Pp	5AT/3AO1-B	Ii Pp	5AT/3AO4-K	Ii Pp
3AX/5BC1-P	ii Pp	5AT/3AO1-C	Ii Pp	5AT/3AO4-L	Ii Pp
3AX/5BC1-Q	II PP	5AT/3AO1-E	II PP	5AT/3AO4-M	ii Pp
3AX/5BC2-B	II PP	5AT/3AO1-F	Ii Pp	5AT/3AO4-N	Ii Pp
3AX/5BC2-D	II PP	5AT/3AO1-G	Ii PP	5AT/3AO4-O	II PP
3AX/5BC2-F	Ii PP	5AT/3AO1-I	Ii Pp	5AT/3AO4-P	Ii Pp
3AX/5BC2-I	Ii Pp	5AT/3AO1-J	Ii PP	5AT/3AO4-Q	II PP
3AX/5BC2-K	ii Pp	5AT/3AO1-K	II PP	5AT/3AO4-R	II PP
3AX/5BC2-L	Ii Pp	5AT/3AO3-A	Ii Pp		
3AX/5BC2-M	Ii Pp	5AT/3AO3-B	ii Pp		

Summary of Table 7.3:

51 F_2 resistant to both imazamethabenz and fenoxaprop-P (I_P_)

0 F_2 resistant to imazamethabenz and susceptible to fenoxaprop-P (I_pp)

9 F_2 susceptible to imazamethabenz and resistant to fenoxaprop-P (iiP_)

1 F_2 susceptible to imazamethabenz and fenoxaprop-P (iipp)

Table 7.4. Derived F_2 genotypes based on $F_{2:3}$ data for flamprop/fenoxaprop-P linkage in UMWO12-03. F, f denote the resistant/susceptible form of the flamprop gene. P, p denote the resistant/susceptible form of the fenoxaprop-P gene.

F_2 Plant	*Genotype*	*F_2 Plant*	*Genotype*	*F_2 Plant*	*Genotype*
3AX/5BC1-A	Ff Pp	3AX/5BC2-N	Ff Pp	5AT/3AO3-A	Ff Pp
3AX/5BC1-D	FF PP	3AX/5BC2-O	Ff Pp	5AT/3AO3-B	ff Pp
3AX/5BC1-E	Ff Pp	3AX/5BC2-P	Ff Pp	5AT/3AO3-C	FF Pp
3AX/5BC1-F	Ff Pp	3AX/5BC2-Q	Ff PP	5AT/3AO4-A	Ff Pp
3AX/5BC1-H	ff Pp	3AX/5BC2-S	ff pp	5AT/3AO4-B	FF PP
3AX/5BC1-I	Ff Pp	3AX/5BC2-T	Ff Pp	5AT/3AO4-C	Ff PP
3AX/5BC1-J	ff Pp	3AX/5BC2-U	Ff Pp	5AT/3AO4-D	ff Pp
3AX/5BC1-K	FF PP	3AX/5BC2-V	FF PP	5AT/3AO4-E	FF PP
3AX/5BC1-M	Ff Pp	3AX5BC2-W	FF PP	5AT/3AO4-F	Ff PP
3AX/5BC1-N	Ff PP	3AX/5BC3-A	ff Pp	5AT/3AO4-G	FF PP
3AX/5BC1-P	ff Pp	3AX/5BC3-B	FF PP	5AT/3AO4-H	FF PP
3AX/5BC1-Q	FF PP	3AX/5BC3-C	Ff Pp	5AT/3AO4-I	Ff Pp
3AX/5BC2-A	FF PP	3AX/5BC3-D	Ff Pp	5AT/3AO4-J	FF Pp
3AX/5BC2-C	Ff Pp	5AT/3AO1-A	FF PP	5AT/3AO4-K	Ff Pp
3AX/5BC2-D	FF PP	5AT/3AO1-B	Ff Pp	5AT/3AO4-L	Ff Pp
3AX/5BC2-F	FF PP	5AT/3AO1-C	Ff Pp	5AT/3AO4-M	Ff Pp
3AX/5BC2-G	FF PP	5AT/3AO1-D	Ff Pp	5AT/3AO4-N	FF Pp
3AX/5BC2-H	Ff PP	5AT/3AO1-E	FF PP	5AT/3AO4-O	FF PP
3AX/5BC2-I	Ff Pp	5AT/3AO1-F	Ff Pp	5AT/3AO4-P	Ff Pp
3AX/5BC2-J	ff pp	5AT/3AO1-G	Ff PP	5AT/3AO4-Q	FF PP
3AX/5BC2-K	Ff Pp	5AT/3AO1-I	Ff Pp	5AT/3AO4-R	FF PP
3AX/5BC2-L	Ff Pp	5AT/3AO1-J	Ff PP		
3AX/5BC2-M	Ff Pp	5AT/3AO1-K	FF PP		

Summary of Table 7.4:

59 F_2 resistant to both flamprop and fenoxaprop-P (F_P_)

0 F_2 resistant to flamprop and susceptible to fenoxaprop-P (F_pp)

6 F_2 susceptible to flamprop and resistant to fenoxaprop-P (ffP_)

2 F_2 susceptible to flamprop and fenoxaprop-P (ffpp)