INHERITANCE AND POLLEN MEDIATED GENE FLOW OF ACETYL-CoA CARBOXYLASE INHIBITOR RESISTANCE IN WILD OAT (*Avena fatua*)

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INHERITANCE AND POLLEN MEDIATED GENE FLOW OF ACETYL-CoA CARBOXYLASE INHIBITOR RESISTANCE IN WILD OAT (*Avena fatua*)

BY

BRUCE GORDON MURRAY

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

ABSTRACT

Murray, Bruce Gordon. Ph.D. The University of Manitoba, 1996
Inheritance and pollen mediated gene flow of acetyl-CoA carboxylase inhibitor resistance in wild oat (*Avena fatua*). Major Professor: Ian N. Morrison.

The inheritance of aryloxyphenoxypropionate (APP) and cyclohexanedione (CHD) resistance was studied in two wild oat populations, UM1 and UM33. Parents, F_1 hybrids, F_2 plants and F_2-derived F_3 families originating from reciprocal crosses between the resistant genotypes and a susceptible wild oat line, UM5, were treated with fenoxaprop-P. When discriminatory dosages of fenoxaprop-P were used, three response types were observed: resistant (R), intermediate, and (I) susceptible (S). Treated F_2 plants and F_2-derived F_3 families segregated in a 1:2:1 ratio indicative of single nuclear gene inheritance. To determine if resistance in UM1 and UM33 resulted from alterations at the same gene locus, F_2 plants derived from reciprocal UM33/UM1 crosses were screened with 150 g ha^{-1} fenoxaprop-P. None of the treated F_2 plants exhibited injury or death, indicating that the resistance traits in UM1 and UM33 did not segregate independently, and were therefore encoded at the same gene locus.

To quantify the degree of out-crossing and pollen-mediated gene flow (PMGF) in wild oat, two separate field experiments were conducted. In both experiments, UM1 was used as a pollen donor and UM5 as the pollen receptor. In the out-crossing experiment, single UM5 plants were surrounded by 20 UM1 plants. By screening seed from the susceptible parent for resistance, out-crossing was determined to range from 0 to 12.3%, with a mean of 5.2%. In the PMGF experiment, single, resistant

UM1 plants were surrounded by UM5 plants arranged in a hexagonal pattern at two densities (19 and 37 plants m^{-2}) over-seeded to wheat or flax. In wheat, mean out-crossing was 0.08 and 0.05% at low and high densities, respectively. In flax, the corresponding values were 0.10 and 0.17%, respectively. By fitting the data from the high density planting in flax to a Weibull function, it was determined that 90% of successful out-crossing events would occur within 56 cm of the pollen donor.

Additionally, a seed bioassay was developed to facilitate the rapid identification of CHD and APP inhibitor resistance in wild oat. Within 5 days of treatment with either 10 µM fenoxaprop-P or 5 µM sethoxydim, susceptible genotypes were dead whereas resistant seedlings were living and produced green leaves. The seed bioassay proved useful for screening resistant populations and the identification of heterozygous (R/S) genotypes in the out-crossing and PMGF studies. **Nomenclature:** Fenoxaprop-P [R-2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid, ethyl ester; Sethoxydim [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one]; wild oat, *Avena fatua* L. # AVEFA; spring wheat, *Triticum aestivum* L. 'Roblin'; flax, *Linum usitatissimum* L. 'Norlin'.

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This research was supported in part by the Natural Sciences and Engineering Research Council (Strategic Grant STRGP 039), the Graminicide Resistance Action Committee, the Herbicide Resistance Action Committee and the Canadian Wheat Board through its postgraduate fellowship program.

I would like to expresses my sincere thanks and appreciation to the following individuals for their contributions to this research project.

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Finally, I would like to express my love and appreciation to my family, Cindy, Scott, Mom and Dad, who contributed a great deal of time, expense and patience; and

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FORWARD

This thesis has been written in manuscript style. Three of the manuscripts were prepared in accordance with the style requirements of Weed Science. The bioassay manuscript (chapter 5) was prepared according to Weed Technology style requirements.

The paper entitled "Resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in wild oat (*Avena fatua*)", by Heap et al. 1993, Weed Science 41:232-238, was a collaborative effort between the coauthors and myself. The research explicitly undertaken by me included the growth-room dose-response experiments for UM1, UM2, UM3, UM33 and UM5 using diclofop-methyl, fenoxaprop-P, and sethoxydim, and all dose-response experiments conducted in the field.

1. INTRODUCTION

In Canada, wild oat (*Avena fatua* L.) is widely distributed on cropland, ranging from British Columbia to Newfoundland, and from the U. S. border to the Northwest Territories (Sharma and Vanden Born 1978; Raju 1990). Weed surveys of cereal and flax fields in Manitoba, Saskatchewan, and Alberta indicated wild oat occurred in 75, 71 and 61 percent of the fields, respectively (Thomas 1983). The total area infested with wild oat in North America was estimated at over 25 million ha (Nalewaja 1970). This weed is one of the most serious and abundant of the Canadian prairie provinces, and is responsible for economic losses through reductions in crop yields, increased dockage, increased seed cleaning costs, and lowered crop quality and grade (Sharma and Vanden Born 1978; Thomas and Wise 1985, 1987, 1988). Characteristics that enhance the persistence and weediness of wild oat include periodicity of germination, rapid development, early maturity, ready shattering ability and a high degree of seed dormancy (Sharma and Vanden Born 1978).

Prior to the advent of selective herbicides, traditional wild oat control practices included cultural controls such as summer fallow, crop rotations, delayed seeding, mowing, and spring and autumn tillage. In general, these methods were moderately effective (Friesen 1974; Hunter et al. 1990). Following the introduction of barban in 1960, producers became heavily reliant on selective herbicides for the control of wild oat and other grassy weeds in both cereal and oilseed crops (Morrison et al. 1992). Through the 70's and 80's more than 10 new wild oat herbicides were registered for

use in western Canada (Morrison et al. 1992). From 1982 through 1989 the area treated in western Canada with wild oat herbicides was between eight to ten million ha annually (Morrison et al. 1992). This represents approximately 20 to 25% of the seeded area in the western provinces.

Herbicide resistance evolution in weeds is a relatively recent development. In Manitoba, resistance to the acetyl-CoA carboxylase (ACCase) inhibitors in wild oat was first suspected in July 1990, and later confirmed in growth room studies that same year (Heap et al. 1993; Goodwin 1994). This was the first reported instance of herbicide resistance in this species. The evolution of ACCase inhibitor resistance in wild oat poses a serious threat to crop production in western Canada, because these herbicides are used extensively to control wild oat and other grassy weeds, including green foxtail (*Setaria viridis*).

The occurrence of herbicide resistant weeds pointed to many short-comings in our understanding of weed biology and those variables that influence the occurrence and rate of resistance evolution. This thesis was undertaken to address a number of these short-comings. The principal objectives were to characterize a number of resistant populations in terms of levels and patterns of cross-resistances to various ACCase inhibitors; to determine the mechanism of inheritance for ACCase inhibitor resistance; and to identify the role pollen movement plays in the evolution and spread of resistance in wild oat populations. Additionally, a seed bioassay was developed to aid in the timely identification of ACCase inhibitor resistant wild oat. The overall purpose of the study was to provide a better understanding of selected variables that

influence the occurrence and spread of herbicide resistance. This, in turn, will aid in the development of effective strategies aimed at delaying the onset of resistance or managing existing herbicide resistant populations.

2.0 LITERATURE REVIEW

2.1 Introduction

This chapter is a review of the literature pertaining to the occurrence and inheritance of herbicide resistance in weeds, as well as on the reproductive biology of selected species and its impact on herbicide resistance evolution. The review is divided into four main sections. The first is a general overview of herbicide resistance and resistance evolution. The second is a discussion of the occurrence of ACCase[1] inhibitor resistance worldwide with a focus on resistant wild oat in western Canada. The third is a review of inheritance mechanisms for herbicide resistance and their impact on the rate of resistance evolution. The fourth, and final, section outlines current information regarding pollen-mediated gene flow and reproductive biology of weeds, and their putative role in herbicide resistance evolution.

2.2 Herbicide Resistance, Overview

2.2.1 Herbicide Resistance Definitions and Status

Many definitions of herbicide resistance have been proposed. For the purpose of this thesis, resistance will be defined as a heritable change in a weed population in response to selection by a toxicant that reduces control in the field (Sawicki 1987). Accordingly, herbicide resistant weeds are those that survive and reproduce at the usually effective field dosage of a herbicide (Holt and LeBaron 1990). Throughout

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[1]Abbreviations: ACCase, acetyl coenzyme A carboxylase; ALS, acetolactate synthase; AHAS, aceto-hydroxyacid synthase; R:S, resistant to susceptible ratio often based on GR_{50} values; GR_{50}, herbicide dosage required to inhibit plant growth by 50% relative to untreated controls; APP, aryloxyphenoxypropionates; CHD, cyclohexanedione

this thesis, resistance will only refer to those instances where a normally susceptible weed population has evolved resistance through selection, and not to situations where weeds are naturally able to withstand herbicide treatment.

Pest resistance to toxicants is not a new phenomenon, although the appearance of herbicide resistant weed populations is relatively recent. The first reported case of a weed evolving resistance to a herbicide was common groundsel (*Senecio vulgaris*), to the triazine herbicides simazine and atrazine (Ryan 1970). Following this initial occurrence, the incidence of herbicide resistance has increased markedly worldwide. In 1990, Holt and LeBaron (1990) reported that resistance had been documented in over 99 weed species. Of these, over half were resistant to the triazine herbicides (55 species, 40 dicots and 15 monocots), with the remainder being resistant to 15 other herbicide families. A more recent estimate by Rubin (1996), places the number of resistant populations at well over 13,000 sites. In western Canada alone, six weed species have evolved resistance to six herbicide groups[2], and well over 450 western Canadian sites have been confirmed to be infested with herbicide resistant weed populations since 1990 (Morrison and Devine 1994).

2.2.2 Herbicide Resistance Mechanisms

Resistance is a consequence of basic evolutionary processes. The selection for resistance to metabolic poisons is a powerful process affecting many, if not all, organisms in nature. Selection for resistance to toxins takes place for naturally

[2]See Table 2-1 for characterization of herbicide groups.

Table 2-1. Herbicide groups.[a]

GROUP 1 (ACCase INHIBITORS)	**GROUP 2 (ALS/AHAS INHIBITORS)**
diclofop methyl	chlorsulfuron
fenoxaprop-p-ethyl	ethametsulfuron
fluazifop-p-butyl	metsulfuron
quizalofop	thiofensulfuron
	tribenuron
clethodim	imazethapyr
sethoxydim	imazamethabenz
tralkoxydim	
clodinafop-propargyl	
GROUP 3 (DINITROANILINES)	**GROUP 4 (GROWTH REGULATORS)**
ethafluralin	clopyralid
trifluralin	dicamba
	dichloroprop
	MCPA
	MCPB
	mecoprop
	picloram
	2,4-D
GROUP 5 (TRIAZINES)	**GROUP 6**
atrazine	bromoxynil
cyanazine	
metribuzin	
GROUP 7	**GROUP 8**
linuron	difenzoquat
	triallate
OTHER HERBICIDES[b]	
EPTC, flamprop-methyl, propanil, TCA, bentazon, glyphosate	

[a] Modified from Guide to Crop Protection 1996, Manitoba Agriculture. Within each group there are several commercial products; this table lists the active ingredients.

[b] Herbicides in this group each have unique modes of action.

occurring earth substances (eg. heavy metals), toxins produced by other organisms (eg. plant diseases) and for man made xenobiotics. Inorganic and organic compounds that impede cellular metabolism create circumstances favourable for individuals in a population that are capable of withstanding the negative effects of the compounds (Duesing 1983).

In order for any organism to overcome toxins, two constituents must be present: heritable variation within the population and an appropriate selection pressure (Maxwell and Mortimer 1994; Cousens and Mortimer 1995). The main selection force driving the development of herbicide resistance in weed populations is the repeated applications of a particular herbicide, or herbicides, with the same mode of action. In Manitoba, a herbicide classification system (see Table 2-1) was developed where herbicides are grouped according to mechanism of action, or where there is evidence that resistance to one herbicide confers resistance to another (Morrison and Devine 1994).

Plants have evolved a number of mechanisms to overcome the toxic effects of herbicides. Potential mechanisms may include enhanced metabolism, altered site of action, sequestration of the herbicide away from the site of action, non-entry, enhanced production of metabolic substrates that compete for a common binding site with the herbicide, and gene amplification of the target site protein (Duesing 1983; LeBaron and McFarland 1990; Warwick 1991). Initially, researchers considered that evolved herbicide resistance would be due to enhanced metabolism (LeBaron and McFarland 1990). Contrary to early speculation, most resistance cases studied to date have been

due to altered sites of action. Resistance to the triazines, dinitroanilines, ALS[1] (AHAS[1]) inhibitors, and ACCase inhibitors, are primarily due to altered sites of action (Devine and Shimabukuro 1994; Gronwald 1994; Saari et al. 1994; Smeda and Vaughn 1994).

Although the majority of resistant weeds have evolved altered target sites, other mechanisms have been identified. In three weed species, velvetleaf *(Abutilon theophtasti* L.), annual ryegrass *(Lolium rigidum* Gaud.), and black-grass *(Alopecurus myosuroides)*, resistance to triazine or urea herbicides is due to enhanced metabolism (Ritter 1986; Powles and Howat 1990; Moss and Cussans 1991; Burnet et al. 1993; Gronwald 1994; Hall et al. 1995). Enhanced metabolism has also been implicated as a mechanism of resistance for a number of ACCase inhibitor, ALS inhibitor and dinitroanaline resistant weed populations (James et al. 1995; Powles and Preston 1995). Additionally, reduced translocation has been observed in 5 weed species resistant to paraquat and two species are thought to have evolved resistance to this herbicide through a sequestration mechanism (Preston 1994). Although other mechanisms have been proposed, to date none have been confirmed (Powles and Preston 1995). This is not to say that alternative mechanisms have not already occurred and/or will not occur in the future.

2.2.3 Factors Affecting the Occurrence and Spread of Herbicide Resistance

Numerous factors are believed to influence the rate of resistance evolution, including the intensity of selection, the relative fitness of the resistant and susceptible genotypes; the initial mutation frequency for the resistance allele(s); the mode of

inheritance of the resistance trait; gene flow via seed, vegetative propagules and pollen; seed longevity in the soil; and the mating system of the weed (Warwick 1991; Maxwell and Mortimer 1994; Cousens and Mortimer 1995; Jasieniuk et al. 1996). A few simple population genetics models have been proposed to estimate the rate of resistance evolution and subsequent geographic spread of resistant individuals and alleles (Gressel and Segel 1990a; Maxwell et al. 1990). These models assume that resistance alleles are initially rare in the population, being maintained through the systematic force of recurrent mutation. Resistant individuals only dominate the population when an appropriate selection pressure (i.e herbicide treatment) is present, giving the resistant genotypes a fitness advantage over the wild type (susceptible). The rate at which resistant genotypes increase is then dependent on the interplay of all the variables listed above.

So far, mathematical models have had limited application as predictive tools because quantitative data for most of the key variables is lacking (Jasieniuk et al. 1996). To utilize the models, it is presently necessary to make numerous assumptions such as the mode of inheritance, initial mutation frequency, degree of selection, relative fitness of the resistant and susceptible genotypes.

Gressel and Segel (1978, 1982) developed a simple population model to predict the rate of enrichment of resistant plants in a population. This model assumes the weed population is grown in monoculture cropping under mono-herbicide usage, and is as follows:

$$N_n = N_0 \, (1 + fa/\beta)^n.$$

N_n is the proportion of R individuals in the population after n generations of herbicide use, N_0 is the initial frequency of R plants prior to herbicide use, f is the relative fitness of R individuals as compared to S genotypes. β is the average seed bank longevity, and a is the degree of selection pressure of the herbicide. Output from this model indicates there is an initial lag period where the resistance alleles increase very slowly, followed by an exponential increase and ultimately a levelling off where resistant allele frequencies in the population are very high.

The intensity of selection pressure has proven to be the most influential agronomic variable operating in this model and subsequent models (Gressel and Segel 1978, 1982, 1990a; Maxwell et al. 1990) that include additional parameters such as mode of inheritance, seed rain, and the application of alternate mode of action herbicides.

The selection pressure exerted by a herbicide is a function of both its efficacy and duration (Mortimer 1993; Maxwell and Mortimer 1994; Cousens and Mortimer 1995; Jasieniuk et al. 1996). The extent to which resistant and susceptible genotypes in treated populations differ in their response to herbicide application determines the degree of selection intensity. Differences in relative seed yield between R and S genotypes as a result of herbicide application, measured at the end of the growing season (effective kill), are generally agreed to provide the best quantification of selection intensity (Gressel and Segel 1978, 1982; Beckie an Morrison 1993; Cousens and Mortimer 1995; Jasieniuk et al. 1996).

Selection duration is a measure of the period of time over which phytotoxicity

is imposed by a herbicide. Duration is a result of the persistence of the herbicide coupled with the number of applications made over time. Unfortunately, many of the attributes that make a herbicide effective and desirable as a weed control agent (i.e. high level of efficacy and long durations of control) also lead to the rapid development of resistance (Mortimer 1993).

2.3 ACCase inhibitor resistance in western Canada

Herbicide resistance was first reported on the Canadian prairies in 1988 when trifluralin resistant green foxtail (*Setaria viridis*), and chlorsulfuron resistant chickweed (*Stellaria media*) and kochia (*Kochia scoparia*) populations were identified in Manitoba, Alberta and Saskatchewan, respectively (Morrison and Devine 1994). Two years later three producers in northwestern Manitoba and one in south central Saskatchewan reported unsatisfactory control of wild oat populations treated with the ACCase inhibitors diclofop-methyl and/or the recently released herbicide fenoxaprop-P. Field histories indicated that in each case ACCase inhibitors had been used repeatedly over the previous nine growing seasons (Heap et al. 1993).

To calculate resistance factors, i.e. R/S ratios, experiments were undertaken to confirm ACCase inhibitor resistance in all four populations and to determine patterns of cross resistance (Heap et al. 1993). A standard susceptible wild oat population (UM5) was included as a reference in all experiments. UM1, the first resistant wild oat population identified, was screened with seven ACCase inhibitors, including four APP[1] and three CHD[1] herbicides (Heap et al. 1993). UM1 exhibited different levels of resistance to the various herbicides. It was most resistant to the CHD herbicide

sethoxydim (R:S = > 152) (Heap et al. 1993).

The field where UM1 was collected had been sprayed mainly with diclofop-methyl but also with two treatments of sethoxydim and one of fenoxaprop-P. Four of the ACCase herbicides included in these initial experiments had not been used in the field. Despite this, UM1 exhibited resistance to the APP herbicides, fluazifop-p-butyl and quizalofop and the CHD herbicides, tralkoxydim and clethodim. Three of these herbicides (quizalofop, tralkoxydim and clethodim) were not registered for commercial use at that time.

To ensure that resistance was expressed under field conditions, three experiments were initiated at Portage La Prairie where diclofop-methyl, fenoxaprop-P and sethoxydim were applied at up to eight times the recommended dosage to both UM1 and the susceptible genotype UM5. As in the growth-room experiments, UM1 exhibited variable levels of resistance to the three herbicides, but was most resistant to sethoxydim (Heap et al. 1993). In all cases UM1 survived treatment at the recommended rates, whereas UM5 was killed.

Additional growth-room dose-response experiments were conducted in which the three remaining resistant populations, UM2, UM3, and UM33, were compared to the susceptible population, UM5. The herbicides used in these studies were the same as those occurring in their field histories, i.e. diclofop, fenoxaprop-P and sethoxydim. Results from these experiments indicated that each population expressed distinct levels of resistance and patterns of cross resistance (Heap et al 1993).

After screening a total of four resistant populations (UM1, UM2, UM3,

UM33), four different cross resistance patterns were evident. Differences in the patterns of cross resistance, coupled with the fact that these populations originated from locations separated by many kilometres, suggested that all four populations evolved resistance independently (Heap et al. 1993). Additionally, it was concluded that resistance in these four populations resulted from different mutations, either at the same gene locus or at distinctly separate loci (Heap et al. 1993; Cousens and Mortimer 1995)

Since the initial confirmation of ACCase inhibitor resistance, hundreds of wild oat populations collected throughout the prairie provinces have been identified as being resistant to various APP and CHD herbicides (Morrison and Bourgeois 1995). Recently four wild oat populations have exhibited multiple resistance to herbicides belonging to different groups with different modes of action. Multiple resistance in these populations include resistance to certain ACCase and ALS inhibitors as well as to flamprop-methyl which has a different mode of action (Morrison and Bourgeois 1995) (Table 2-1). The mechanism of resistance in these populations is currently under investigation (I. N. Morrison, personal communication[3])

Worldwide, ten weed species have been reported to have evolved resistance to the ACCase inhibitors (see Table 2-2). Different populations have exhibited distinct levels of resistance and patterns of cross resistance to a wide range of these herbicides. In four cases, annual ryegrass (*Lolium rigidum*), blackgrass (*Alopecurus myosuroides*),

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green foxtail (*Setaria viridis*) and wild oat (*Avena fatua*), multiple resistance to more than one herbicide group has been identified (Heap and Knight 1986; Moss 1990; Morrison and Devine 1994; Morrison and Bourgeois 1995).

A mechanism of ACCase inhibitor resistance has been identified for three of the weed species listed in Table 2-2 including the Canadian wild oat population UM1. These are diclofop-methyl resistant Italian ryegrass (*Lolium multiflorum*), and APP and CHD resistant winter wild oat (*Avena sterilis* ssp. ludoviciana) and wild oat (*Avena fatua*). In all three instances resistance is due to a modified target site (Betts et al. 1992; Mansooji et al. 1992; Marles and Devine 1995).

2.4 The Inheritance of Herbicide Resistance

As discussed previously, two critical factors necessary for the evolution of herbicide resistance are heritable variation and an appropriate selection pressure. Genetic variation is maintained in populations via recurrent random mutation events and subsequent recombination. The genesis of resistance, regardless of mechanism, resides in mutations of the genetic information of the cell (Duesing 1983; Darmency 1994; Jasieniuk et al. 1996). Given the existence of genetic variation, then the mode of inheritance of the trait, coupled with natural selection and random genetic drift, are the primary determinants of the frequency at which resistance alleles will be maintained in a population prior to the extensive use of herbicides (Roush and Daley 1990; Mortimer 1993; Jasieniuk et al. 1996). Estimates of initial mutation frequencies range from 10^{-2} to 10^{-13} (Roush and Daley 1990; Jasieniuk et al. 1996).

The mode of inheritance of the resistance trait influences the rate of resistance

Table 2-2. Weed species with populations confirmed as being resistant to ACCase inhibitors.

Weed Species	ACCase Inhibitors Tested	References
Annual ryegrass *Lolium rigidum*	diclofop, fluazifop, CGA-82725	Heap and Knight 1986
Italian ryegrass *Lolium multiflorum*	diclofop	Stanger and Appleby 1989
Slender foxtail *Alopecurus myosuroides*	diclofop	Moss 1990
Winter wild oat *Avena sterilis*	diclofop, fluazifop haloxyfop, fenoxaprop, quizalofop, propaquizafop, quinfurop, sethoxydim, tralkoxydim, cycloxydim	Mansooji et al. 1992
Wild oat *Avena fatua*	diclofop, fenoxaprop, haloxyfop, quizalofop, fluazifop, quinfurop, propaquizafop, quizalofop, sethoxydim, cycloxydim, tralkoxydim, clethodim,	Mansooji er al. 1992 Heap et al. 1993
Johnsongrass *Sorghum halepense*	fluazifop, sethoxydim, quizalofop	Barrentine et al. 1992
Giant foxtail *Setaria faberi*	sethoxydim, fluazifop, diclofop, quizalofop, fenoxaprop, clethodim	Stoltenberg and Wiederholt 1995
Green foxtail *Setaria viridis*	diclofop, fenoxaprop, sethoxydim, tralkoxydim, clethodim, clodinafop, fluazifop, quizalofop	Heap and Morrison 1996
Large crabgrass *Digitaria sanguinalis*	sethoxydim, fluazifop, fenoxaprop, quizalofop, diclofop, clethodim	Wiederholt and Stoltenberg 1995
Goosegrass *Eleusine indica*	clethodim, tralkoxydim, sethoxydim, fluazifop, fenoxaprop, diclofop	Marshall et al. 1994

evolution by affecting the frequency of resistant genotypes in the population. The inheritance of resistance determines, in concert with selection, the success that a resistance allele will have in becoming established in a population and the equilibrium frequency at which it will be maintained. If the allele is established at a high equilibrium frequency, then resistance evolution will occur more quickly. Conversely, if the equilibrium frequency is low then the rate of evolution will be retarded. The equilibrium frequency, or the initial resistant plant/individual frequency, can be estimated using mutation-selection equilibrium theory. This approach incorporates the degree of selection on the resistant genotype, the initial mutation frequency and the mode of inheritance of the resistance trait (Jasieniuk et al. 1996).

Additionally, the inheritance of resistance will also influence the rate of resistance evolution by altering the success at which the alleles are spread via seed and pollen dispersal. Dominant traits will be expressed in the initial heterozygotes, leading to a higher success rate for the movement of the resistance trait via seed and pollen. If the resistance trait is recessive then the trait will be masked in the heterozygous state (Jasieniuk et al. 1994; Cousens and Mortimer 1995; Jasieniuk et al. 1996).

When appraising inheritance mechanisms and considering their influence on evolution, three attributes are of primary interest: the number of genes involved (monogenic vs. polygenic traits); gene(s) location, (cytoplasmic vs. nuclear); and the gene(s) expression (Mortimer 1993; Jasieniuk et al. 1996). Each of these attributes will be considered in turn.

2.4.1 Gene Number

The number of gene mutations required to confer resistance is considered the most important genetic factor in determining the likelihood of recovering a resistant individual (Duesing 1983). If the average initial mutation frequency for one gene is 10^{-6} then the frequency of mutation for a two gene system is 10^{-6} x 10^{-6} or 10^{-12}. With the requirement of additional gene mutations for successful expression of resistance, the probability of an individual possessing all necessary genes is exceedingly low. The low probability of successfully recovering an individual with polygenic traits, combined with the fact that many recently registered herbicides are target site specific and that the level of selection imposed by these new herbicides is extremely high, creates conditions where herbicide resistance is most likely to be encoded by a monogenic alteration (Duesing 1983; MacNair 1991; Cousens and Mortimer 1995).

Traditional population genetics theory assumes that adaption would normally be achieved through the accumulation of many genes of small effect (polygenes) (Lande 1983). In fact, adaption to pollutants, heavy metals and xenobiotics by many organisms has been accomplished through the selection of single major genes. These toxins exert such high selection pressures that often populations can only acquire resistance through major gene inheritance (MacNair 1991, Jasieniuk et al. 1996).

With polygenic traits, recombination among individuals for many generations is required to produce highly resistant individuals (Jasieniuk et al. 1996). Therefore, a polygenic based resistance would require a reduction in the selection pressure to allow

for the adequate accumulation of enough minor genes (polygenes) for adequate expression of resistance (Cousens and Mortimer 1995). If the selection pressure is too high early in the selection process, those individuals with low levels of resistance, i.e. an inadequate number of polygenes, would be lost from the population and the probability of subsequent recombination of minor genes would be reduced.

In the event that a polygenic resistance trait were to establish, the subsequent spread of resistance via out-crossing and seed dispersal would be slow due to the reduced probability of all necessary resistance genes being transmitted and expressed following independent assortment of the genes and recombination at the time of gamete formation and reproduction.

2.4.2 Gene Location

Gene location (nuclear or cytoplasmic) also influences the rate of resistance development. Cytoplasmically encoded traits are controlled by many levels of compartmentalization. To illustrate the complexity, Duesing (1983) calculated the probability of a chloroplast mutation surviving and being transmitted to the next generation, i.e. there are multiple chloroplast genomes and photosynthetic complexes in each chloroplast, multiple chloroplasts in each cell, multiple cells in each tissue layer, multiple tissue layers per leaf, and multiple leaves per plant. In one plastid alone there are 10-50 copies of chloroplast DNA. Therefore, the probability that a herbicide resistant mutation would survive, be expressed and then transmitted onto the next generation is small. Duesing (1983) determined that the probability of a cytoplasmic mutant being established was approximately 10^{-10} to 10^{-12}. In contrast,

nuclear gene mutations are believed to occur at significantly higher frequencies with estimates often ranging from 10^{-6} to 10^{-8} (Gressel and Segel 1990b).

Once initial mutants are established in a population, cytoplasmically inherited traits would evolve marginally faster than nuclear traits, since resistance alleles are not lost or hidden in heterozygotes (Mortimer 1993). The subsequent spread of resistance via pollen transmission would not be a factor since there is little chance of cytoplasmic genes being carried and passed via pollen.

2.4.3 Gene Expression

In situations where resistance is encoded on a nuclear gene, its expression (dominant, partially dominant, or recessive) is an additional variable that influences the rate of evolution. Dominance is merely the relative phenotypic resemblance between the heterozygote and its homozygous parents. The degree of dominance has a serious impact on the eventual fate of the resistance allele. Initially when an allele is rare, it occurs almost exclusively in the heterozygous state (Georghiou and Taylor 1977). Thus, during the early stages of evolution, the susceptibility of heterozygous genotypes is the main determinant of the rate of resistance evolution. If the resistance allele is recessive, there is an increased probability that the hidden allele would be lost, especially if the population was treated with herbicide (Duesing 1983). Once established, the degree of dominance also influences the success at which a resistance trait will spread within a population via out-crossing and seed movement. Again, recessive traits are not expressed in the heterozygote, resulting in a high probability of loss following herbicide treatment.

Employing a deterministic model based on resistance evolution in insect populations, Georghiou and Taylor (1977) established that gene expression influenced changes in both gene frequency and population size. Dominant resistance traits reached economically important levels almost twice as rapidly as intermediate or recessive traits. This model assumed the initial mutation frequency was the same for each type of gene expression, ignoring the probability of successful establishment. Had the initial frequency of resistant plants in the population been considered, e.g. recessive traits 10^{-10}, dominant traits 10^{-6}, then differences in the rate of resistance evolution would be even larger.

Dominance appears to play a significant role in adaptive evolution for traits other than resistance. Often when a high degree of selection is applied, organisms adapt through dominant alleles (Merrell 1981; Roush and Daley 1990; Jasieniuk et al. 1996).

2.4.4 Ploidy Level and Number of Potential Mutations

Other genetic factors that may influence the initial frequency and subsequent success of establishment of resistance in a population include the ploidy level of the species, the number of resistance mechanisms and the number of functional resistant mutations for each mechanism. Many of our weeds are polyploid (Gould 1995). The effect that this has on the rate of resistance evolution is still open to debate. Some suggest that polyploidy would serve to reduce the probability that a mutant resistance allele would be expressed due to the abundance of wild type (susceptible) alleles in the remaining genomes (Duesing 1983; Gould 1995). These authors assume that a

single allele would not be sufficient to overcome the toxic effects of the herbicide when the remaining alleles would be encoding for a susceptible gene product. It now appears that herbicide resistance can be encoded at a single gene locus in polyploid weed species [e.g. ACCase inhibitor resistance in wild oat, a hexaploid (Murray et al. 1994)]. If this is the case then polyploidy may, in fact, serve to increase the rate of occurrence of initial mutants through increasing the number of gene loci available to undergo random mutation.

As indicated previously, plants have evolved a number of ways to overcome the toxic effects of herbicides. Thus if multiple resistance mechanisms occur, repeated use of a particular herbicide may select for more than one mechanism simultaneously. With the simultaneous selection of multiple mechanisms (e.g. altered target site and enhanced metabolism) (Gronwald 1994) the chances of selecting a resistant genotype is increased, and therefore the rate of evolution will increase. Additionally, if more than one specific mutation for any gene locus encodes for normal gene function and resistance to the herbicide, then the chances of a resistant mutant being established in a population is greater; and, therefore, so is the rate of resistance development.

The molecular basis of ALS inhibitor resistance in a number of weed and crop species have been studied (Saari et al. 1994; Guttieri et al. 1995). The results indicate that multiple locations along the amino acid sequence encoding for the ALS gene can be altered to express resistance. Additionally, there are also multiple amino acid substitutions at each location that will encode for resistance and leave the ALS protein functioning normally.

The inheritance of resistance is an important variable in evolution, but it is crucial to remember that this variable is not operating alone. As indicated earlier, selection is paramount for the development of resistant populations. Also important are the number of individuals in the selected population, the mating system of the weed, and various other factors. For a complete review of many of these variables and a discussion on their interactions with regards to resistance evolution, see the recent review article by Jasieniuk et al. (1996).

2.4.5 Inheritance Studies

Given the importance of inheritance mechanisms and their role in determining the rate of resistance evolution, there is surprisingly little information available on the inheritance of resistance in weeds. In those cases where inheritance studies have been conducted, resistance to only one herbicide group has shown cytoplasmic inheritance. (Cousens and Mortimer 1995). The exception is triazine resistance. In all but one case triazine resistance was confirmed to be encoded in the chloroplast genome. The point mutation that confers triazine resistance involves a single base substitution in the psbA chloroplast gene that encodes for a photosystem II membrane protein to which triazines bind (Hirschberg et al. 1984; Darmency and Gasquez 1990). Although other point mutations in the psbA gene are known to impart triazine resistance, in plants it appears that a specific mutation in position 264 is necessary (Darmency and Gasquez 1990).

Over 75 weed species have evolved resistance to the triazine herbicides (rubin 1996). It is surprising that so many triazine resistant weeds have occurred given the

maternal inheritance of this trait. Duesing (1983) speculated that a recessive nuclear plastome mutator gene system may be responsible for the increased mutation frequency observed for triazine resistances. Plastome mutators are a means by which plants may increase chloroplast mutation frequencies by 100-1000 fold. In studies with common lamb's quarters (*Chenopodium album*) Darmency and Gasquez (1990), observed spontaneous mutation frequencies for triazine resistance as high as 3.3 percent. This mutation frequency is much higher than expected, and adds support to Duesing's speculation. In addition, Darmency and Gasquez (1990), determined that all of the psbA gene copies in the mutant plants were triazine resistant. The lack of heteroplasmicty implies that triazine resistance may be due to an alteration in a chloroplast DNA replication system.

All other reports concerned with the inheritance of weed resistance concluded that resistance is encoded on nuclear gene(s). With two exceptions, [blackgrass (*Alopecurus myosuroides*) and foxtail barley (*Hordeum jubatum*)], herbicide resistance is governed by single gene alterations (Cousens and Mortimer 1995; Jasieniuk et al. 1996). In addition, all but one of these studies have determined that resistance is expressed as either a dominant or partially dominant trait (Jasieniuk 1994; Cousens and Mortimer 1995).

There have been three studies concerned with the inheritance of ACCase inhibitor resistance in plants. These have focused on maize (*Zea maize*) (Parker et al. 1990), Italian ryegrass (*Lolium multiflorum*) (Betts et al. 1992), and winter wild oat (*Avena sterilis*) (Barr et al. 1992). In all three studies, resistance was governed by a

single, partially dominant, nuclear gene. The mechanism of resistance for all three species has been determined to be an altered site of action (i.e. altered ACCase) although the exact nature of the mutation has not been identified. Given the mode of inheritance one would expect the rate of ACCase inhibitor resistance evolution for the two weed species to be rapid.

2.5 Gene flow

Slatkin (1985) defines gene flow as a collective term that includes all mechanisms resulting in the movement of genes from one population to another. In plants the level of out-crossing and the degree of gene migration via seed, pollen and vegetative propagules are all factors that influence the rate of resistance evolution. Gene flow from resistant to susceptible populations may provide an initial source of resistance genes to susceptible populations, similar to mutation (Jasieniuk et al. 1996). Rates of gene flow are believed to be higher than rates of mutation, and, therefore, may serve to increase the rate of resistance evolution in previously unselected populations. Therefore, gene flow can be a potent evolutionary force. Despite this, there is little information currently available regarding the basic reproductive biology for many of our economically important weeds, including wild oat.

In contrast, gene dispersal, within and between domesticated crop plant populations has been of interest to plant breeders and seed producers for decades (Harrington 1932). The economic considerations associated with contamination of seed crops via pollen migration has stimulated studies of gene flow with regards to distance, breeding systems, pollinator agents and planting designs. More recently, the

production of transgenic crops has stimulated research into crop-weed interactions and the risk of gene escape into natural weed populations (Kareiva et al. 1991; Manasse and Kareiva 1991; Manasse 1992; Kareiva et al. 1994)

The degree of gene dispersal within and between populations is determined by a multitude of factors including population size and shape, plant height, wind direction and velocity, breeding system of the plant, the foraging behaviour of pollen and seed animal vectors and the distance between populations or between plants within the same population (Levin and Kerster, 1974; Handel 1983). The following discussion will be limited to pollen-mediated gene flow, since research conducted for this thesis was concerned with this topic. Additionally, this review is further restricted to wind-mediated pollen flow due to the apparent limited involvement of insect and animal vectors in the pollination of *Avena* species (Raju et al. 1985).

2.5.1 Pollen-Mediated Gene Flow

The degree of pollen-mediated gene flow is influenced by numerous factors, as mentioned above. Pollen is dispersed by animals or wind. Pollination by animals is more efficient than wind pollination since the former requires the production of fewer pollen grains to attain the same level of successful fertilization (Levin and Kerster 1974). Biologists have traditionally considered wind movement the most wasteful strategy for pollen dispersal where as few as 1 in 1000 pollen grains reaches the female organ of the target plant to produce a seed (Niklas 1987).

The movement of pollen has been studied extensively. Pollen grains are released and lifted into the air by turbulence, following wind currents which often

move in a horizontal direction. Variables that are believed to affect the duration and distance pollen grains are carried include height of release, the terminal velocity of the grains, the velocity of the air current and the degree of air turbulence (Levin and Kerster 1974; Maxwell and Mortimer 1994).

Various measures have been employed to quantify the distance pollen moves. The most common and useful are estimates of the mean pollen flow distance, the standard deviation of the pollen flow distribution and the degree of kurtosis in the gene flow distribution pattern (Wright 1946; Rai and Jain 1982; Tonsor 1985a). The mean pollen flow distance and the standard deviation of the distribution provide a convenient method for comparing pollen movement distances, where the degree of kurtosis affords a description of the distribution pattern. Rai and Jain (1982) outline the calculations required to estimate each of the above parameters.

A number of simple models, based on the movement and deposition of particles over distance have been employed to predict the distribution of pollen grains from a source. Sutton's (1932, 1947) eddy diffusion law describes pollen movement in terms of a suspended pollen cloud, where eddies (turbulence) were the major factor controlling the distribution pattern of the pollen, with terminal velocity of the pollen grain and wind speed being second order interactions. Results from these simulations indicate that the shape of the relative pollen concentration curves is leptokurtic, where the vast majority of the pollen released is deposited near the source. A distribution pattern is considered leptokurtic when β (a measure of kurtosis) is significantly greater than 3.0 (Rai and Jain 1982). Thus, as the distance from the pollen emitting source

increases, fewer grains will be deposited (Sutton 1947).

Pollen-mediated gene flow can be measured as either potential gene flow or as actual gene flow (Levin and Kerster, 1974). Potential pollen-mediated gene flow is a measure of pollen grain deposition, as discussed above. Sutton's diffusion law describes the distribution of potential pollen-mediated gene flow since it is concerned with the distribution of pollen grains. Many indirect measures of pollen movement have been conceived to estimate potential gene flow. Handel (1983) summarized a number of methodologies including the use of dyes and powders on source flowers, chemical labelling of pollen grains, and following pollinator movements. Additionally direct measurements of pollen movement through the use of pollen samplers and by censusing pollen grains on receptor flowers also give measurements of potential gene flow.

In contrast, actual gene flow is a measure of successful fertilizations. Procedures for estimating actual gene flow are limited. One direct procedure is through progeny analysis (Handel 1983). More recently, paternity analysis using multiple polymorphic loci as markers has been employed in natural plant populations (Ellstrand and Marshall 1985). Paternity analysis gives an estimate of gene flow through the elimination of all possible paternal contributors by comparison of maternal and progeny genotypes.

The most accurate and useful measure of actual pollen-mediated gene flow is through the use of progeny analysis (Rai and Jain 1982). This technique has a long history with plant breeders, where a marker gene is used to identify progeny formed

through out-crossing between the pollen source population carrying the marker gene and the receptor population (Rai and Jain, 1982). Progeny collected from receptor plants is then scored for the marker gene and a measure of actual gene flow is attained. For progeny analysis it is preferable that the marker gene be dominant and easily identified in the hybrid offspring.

There have been very few studies conducted that have attempted to describe pollen-mediated gene flow in natural populations. In most instances gene flow has been estimated through indirect measures, giving an indication of potential gene flow (Devlin and Ellstrand 1990). In all cases where potential gene flow has been measured, pollen distribution has been characterized as leptokurtic (Levin and Kerster 1974; Ellstrand and Marshall 1985) Such indirect measures do not take into account that spacial arrangements and genetic relationships of the potential parents can influence the probability of successful fertilization. In addition, indirect measures of pollen flow do not take into consideration the production of pollen on receptor plants and the increased competition for successful fertilization on the stigma by marker and receptor pollen as distance from source plants increases and/or the density of the receptor plants increases. This over-estimation would be most severe for self fertilized plant species (Levin and Kerster 1974).

In a series of studies with common plantain (*Plantago lancellata*), Tonsor (1985a, 1985b) compared pollen dispersal and pollen-mediated gene flow distributions in wind tunnel studies. In all instances pollen dispersal distributions were leptokurtic. These results are in accordance with previous wind borne pollen distribution studies.

Using a male sterile genotype as the pollen acceptor population, Tonsor (1985a, 1985b) established that actual pollen-mediated gene flow patterns in this species was platykurtic with optimum gene flow at intermediate distances.

The most common means of expressing gene flow distances is the standard deviation of the distribution (Wright 1946). In Tonsor's wind tunnel studies the standard deviation for the pollen deposition data was 57.6 cm where the actual gene flow distribution's standard deviation was 68.9 cm. Under field conditions the standard deviation of the gene flow distribution was 133 cm and was also characterized as platykurtic. Tonsor (1985a, 1985b) correlated gene flow distribution to the distribution of pollen clusters containing at least 3 to 6 pollen grains. He speculated that pollen clusters would be more likely to successfully fertilize than single pollen grains, and therefore the distribution of clusters should be considered rather than simply counting pollen grains. The tendency for pollen grains to cluster is widespread among wind pollinated species (Anderson 1970). If these results reflect the situation with other wind pollinated plant species, then indirect measurements of pollen-mediated gene flow may actually be underestimating the actual mean distances travelled. Where progeny analysis has been employed in the field, it has been demonstrated that actual gene flow distances can be significantly greater than those estimated by indirect methods (Ellstrand and Marshall 1985; Tonsor 1985b).

A potential flaw in Tonsor's experiments is that a male sterile genotype was employed as the pollen acceptor population. The use of a male sterile receptor may cause an over-estimation of gene flow distances since there is no production of pollen

on these plants and, therefore, no pollen competition originating from acceptor plants. (Levin and Kerster 1974). It would have been more accurate to use a marker gene system to identify out-crossed seed rather than a male sterility system where the production of any seed indicates an out-crossing event. Later studies by Bos et al. (1986) also with *Plantago lanceolata* confirm gene flow distribution patterns (platykurtic) in this species, although no explanation is offered for this distribution pattern based on pollen cluster size.

To date there have not been any reported studies describing gene flow distribution patterns of *Avena fatua.* Rai and Jain (1982) reported on pollen and seed mediated gene flow patterns for a related wild oat species, *Avena barbata.* This tetraploid species is believed to be highly selfed with out-crossing rates similar to that of *Avena fatua* (1-5%)(Raju 1990; Rai and Jain 1982). Using three dominant genetic markers (lemma colour, lemma pubescence and leaf sheath pubescence) the authors attempted to estimate gene flow for two planting designs. The first design was a cross planting where the dominant marker plants were placed in the centre of four linear transects of recessive receptor plants. The second design was a spiral pattern where the dominant marker plants were placed in the centre of a spiral grid of recessive receptor plants.

The mean pollen flow distance differed for the two planting designs. It was 0.98 m for the first design and 2.94 m for the second design. The average percent out-crossing was affected by prevailing wind direction. For example the mean out-crossing rate in the eastern transect arm of the first design was 15.6 percent as

compared to 1.5 percent in the western direction. The distribution patterns of pollen-mediated gene flow were separated based on direction and were determined to be platykurtic. Had the pollen flow data been grouped for each design over all directions it is likely that the distribution pattern for the first design would have been considered leptokurtic and for the second design, platykurtic.

Additional gene flow studies where actual pollen-mediated gene flow has been estimated for wind pollinated plant species is very limited. In out-crossing studies with Italian ryegrass, (*Lolium multiflorum*), Maxwell and Ristau (1992) determined that actual gene flow distances were further than those estimated by pollen dispersal patterns. Additionally, increased wind speed served to expand pollen-mediated gene flow distances in wind tunnel studies. Working with the same plant species, Copeland and Hardin (1970) determined that out-crossing between marker and receptor populations was negligible at 6 m and undetectable 12 m from the pollen source. Plots of the gene distribution versus distance from pollen source appeared to be platykurtic, although a formal quantification of the degree of kurtosis was not reported. Copeland and Hardin (1970) observed a great deal of crossing in both down- and up-wind directions. These observation are in agreement with those reported by Maxwell and Mortimer (1994). Significant movement of pollen in the upwind direction may point to the involvement of insect or animal vectors, or due to turbulence. Neither was substantiated.

2.5.2 Out-crossing

Very little is known about the most basic aspects of pollination biology for

many of our economically important weeds, including the level of out-crossing in wild oat. To date there have been few reports that have estimated the degree of out-crossing in *Avena fatua.* Such estimates have been made by plant breeders where the level of introgression between wild oat and domesticated oat (*Avena sativa*) was reported. The degree of out-crossing will directly affect actual pollen-mediated gene flow, where highly out-crossing species will have a higher degree of success at spreading a gene via pollen movement. Additionally the mating system will influence the success at which an initial mutant allele will become fixed in a population. For recessive traits, selfing has the effect of greatly increasing the probability of expression and the rate of evolution of the trait (Jasieniuk et al. 1994). Therefore, mating system is an important variable in determining the rate of resistance evolution and the subsequent geographic spread of resistant individuals.

The first report on the level of introgression between wild and domesticated oat was made by plant breeders concerned with the contamination of seed oat crops. As early as 1930, Coffman and Wiebe reported an average introgression rate of 1.28 percent between light (domesticated) and dark (wild) kernelled oats. In 1932 Harrington reported a 9.82 percent out-crossing rate in 'Liberty' a hulless domesticated oat variety, as compared to an average out-crossing rate of 0.07 percent for the hulled cultivar 'Golden Rain'. Harrington's studies reported on domesticated oat by domesticated oat crosses. In 1933, Derrick reported a 0.1 percent introgression rate between wild oat and the domesticated oat cultivar, 'Banner' taken from a seed producers field. In further controlled field studies Derrick (1933) reported an out-

crossing rate of 0.31 percent between the domesticated oat cultivars 'Banner' and 'Old Island Black' when plots were sown in an east-west orientation. When sown in a north-south direction there was a 0.1 percent out-crossing rate.

The most recently reported introgression studies were those conducted by Bickelmann and Leist (1988). Using electrophoresis patterns to identity hybrids, Bickelmann and Leist (1988) observed out-crossing rates between domesticated oat cultivars ranging from zero to over 9% and introgression levels ranging from 0 to over 6% between wild and domesticated oat. Levels of out-crossing were dependent on genotype and year for inter- and intra-specific crosses. In these studies all introgression rates were determined using the domesticated oat cultivar as the maternal parent. The author of this thesis is unaware of any study where the level of introgression between wild and domesticated oat has been determined in the opposite direction where the wild oat serves as the maternal parent. There is a possibility that introgression rates in the opposite direction could be reduced due to height differences between the two species, since wild oat is often taller than domesticated oat.

In 1964, Imam and Allard estimated out-crossing rates for seven California wild oat (*Avena fatua*) populations to range from 1.24 to 11.78 %. The authors scored plants on the basis of two simply inherited, phenotypic characteristics, lemma colour and rachilla pubescence. Out-crossing rates were then estimated for each population based on the variability of each of the two traits. Although this is not a direct measure of mating behaviour in wild oat, this is the only report of out-crossing rate for this species in natural populations. The upper end estimate of 12 % out-crossing is

much higher than previously assumed for *Avena fatua*, although Rai and Jain (1982) reported out-crossing rates as high as 15 percent in the related tetraploid wild oat species *Avena barbata.*

Relatively high out-crossing rates in predominantly selfing species may be observed under some instances. Mating system has been reported to be influenced by a number of variables, including genotype, environmental conditions at the time of anthesis, population size and structure (Levin and Kerster 1974). Analysis of several wild oat (*Avena fatua* and *Avena barbata*) populations in California by Jain and Marshall (1967) indicated a high degree of polymorphism for three marker allele systems. The high degree of polymorphism suggests a substantial degree of out-crossing among these populations.

With the introduction of transgenic crops to the market, there has been renewed interest in the pollination biology of many of our crops and related weed species. One of many concerns regarding the release of transgenic crops is the risk of gene escape into related weed populations. Genes will escape to related weed species via pollen transfer, and therefore pollen-mediated gene flow is of interest. As mentioned above, the only accurate representation of gene movement will be through progeny analysis (Rai and Jain 1982). Often the gene being introduced to the crop genome is a herbicide resistance gene, and this trait is an excellent marker gene system since often it is encoded by a simply inherited, dominant gene systems.

2.6 Summary

Herbicide resistant weeds are being reported in an increasing number of species

and to an increasing number of herbicides (Holt and LeBaron 1990; Matthews 1994). A heavy reliance on highly effective, site specific herbicides has been the main driving force behind this phenomenon (Cousens and Mortimer 1995; Jasieniuk et al. 1996). Although farmers' reliance on these effective herbicides has been the primary force behind the evolution of herbicide resistance, many other variables will ultimately contribute to the enrichment rate and spread of resistance. Such factors as the initial frequency of resistant mutants, the mechanism of inheritance of the trait, the number of individuals in the population, seed bank longevity, migration via seed and pollen, the fitness of the resistant individuals as compared to the wild types, and many others variables will ultimately work in concert with selection to influence the rate of evolution of resistance (Maxwell and Mortimer 1994; Cousens and Mortimer 1995, Jasieniuk et al. 1996).

Research by weed scientists and population geneticists has mainly been limited to estimating many of these variables, and much of the information needed about common weeds is not available. A better understanding of these economically important pests will lead to better management strategies for both delaying the occurrence of new cases of resistance and the management of existing resistant populations (Morrison and Bourgeois 1995).

3. INHERITANCE OF ACETYL-CoA CARBOXYLASE INHIBITOR RESISTANCE IN WILD OAT (*Avena fatua*)[4]

Abstract. Resistance to fenoxaprop-P and other aryloxyphenoxypropionate and cyclohexanedione herbicides in the wild oat population, UM1, is controlled by a single, partially dominant, nuclear gene. In arriving at this conclusion, parents, F_1 hybrids, and F_2 plants derived from reciprocal crosses between UM1 and a susceptible wild oat line, UM5, were treated with fenoxaprop-P over a wide range of dosages. Based on these experiments, a dosage of 400 g ai ha^{-1} fenoxaprop-P was selected to discriminate between three response types. At this dosage susceptible plants were killed and resistant plants were unaffected, whereas plants characterized as intermediate in response were injured but recovered. Treated F_2 plants segregated in a 1:2:1 (R,I,S) ratio, indicative of single nuclear gene inheritance. This was confirmed by selfing F_2 plants and screening several F_3 families. Families derived from intermediate F_2 plants segregated for the three characteristic response types, whereas those derived from resistant F_2 plants were uniformly resistant. Chi-square analysis indicated the F_2 segregation ratios fit those expected for a single, partially dominant, nuclear gene system. In addition F_2 populations from both crosses were screened with a mixture of fenoxaprop-P and sethoxydim. The dosages of both herbicides (150 g ai ha^{-1} fenoxaprop-P and 100 g ha^{-1} sethoxydim) were sufficient to control only susceptible plants. Treated F_2 populations segregated in a 3:1 (R:S) pattern thereby

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[4]This chapter was published as the following: Murray, B. G., I. N. Morrison, and A. L. Brûlé-Babel. 1994. Inheritance of acetyl-CoA carboxylase inhibitor resistance in wild oat (*Avena fatua*). Weed Sci. 43:233-238.

confirming that resistance to the two chemically unrelated herbicides results from the same gene alteration. Nomenclature: Fenoxaprop-P [R-2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid, ethyl ester; sethoxydim, [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one]; wild oat, *Avena fatua* L. #[5] AVEFA.

Additional index words. Fenoxaprop-p-ethyl, aryloxyphenoxypropionate, cyclohexanedione, ACCase, herbicide resistance, weed genetics, AVEFA.

3.1 INTRODUCTION

It is commonly accepted that herbicide resistant weeds occur naturally in populations at very low frequencies through recurrent mutation (Duesing 1983; Gressel and Segel 1978; Jasieniuk et al. 1996). Resistant weeds will predominate in a population only when they have a selective advantage. Repeated applications of a single herbicide, or herbicides with a common mode of action, provide the necessary selection pressure to shift weed populations towards high frequencies of resistant individuals.

For herbicide resistance to evolve to detectable levels in a population, the trait must be heritable. The inheritance of the resistance trait influences the initial frequency of resistant individuals within an unselected population, and subsequently the degree to which a gene will spread among individuals via pollen transfer (Duesing

[5]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds Revised 1989. Available from WSSA, 1508 W. University Av.,Champaign, IL 61820.

1983; Jasieniuk et al. 1996). Both the mode of inheritance and the degree of outcrossing ultimately affect the rate of resistance evolution.

Aside from triazine resistance, which is maternally inherited in most species (Machado 1982), resistance to other herbicides most commonly results from an alteration in a single nuclear gene (Jasieniuk et al. 1996). One notable exception is chlortoluron [N'-(3-chloro-4-methylphenyl)-N,N-dimethylurea] resistance in blackgrass (*Alopecurus myosuroides* Huds.), which is controlled by two additive genes (Chauvel 1991).

In most instances where resistance is encoded by a nuclear gene, it is expressed as either a dominant or partially dominant trait. This is true for sulfonylurea herbicide resistant mutants of prickly lettuce (*Lactuca serriola* L.) (Mallory-Smith et al. 1990), as well as for diclofop-methyl [(±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid, methyl ester] resistant Italian ryegrass (*Lolium multiflorum* Lam.) (Betts et al. 1992), and fenoxaprop [(±)-2-[4-[(6-chloro-2-benzoxazoly)oxy]phenoxy]propanoic acid] and fluazifop [(±)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid] resistant winter wild oat (*Avena sterilis* L.) (Barr et al. 1992). An exception is dinitroaniline resistance in green foxtail (*Setaria viridis* L. Beauv.), which is inherited as a recessive character (Jasieniuk et al. 1994).

The objective of this study was to determine the inheritance of resistance in a wild oat population previously described as UM1, which is resistant to several

aryloxyphenoxypropionate (APP)[6] and cyclohexanedione (CHD)[6] herbicides (Heap et al. 1993).

3.2 MATERIALS AND METHODS

3.2.1 Parental populations.

The two parental wild oat populations selected for this study were UM1 and UM5. UM1 is resistant to diclofop-methyl (R/S[6]=6) and fenoxaprop-P (R/S=14) and extremely resistant to sethoxydim (R/S = >152) whereas UM5 is susceptible to these herbicides (Heap et al. 1993).

To ensure homozygosity for resistance in UM1 and susceptibility in UM5, single seeds from each population were selected and selfed for two generations. The parental lines and all subsequent generations were selfed by enclosing panicles within glassine bags prior to anther dehiscence.

3.2.2 Development of F_1, F_2, and F_3 generations.

General seed production procedures. Seed was dehulled by hand and germinated on two layers of filter paper moistened with 5 ml 0.1% KNO_3 solution in 9 cm-diameter plastic petri dishes. The dishes were kept at 5 C in the dark. After 24 h, seeds were pierced on the dorsal side adjacent to the embryo to aid in breaking dormancy. Seeds were then placed at 5 C for an additional 72 h.

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[6]Abbreviations: APP, aryloxyphenoxypropionate; CHD, cyclohexanedione; R/S, resistant to susceptible ratios based on GR_{50} values; S, susceptible; I, intermediate; R, resistant; GR_{50}, herbicide dosage required to inhibit plant growth by 50% relative to untreated controls.

Germination was considered to have occurred when both the radicle and the coleoptile were visible. Germinated seeds were planted in 15 cm-diameter plastic pots filled with a 2:1:1 by vol, soil/sand/peat mixture. One seed was planted in each pot. The mixture was initially augmented with a single application of nutrient solution containing 100 ppm N, 50 ppm P, 150 ppm K, 25 ppm S, 5 ppm Cu and 10 ppm Zn at a rate of 62.5 ml per kg of soil mixture. Beginning three weeks after emergence, plants were fertilized weekly with a solution, (2.4 g L^{-1}) of water-soluble fertilizer[7].

F_1 generation. To produce F_1 seed, parental plants were grown in growth cabinets at a constant 16 C with a 16/8 h day/night regime. Irradiance was 600 µE $m^{-2}s^{-1}$ PPFD. To ensure asynchronous anther dehiscence of pollen donor and maternal plants, two cabinets were utilized with different lighting regimes. In the cabinet containing the pollen donor plants, the day cycle began 2 h earlier than in the cabinet containing the maternal plants. This time difference allowed for a period during which emasculation and pollen transfer could be performed with minimal risk of the maternal plants being self pollinated. All florets on the main panicle of the maternal plants were emasculated and cross pollinated; all other panicles were selfed. Emasculation and crossing was conducted over a period of 7 d, during which time stigmas on the emasculated panicles remained receptive. Selfed seed from parental plants were later screened to confirm homozygosity for resistance or susceptibility. Two F_1 genotypes, (UM1/UM5 and UM5/UM1) were produced by making reciprocal crosses between the

[7]Peters Professional Water Soluble Fertilizer 20-20-20 (with Chelated Micro-nutrients). W.R. Grace and Co., P.O. Box 238, Fogelsville, PA 18051.

two parental lines. According to accepted convention, F_1 genotypes are designated with the maternal parent preceding the pollen donor, i.e. ♀ x ♂.

F_2 and F_3 generations. F_2 and F_3 populations were produced in a single growth cabinet, under the same temperature and lighting regime as described for the F_1 seed production. F_2 populations were produced by selfing F_1 plants. F_2 plants that exhibited no injury, and those which were injured but survived treatment with 400 g ha^{-1} fenoxaprop-P, were replanted 21 DAT. These were selfed to produce F_3 families.

3.2.3 Screening procedures.

General. F_1, F_2 and F_3 generation screens included plants originating from both reciprocal crosses. Plants were germinated and fertilized as described above.

Plants were grown in a growth-room at 21/15 C with a 16/8 h day/night regime and an irradiance of 480 µE m^{-2} s^{-1} PPFD. All three generations were screened with fenoxaprop-P, 14 days after emergence, at the three-leaf stage. Treatments were applied using a cabinet sprayer equipped with a flat fan nozzle[8] that delivered 117 L ha^{-1} at 275 kPa in a single pass.

F_1 dose-response experiments. F_1 and parental plants were planted into 9 cm-diameter plastic pots, three pre-germinated seeds per pot. Seeds were planted at a depth of 2.5 cm. F_1 and parental plants were treated with fenoxaprop-P at dosages comparable to earlier dose-response experiments reported by Heap et al. (8). The dosages employed for screening F_1 hybrids were: 0, 50 100, 200, 400, 800, and 1600 g

[8]TeeJet SS80015 flat-fan nozzle tip. Spraying Systems Co., Wheaton IL 60188,

ha^{-1}. UM1 plants were also sprayed at these dosages, whereas UM5 plants were treated at 0, 12.5, 25, 50, 100, 200, and 400 g ha^{-1}. The F_1 screening experiment was a randomized complete block, with three replicates. Replicates and treatments within replicates were re-randomized weekly to ensure uniformity of growth.

Twenty-one DAT, shoots were clipped at the soil level, oven dried for 48 h at 80 C and then weighed. Shoot dry matter measurements were averaged for the three plants in each pot and the results expressed as a percentage of the mean of the nine untreated control plants for each genotype.

F_2 segregation experiments. F_2 populations were screened in two steps. The first entailed determining the appropriate discriminatory dosage of fenoxaprop-P. The second involved treating F_2 plants at the selected dosage.

Initially, 90 F_2 plants, along with 5 UM1 and 4 UM5 plants were planted into 46 by 56 by 14 cm wooden flats. In the second step, 99 F_2 plants were planted in each flat with parental lines included in separate flats. Germination and growing conditions were as described previously.

In the initial step, one flat of each F_2 cross was treated at each of the following dosages: 0, 200, 400, 800 and 1600 g ha^{-1} of fenoxaprop-P. Treatment conditions and methods were similar to those described for the F_1 screening experiment.

Twenty-one DAT, flats were removed from the growth-room and individual plants were examined and characterized based on their response to the herbicide. Three response types were identified: Resistant (R)[6], intermediate (I)[6], and susceptible (S)[6]. R plants exhibited no herbicide injury symptoms, while I plants were stunted

with chlorotic and necrotic areas on the foliage. S plants were entirely necrotic and obviously dead. Based on visual observations the 400 g ha^{-1} dosage of fenoxaprop-P gave the best discrimination between the response types.

In the second step of F_2 screening, two flats of each F_2 cross, and one flat of each parental genotype were treated with 400 g ha^{-1} fenoxaprop-P. Additionally, one flat of each F_2 cross and parent were included as untreated controls. Segregation patterns were determined based on the three response types.

F_3 family segregation experiment. Sixty-one F_3 families were produced by selfing F_2 plants that survived treatment. The 61 surviving F_2 plants were transplanted into 15 cm-diameter plastic pots 21 DAT. These plants were then selfed to produce F_3 families. Forty-one of the transplanted F_2 plants were visually assessed as being R and 20 as I.

Eighteen germinated seeds per F_3 family were sown into flats. Each flat contained five F_3 families (two rows, each with 9 plants) and one row (9 plants) of either UM1 or UM5 parental plants. All other germination and growth conditions were as described for the previous F_2 segregation experiments.

The F_3 families and parental plants were treated with 400 g ha^{-1} fenoxaprop-P. Twenty-one DAT, the F_3 families were characterized as either segregating or homozygous R.

3.2.4 CHD inheritance confirmation.

F_2 populations as well as parental plants were screened with fenoxaprop-P, sethoxydim and a mixture of the two herbicides to determine if APP and CHD

resistance in UM1 was encoded for by the same or separate gene alterations. Ninety nine F_2 plants (UM5/UM1) were planted into 46 by 56 by 14 cm wooden flats with parental plants (49 plants each) included in separate flats. Individual F_2 and parental flats were treated with each herbicide (150 g ha^{-1} fenoxaprop and 100 g ha^{-1} sethoxydim). In addition, two F_2 flats (99 plants of each cross) and a single parental flat were also treated with a mixture of fenoxaprop and sethoxydim at the same dosages. Twenty-one DAT F_2 and parental flats were assessed and F_2 segregation patterns determined from visual ratings of individual plants.

3.2.5 Statistical analysis.

Dose response curves from the F_1 experiment best fit a logistic model described by Brain and Cousens (Brain and Cousens 1989). This model has had utility in comparative bioassay experiments (Streibig 1980; Brain and Cousens 1989). The model fitted was

$$y = k/(1+e^{bg}x^{b})+d$$

where y is the dependent variable, mean shoot dry matter per plant (% of control), x is the herbicide dosage, d is the lower asymptote, $k + d$ is the upper asymptote, e is the base of the natural logarithm, and b and g determine the slope of the inflection region of the curve. Regression analysis was performed using SAS[9].

Chi-square analysis was used to determine the best fit for F_2 segregation ratios based on response types (Steele and Torrie 1980). Homogeneity chi-square tests were performed to determine if the data could be pooled within crosses and between

[9]SAS Version 5. 1985. SAS Inst. Inc., Box 8000, Cary, NC 27511-8000.

reciprocal crosses (Strickberger 1976).

3.4 RESULTS AND DISCUSSION

3.4.1 F_1 dose-response curves.

The response of both F_1 genotypes to increasing dosages of fenoxaprop-P was intermediate to those of the parents (Figure 3-1, Table 3-1). Since the parental UM1 response curve had only one point substantially below the fitted upper asymptote, it was not possible to accurately describe the inflection (*b* and *g*) and the lower asymptote (*d*) regions of this curve. However, the results from the F_1 dose-response experiment were useful in determining gene location (nuclear vs. cytoplasmic) and expression type.

Results of the F_1 experiment indicate that fenoxaprop-P resistance in UM1 is governed by a partially dominant, nuclear gene(s) system. The fact that UM1 resistance is encoded for on a nuclear gene is evident in the similarity in responses between the two reciprocals (Figure 3-1). Had the trait been due to an altered cytoplasmic gene, the response curve of the UM5/UM1 cross would be similar to that of its susceptible maternal parent (UM5).

Both crosses resulted in F_1 progeny with a six to seven-fold increase in resistance relative to the susceptible genotype (Table 3-1). Had the expression type been either recessive or fully dominant, the F_1 dose-response curves would have closely paralleled the susceptible or resistant genotype responses, respectively. At 200

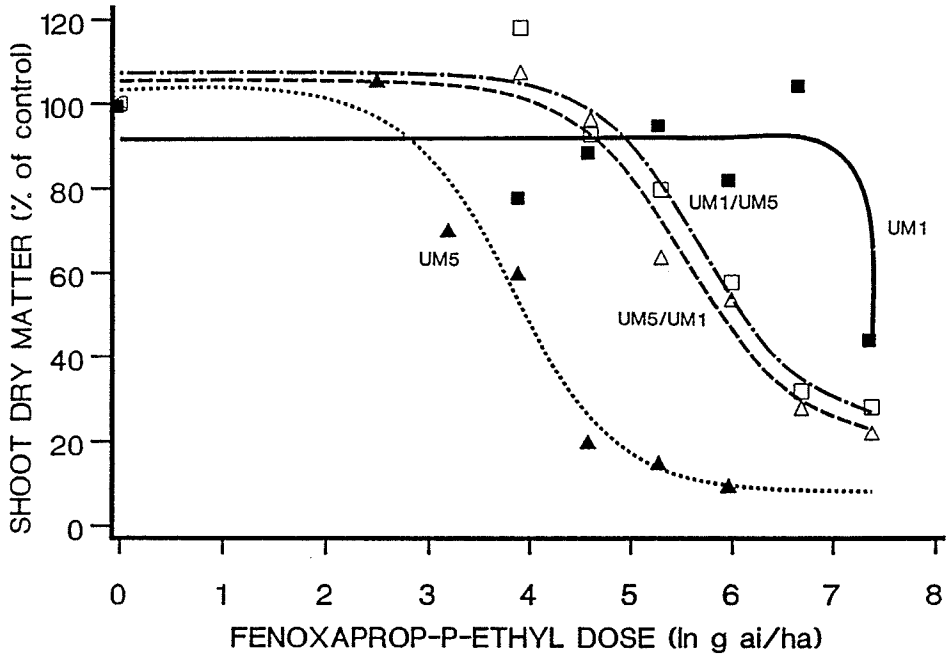

Figure 3-1. Response of parental and F_1 genotypes to increasing dosages of fenoxaprop-P. Refer to Table 3-1 for parameter estimates, GR_{50} values, and R/S ratios.

Table 3-1. Parameter estimates[a] (standard errors in parenthesis) describing the response of resistant (UM1), susceptible (UM5) and F_1 (UM5/UM1, UM1/UM5) plants treated with fenoxaprop-P under growth room conditions (Figure 3-1).

Genotype	*g*	*b*	*d*	*k*	R^2	Shoot growth GR_{50}[4]	GR_{50} ratio R/S
UM1 (R)[b]	-	-	-	91.5 (5.3)	-	>800	>17
UM5 (S)	-3.8 (0.3)	1.9 (0.8)	8.1 (10.2)	95.2 (15.2)	0.96	46	*na*[c]
UM5/UM1 (F_1)	-5.6 (0.3)	1.8 (0.7)	19.7 (11.3)	85.8 (15.1)	0.97	260	6
UM1/UM5 (F_1)	-5.7 (0.3)	1.9 (1.0)	23.6 (13.8)	83.7 (17.9)	0.96	311	7

[a]Refer to Material and Methods for a description of the model fitted.
[b]Parameters describing the inflection and lower asymptotes of the UM1 dose-response curve could not be accurately estimated due to the limited number of data points in these regions.
[c]Not applicable

or 400 g ha^{-1} fenoxaprop-P it was possible to visually distinguish the F_1 hybrids from either parent.

3.4.2 F_2 segregation for resistance trait.

Observations from the first step in the F_2 segregation experiments indicated that 400 g ha^{-1} fenoxaprop-P provided the best discrimination between response types (data not shown). At this dosage, three distinct plant responses could be identified among the F_2 progeny (Figure 3-2). At the lower dosage of 200 g ha^{-1} it was difficult to separate R from I plants since I plants exhibited little injury. This is in contrast to F_1 dose-response results, where hybrids could be visually distinguished at this low dosage. This discrepancy is probably due to differences in growing conditions, where F_1 plants were screened in 9 cm-diameter plastic pots and F_2 populations were screened in large wooden flats.

At dosages greater than 400 g ha^{-1}, UM1 plants were injured and it was not possible to clearly distinguish R from I plants. When treated with 400 g ha^{-1}, UM1 parental plants were unaffected (R), whereas UM5 plants died (S).

Segregation patterns, based on F_2 plant response types, were determined by visual assessment of those flats sprayed with 400 g ha^{-1} in both F_2 screening experiments. In every instance, the F_2 populations segregated in a 1:2:1 (R:I:S) pattern, further indicating that resistance is governed by a single, partially dominant, nuclear gene (Table 3-2). Untreated F_2 and parental flats produced only healthy plants and, therefore, response types observed for treated flats were the result of herbicide treatment.

Figure 3-2. F_2 plant response types 21 DAT with 400 g ha^{-1} fenoxaprop-P. R = resistant, I = intermediate and S = susceptible. Refer to Material and Methods for a description of the three response types.

Table 3-2. Segregation for ACCase inhibitor resistance in the F_2 generation treated with 400 g ha^{-1} fenoxaprop-P. Chi-square values (X^2) and associated probabilities (P) are the result of tests for goodness of fit to a 1:2:1 (resistant:intermediate:susceptible) segregation ratio. Resistant (R) = no injury symptoms; intermediate (I) = stunted plant with some chlorotic and necrotic foliage; susceptible (S) = dead, 100% necrosis.

F_2 population	Phenotype				
	R	I	S	X^2	P
UM5 / UM1	——	no. of plants	——		
Expt. 1, Flat 1[a]	22	44	21	0.04	0.98
Expt. 2, Flat 1	19	41	24	0.64	0.73
Expt. 2, Flat 2	25	55	17	3.06	0.22
Total (UM1/UM5)	66	140	62	0.66	0.72
Homogeneity				3.08	0.54
UM1 / UM5					
Expt. 1, Flat 1	18	41	20	0.22	0.90
Expt. 2, Flat 1	28	42	26	1.58	0.45
Expt. 2, Flat 2	23	41	24	0.43	0.81
Total (UM5/UM1)	69	124	70	0.86	0.65
Homogeneity				1.37	0.85
Total (overall)	135	264	132	0.05	0.98
Homogeneity				5.92	0.82

[a]Expt. no., Flat no. = F_2 experiment step and flat number.

Results from individual flats were tested for homogeneity using a chi-square test, and in all instances the data could be pooled, both within and between reciprocal crosses. Analysis of the pooled data over all flats (531 F_2 plants) yielded a chi-square value of 0.05 and a probability of 0.98 for a 1:2:1 segregation pattern (Table 3-2). These results confirm the initial conclusions drawn from the F_1 dose response experiment, and further indicate that resistance is due to a single gene alteration.

3.4.3 F_3 family segregation

Seed from each of the F_2 plants transplanted after treatment constituted a single F_3 family. In all cases, the F_3 families segregated as expected (data not shown). Families originating from I F_2 plants produced progeny exhibiting all three response types, indicating that I F_2 plants were heterozygous for the resistance trait. Without exception, families originating from R F_2 plants produced all R F_3 offspring, indicating that R F_2 plants were homozygous for the resistance trait. F_3 screening re-confirmed the accuracy of classification of F_2 plants into the three reaction groups, and further supports the conclusion that resistance is governed by a single, partially dominant nuclear gene.

3.4.4 CHD inheritance confirmation.

UM1 is resistant to a number of APP and CHD herbicides (Heap et al. 1993). Additional studies indicated that resistance to sethoxydim was also inherited as a single "dominant" gene (data not shown). To ascertain if resistance to both fenoxaprop-P and sethoxydim are encoded for at the same or separate gene loci, F_2 populations were treated with a mixture of fenoxaprop-P and sethoxydim. The

dosages employed (150 g ha^{-1} fenoxaprop-P and 100 g ha^{-1} sethoxydim), were sufficient to control only homozygous susceptible genotypes (Figure 3-3). To confirm that the dosages selected would only control susceptible genotypes (those not possessing a resistance allele), F_2 populations were screened with single application of both herbicides. In both instances, treatment with either herbicide alone caused F_2 populations to segregate in a 3:1 (R:S) ratio (Table 3-3). When parental flats were treated with single applications of either herbicide or the tank mixture, UM1 plants were unhurt and UM5 plants died. The 3:1 segregation ratios observed for the F_2 populations treated with either fenoxaprop-P or sethoxydim confirm that the dosages employed were sufficient to control only susceptible genotypes and not kill homozygous resistant or heterozygous genotypes.

F_2 flats that were treated with the tank-mixture exhibited two response types, R and S. Segregation patterns for both crosses fit a 3:1 (R:S) model (Table 3-3). Results from individual flats treated with the tank-mixture were tested for homogeneity using a chi-square test, and then pooled. Analysis of the pooled data (190 F_2 plants) yielded a chi-square value of 0.568 and a probability of 0.451 for a 3:1 segregation pattern. None of the tank-mix treated F_2 flats fit a 9:7 segregation model (Table 3-3). These results indicate that APP and CHD resistance in UM1 is controlled by the same single gene alteration, i.e. that a single mutation conferred resistance to both herbicides.

3.4.5 Summary

Results from the fenoxaprop-P inheritance studies illustrate that caution must

ONE GENE SYSTEM

(3:1, Alive:Dead)

ALLELES	R[a]	r
R	RR	Rr
r	Rr	rr

TWO GENE SYSTEM

(9:7, Alive:Dead)

ALLELES	F S[b]	F s	f S	f s
F S	FFSS	FFSs	FfSS	FfSs
F s	FFSs	Ffss	FfSs	Ffss
f S	FfSS	FfSs	ffss	ffss
f s	FfSs	Ffss	ffss	ffss

[a] R = resistant allele for both herbicides, r = susceptible allele for both herbicides.
[b] F = resistant allele for fenoxaprop-P, S = resistant allele for sethoxydim, f = susceptible allele for fenoxaprop-P, s = susceptible allele for sethoxydim.

Figure 3-3. Segregation patterns for single gene and two gene models for F_2 populations treated with a mixture of fenoxaprop-P and sethoxydim (150 g ha^{-1} and 100 g ha^{-1}, respectively). Shaded cells indicate genotypes expected to die when treated.

Table 3-3. Segregation for ACCase inhibitor resistance in the F_2 generation treated with 150 g ha^{-1} fenoxaprop-P, 100 g ha^{-1} sethoxydim or both in a tank mixture application. Chi-square values (X^2) and associated probabilities (P) are the result of tests for goodness of fit to a 3:1 and 9:7 (resistant:susceptible) segregation ratios. Resistant (R) = no injury symptoms; susceptible (S) = dead, 100% necrosis.

F_2 population	Phenotypes			
	R	S	X^2	P
UM5/UM1 (Fenoxaprop-P)	70	27		
(3:1) segregation			0.416	0.519
UM5/UM1 (Sethoxydim)	70	28		
(3:1) segregation			0.670	0.412
Fenoxaprop-P + Sethoxydim				
UM5/UM1	71	22		
(3:1) segregation			0.089	0.765
(9:7) segregation			15.25	<<<0.01
			9	
UM1/UM5	67	30		
(3:1) segregation			1.818	0.178
(9:7) segregation			6.480	0.011
Total	138	52		
(3:1) segregation			0.568	0.451
(9:7) segregation			20.71	<<<0.01
			9	
Homogeneity (3:1)			1.339	0.512
Homogeneity (9:7)			1.020	0.601

be exercised when assessing the gene expression for herbicide resistance traits. Unlike other qualitative characters, herbicide resistance and its expression is highly dependent on the dosage applied (i.e. the intensity of the selection pressure). For this study, both the F_1 and F_2 generations were screened over a range of dosages of fenoxaprop-P to accurately characterize phenotypes. If the F_2 generation had been screened at the recommended field application rate (90 g ha^{-1} fenoxaprop-P), the resistance would appear to be expressed as a fully dominant trait, because the dosage would not be high enough to distinguish homozygous resistant individuals from heterozygotes. At excessive dosages (>800 g ha^{-1}), the results would indicate that UM1 resistance is expressed as a recessive trait, since heterozygous genotypes would give an S response at these extreme dosages, i.e. they would die when treated at these dosages. It is, therefore, imperative that studies on the inheritance of herbicide resistance in weeds are preceded by dose-response experiments including a range of dosages for both parental and F_1 genotypes.

The mechanism of inheritance is one of a number of variables that influence the rate of resistance evolution in a weed population. Mathematical models predicting the rate of evolution over time have been developed which incorporate many of these variables (Gressel and Segel 1990b; Maxwell et al. 1990). However, in considering the mechanism of inheritance and its influence on the evolution of resistance, the expression of resistance under normal environment/selection pressures must not be overlooked. For example, as this study has demonstrated, the recommended field dosage of fenoxaprop-P is much lower than that required to distinguish homozygous

resistant plants from heterozygotes. At this dosage, heterozygotes in the population would not be injured and theoretically would not suffer a subsequent loss in fitness (reduced fecundity) compared to homozygous resistant individuals. Therefore, under normal field selection conditions, populations like UM1 would function as fully dominant, which would increase the rate of resistance evolution.

It is important that the long-term utility of ACCase inhibitor herbicides, which are highly effective and can be used in a variety of crops, be preserved. Based on the functional mode of inheritance for the resistance trait, one would expect that a UM1 genotype would evolve rapidly in the field given successive applications of APP's and CHD's. Growers can minimize the risk of establishing substantial numbers of resistant wild oat plants within field populations by using alternative herbicides with different modes of action in rotation, implementing non-herbicidal control measures, and practising effective sanitation, particularly of harvesting equipment (Gressel and Segel 1990b; Maxwell et al. 1990).

4. TWO DISTINCT ALLELES ENCODE FOR ACETYL-CoA CARBOXYLASE INHIBITOR RESISTANCE IN WILD OAT (*Avena fatua*).[10]

Abstract. The objectives of this study were to determine the inheritance of aryloxyphenoxypropionate (APP) resistance in the wild oat population UM33 and to determine the genetic relationship between resistance in UM33 and another population, UM1, which has a different cross-resistance pattern. Reciprocal crosses were made between UM33 and a susceptible population UM5, and between UM33 and UM1. Initial screenings of F_1 and F_2 populations derived from crosses between UM33 and UM5 were conducted over a range of fenoxaprop-P rates to determine a discriminatory dosage. F_2 populations and F_2-derived F_3 families were then screened at this dosage (1200 g ai ha^{-1}) to determine segregation patterns. Results from reciprocal UM33/UM5 F_1 dose-response experiments, and F_2 and F_2-derived F_3 segregation experiments indicated that UM33 resistance to fenoxaprop-P was governed by a single, partially dominant nuclear gene system. To determine if resistance in UM1 and UM33 results from alterations at the same gene locus, 584 F_2 plants derived from reciprocal UM33/UM1 crosses were screened with 150 g ha^{-1} fenoxaprop-P. This dosage was sufficient to kill susceptible plants (UM5), but was not sufficient to kill plants with a resistance allele from either parent. None of the treated F_2 plants exhibited injury or death, indicating that UM1 and UM33 resistance genes did not segregate

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independently. From this it was concluded that resistance in both populations is encoded at the same gene locus. Nomenclature: Fenoxaprop-p-ethyl, (±)-ethyl 2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoate; wild oat, *Avena fatua* L. #[11] AVEFA.

Additional index words. Fenoxaprop-p-ethyl, aryloxyphenoxypropionate, cyclohexanedione, ACCase inhibitor, herbicide resistance, weed genetics, AVEFA.

4.1 INTRODUCTION

In western Canada, wild oat populations have evolved resistance to the aryloxyphenoxypropionate (APP)[12] and cyclohexanedione (CHD)[12] herbicides, commonly referred to as the acetyl-CoA carboxylase (ACCase)[12] inhibitors (Morrison et al. 1992; Morrison and Devine 1994). Dose-response experiments indicate that many of these resistant populations differ significantly in levels of resistance and patterns of cross-resistance to these herbicides (Heap et al. 1993; Morrison and Bourgeois 1995). Two of the wild oat field populations collected in Manitoba with distinctive cross-resistance patterns are UM1 and UM33 (Heap et al. 1993). UM1 is resistant to both APP and CHD herbicides, whereas UM33 is resistant

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[11]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds Revised 1989. Available from WSSA, 309 W. Clark St.,Champaign, IL 61820.

[12]Abbreviations: ACCase, acetyl-CoA carboxylase; APP, aryloxyphenoxypropionate; CHD, cyclohexanedione; ALS, acetolactate synthase; DAT, days after treatment; R/S, resistant to susceptible ratios based on GR_{50} values; R, resistant; I, intermediate; S, susceptible.

to only the APP herbicides (Heap et al. 1993). Such differences can be taken as evidence that these populations evolved independently and that different mutations, either at the same or different loci, encode for resistance (Morrison et al. 1992).

The inheritance of ACCase inhibitor resistance has been studied in relatively few plant species: maize (*Zea mays* L.), Italian ryegrass (*Lolium multiflorum* Lam.), winter wild oat (*Avena sterilis* L. ssp. *ludoviciana*) and wild oat (UM1) (Parker et al. 1990; Barr et al. 1992; Betts et al. 1992; Murray et al. 1994). In each instance, resistance was governed by a single, partially dominant, nuclear gene. Likewise, in each instance, physiological studies indicated that resistance was due to an altered site of action, i.e. ACCase (Parker et al. 1990; Barr et al. 1992; Betts et al. 1992; Marles and Devine 1995).

A comparable situation exists for acetolactate synthase (ALS)[12] inhibitor resistant weeds. For example, limited dose-response studies indicate that cross-resistance patterns to sulfonylurea and imidazolinone herbicides vary among populations (Saari et al. 1994). Inheritance studies with ALS inhibitor resistant populations of prickly lettuce (*Lactuca serriola* L.) and kochia (*Kochia scoparia* L.) indicated that resistance was governed by single, nuclear partially dominant, and dominant gene alterations, respectively (Mallory-Smith et al. 1990; Mulugeta et al. 1991). Although ALS inhibitor resistant mutants of rigid ryegrass (*Lolium rigidum* Gaud.) with enhanced metabolism have been identified, ALS inhibitor resistance in most weed populations was associated with modified target site (ALS) (Saari et al. 1994). Specific mutations encoding ALS inhibitor resistance in bacteria, yeast and

higher plants have been identified. Molecular genetic studies indicated that a number of mutations at various locations along the DNA sequence encoding for the ALS enzyme result in resistance (Saari et al. 1994). In kochia, several different mutations in the gene encoding for acetolactate synthase have been identified in resistant populations (Guttieri et al. 1995).

The objectives of the present study were: 1) to determine the mode of inheritance of APP resistance in the wild oat population UM33; and 2) using classical breeding techniques determine whether or not the mutations conferring resistance in UM33 and another resistant population UM1 are located at the same or separate gene loci.

4.2 MATERIALS AND METHODS

4.2.1 UM33 inheritance studies.

Parental populations. The two parental wild oat populations were UM33 and UM5. UM33 is resistant to the APP herbicides diclofop-methyl [(±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid, methyl ester] (R/S^{12}>20), and fenoxaprop-P (R/S>74), but is susceptible to the CHD herbicide sethoxydim, [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one] (Heap et al. 1993). In contrast, UM5 is susceptible to both APP and CHD herbicides. To ensure homozygosity for resistance in UM33 and susceptibility in UM5, single seeds from each population were selected, selfed and screened for two generations prior to genetic analysis. Selfing was ensured by enclosing panicles within glassine bags prior to

anther dehiscence. Except where noted, the growing conditions, generation development, and screening procedures for all controlled environment studies were identical to those previously described by Murray et al. (1994). The UM33 inheritance studies conducted in the controlled environments involved evaluations of reciprocal crosses for all three generations. F_2 field evaluations were carried out with the UM5/UM33 cross only.

F_1 dose-response experiment. Three pre-germinated seeds were planted in 9 cm-diameter pots for the parental and UM33/UM5 F_1 populations, whereas two pre-germinated seeds of the UM5/UM33 F_1 population were planted per pot. The experimental design was completely randomized, with three replicates (pots) for the UM33/UM5 F_1 and the parental populations, and two replicates for the UM5/UM33 F_1 population. Limited success in the production of UM5/UM33 F_1 seed accounted for the reduced number of seeds per pot and number of replicates used for this cross. Replicates and treatments within replicates were re-randomized weekly to ensure uniformity of growth.

F_1 and parental plants were treated with commercial formulations of fenoxaprop-P at dosages comparable to those used by Heap et al (1993); F_1 hybrids and UM33 were treated at 0, 20, 55, 150, 400, 1100, 3000 and 8100 g ai ha^{-1}, whereas UM5 plants were treated at 0, 3, 7, 20, 55, 150, 400 and 1100 g ai ha^{-1}. Reciprocal F_1 and parental genotypes were screened over a range of dosages to give an initial indication of the gene expression type (i.e. dominant or recessive), and location (nuclear vs. cytoplasmic), and to help select an appropriate range of dosages for

further F_2 and F_3 family segregation experiments.

F_2 segregation experiments. F_2 populations were screened in two steps as previously described by Murray et al. (1994). The first determined the appropriate discriminatory dosage of fenoxaprop-P. The second step involved treating F_2 plants at the selected dosage. In the first step, 90 F_2 plants, 5 UM33 and 4 UM5 plants were planted into 46 by 56 by 14 cm wooden flats. In the second step 99 F_2 plants were sown into each flat, with parental genotypes included in separate flats (99 plants of each parental genotype per flat).

In the first step, one flat of each cross was treated at each of the following dosages: 0, 600, 800, 1000, 1200 and 1400 g ha^{-1} of fenoxaprop-P, and placed in a controlled environment chamber as previously described (Murray et al. 1994). Twenty-one days after treatment (DAT)[12], flats were removed from the controlled environment chamber and individual plants were characterized based on visual appearance. Three response types were identified: resistant (R)[12], intermediate (I)[12], and susceptible (S)[12]. R plants exhibited no injury symptoms. I plants recovered from herbicide treatment but were stunted and had necrotic and chlorotic areas on the foliage. S plants were entirely necrotic and obviously dead.

Based on visual observations, the dosage of fenoxaprop-P that gave the best discrimination between response types was 1200 g ha^{-1}. In the second step, three flats of each cross and one flat of each parental genotype were treated at this dosage. Additionally, one flat of each F_2 cross and parent were included as untreated controls.

Segregation patterns were determined based on response types.

F_3 family segregation experiment. Two hundred untreated F_2 plants were selected at random to produce F_2-derived F_3 families. In total, 195 F_2 plants survived to maturity and produced selfed F_3 families (100 UM5/UM33 and 95 UM33/UM5).

F_3 seeds were sown in flats, with each flat containing 5 families (two rows per family, each with 9 plants) and one row (9 plants) of either UM33 or UM5 parental plants. F_3 families were treated with 1200 g ha^{-1} fenoxaprop-P. Twenty-one DAT each family was characterized as being homozygous R, segregating, or homozygous S. F_3 families displaying only R responses were characterized as being homozygous resistant, whereas segregating F_3 families exhibited all three response types. Homozygous S F_3 families were entirely dead. Segregation patterns for the F_3 families were determined based on these responses.

Field F_2 segregation analysis. Field plots were established at Portage La Prairie, Manitoba, to determine the expression of resistance in UM33 under typical field growing conditions. The soil was a Neuhorst clay loam, [Edenburg (Udic Haploboroll) series], with 7.5% organic matter and a Ph of 7.4. Pre-germinated seeds were hand planted into 2 m^2 plots on June 3, 1993. Each plot consisted of four, 2 m rows (50 seeds per row), with seeds planted at a depth of 2.5 cm. Three F_2 plots (200 UM5/UM33 F_2 plants) and three parental plots (100 UM33 and 100 UM5 plants) were planted .

All genotypes emerged within 5 d. At this time, non-vigorous plants were removed and the total number of plants per plot was recorded. When the plants

reached the three leaf stage, individual F_2 and parental plots were treated with one of the following dosages of a commercial formulation of fenoxaprop-P: 0, 100, and 1200 g ha^{-1}. Treatments were applied using a bicycle sprayer that delivered 105 L ha^{-1} of spray solution at 275 Kpa. Plants were characterized 21 DAT according to their response. F_2 segregation patterns were determined based on response types as previously described.

4.2.2 UM1/UM33 F_2 segregation analysis.

Parental populations. To ensure homozygosity for resistance, single seeds from the resistant populations, UM1 and UM33 were selected, self pollinated and screened for two generations prior to genetic analysis. Reciprocal F_1 hybrids were produced by crossing the selfed lines, and F_2 seed was produced for segregation analysis.

F_2 segregation experiment. F_2 seeds from reciprocal crosses were planted into 46 by 56 by 14 cm wooden flats at a rate of 99 seeds per flat. Both parental populations (36 plants of each) and a known susceptible wild oat population (UM5) (27 plants) were planted into separate flats. Three flats of each F_2 cross and one flat containing parental lines were treated with 150 g ha^{-1} of fenoxaprop-P, a dosage sufficient to kill susceptible genotypes.

Twenty-one DAT, plants were characterized based on visual appearance. F_2 segregation patterns were determined based on response types as previously described.

4.2.3 Statistical analysis.

Dose-response curves generated in F_1 screenings for the UM33 genetics study were fitted to a logistic model described by Brain and Cousens (1989). This model

has utility in comparative bioassay experiments and is useful in describing dose-response data (Streibig 1980; Brain and Cousens 1989). The model is

$$y = k/(1+e^{bg}x^{b})+d$$

where y is the dependent variable, mean shoot dry matter per plant expressed as % of control, x is the herbicide dosage, d is the lower asymptote, $k + d$ is the upper asymptote, e is the base of the natural logarithm and b and g determine the slope of the inflection region of the curve. Regression analysis was performed using SAS[13].

Chi-square analysis was used to determine the best fit for F_2 and F_3 segregation ratios for all studies based on F_2 plant response types and F_3 family responses (Steele and Torrie 1980). Homogeneity chi-square tests were conducted to determine if F_2 and F_3 segregation data could be pooled both within crosses and between crosses for each experiment (Strickberger 1976).

4.3 RESULTS AND DISCUSSION

4.3.1 UM33 inheritance studies.

F_1 dose-response experiment. The responses of UM5/UM33 and UM33/UM5 F_1 hybrids to increasing dosages of fenoxaprop-P were intermediate to those of the parents (Figure 4-1, Table 4-1). The fact that both F_1 hybrids exhibited an intermediate response to the parental genotypes indicates that APP resistance could be inherited as a partially dominant, nuclear trait (Murray et al. 1994).

The F_1 dose-response results were useful in determining the range of dosages

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[13]SAS Version 5. 1985. SAS Inst. Inc., Box 8000, Cary, NC 27511-8000.

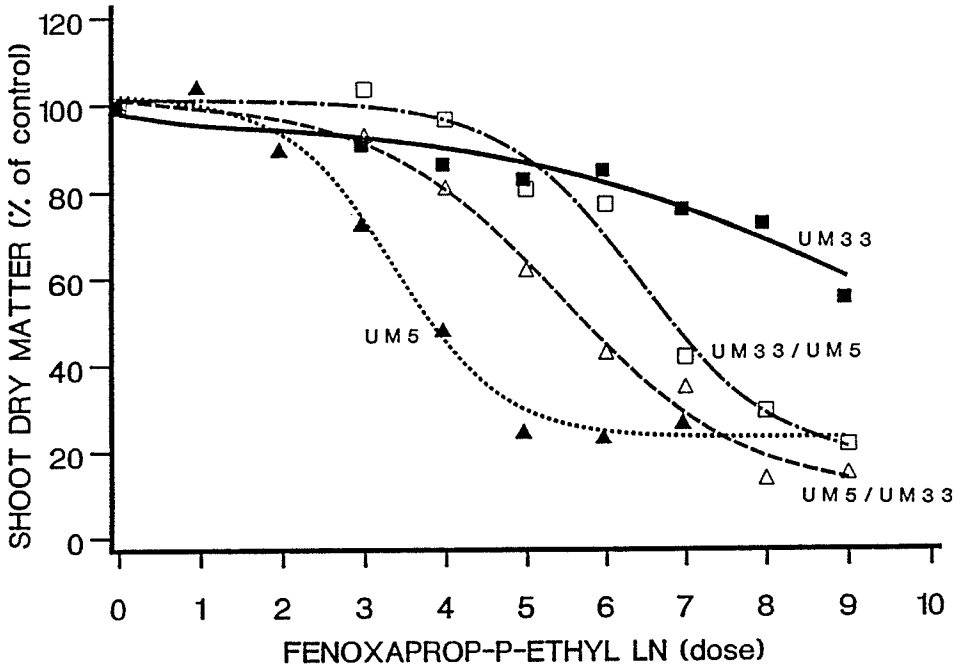

Figure 4-1. Response of parental and F_1 genotypes to increasing dosages of fenoxaprop-P. Refer to Table 4-1 for parameter estimates, GR_{50} values and R/S ratios.

Table 4-1. Parameter estimates (standard errors in parenthesis) describing the response of resistant (UM33), susceptible (UM5) and F_1 (UM5/UM33, UM33/UM5) plants treated with fenoxaprop-P under growth room conditions.

Genotype	*g*	*b*	*d*	*k*	R^2	Shoot growth GR_{50}	GR_{50} ratio R/S
UM33 (R)	-10.2	0.4	*na*[a]	98.0	0.94	>8100	>277
	(0.5)	(0.1)	*na*	(3.5)			
UM5 (S)	-3.4	1.5	23.2	78.8	0.99	29	*na*
	(0.2)	(0.3)	(3.5)	(5.4)			
UM5/UM33 (F_1)	-5.5	0.9	9.4	91.7	0.99	233	8
	(0.3)	(0.2)	(6.2)	(8.4)			
UM33/UM5 (F_1)	-6.5	1.2	16.5	84.7	0.98	637	22
	(0.3)	(0.4)	(9.4)	(11.5)			

[a]not applicable.

required for the first step of the F_2 screening experiment. At dosages of 400 and 1100 g ha^{-1} it was possible to visually distinguish F_1 plants from either parent. At these dosages UM33 plants were not visibly affected, whereas F_1 plants were visibly injured but still living, and UM5 plants were dead.

Growthroom F_2 segregation experiments. Results from the first step in the F_2 segregation experiments indicated that 1200 g ha^{-1} fenoxaprop-P provided the best discrimination between response types (data not shown). At this dosage, R, I and S phenotypes could be identified among the F_2 progeny. At dosages lower than 1200 g ha^{-1} it was difficult to separate R from I responses, since I plants exhibited little injury. When treated with 1400 g ha^{-1}, UM33 plants were injured and therefore it was not possible to separate I from R responses. At a discriminatory dosage of 1200 g ha^{-1} fenoxaprop-P, UM33 plants were unaffected (R), whereas UM5 plants were dead (S).

Only 1200 g ha^{-1} fenoxaprop-P was applied in the second step of the F_2 screening. Segregation patterns, based on F_2 plant response types, were determined by visual assessment of plants treated with 1200 g ha^{-1} in both F_2 screening steps. In every instance, F_2 populations segregated in a 1:2:1 (R:I:S) pattern, which indicated that resistance is governed by a single, partially dominant nuclear gene (Table 4-2). Results from individual flats were tested for homogeneity using a chi-square test, and in all instances the data could be pooled, both within and between crosses. Analysis of the pooled data over all flats (694 plants) yielded a chi-square value of 0.26 and an associated probability of 0.88 for a 1:2:1 segregation pattern (Table 4-2). The results from the F_2 segregation analysis support the conclusions drawn from the F_1 dose-

Table 4-2. Segregation for ACCase inhibitor resistance in the F_2 generation treated with 1200 g ha^{-1} fenoxaprop-P. Chi-square values (X^2) and associated probabilities (P) are the result of tests for goodness of fit to a 1:2:1 (resistant:intermediate:susceptible) segregation ratio. Resistant (R) = no injury symptoms; intermediate (I) = stunted plant with some chlorotic and necrotic foliage; susceptible (S) = dead, 100% necrosis.

F_2 population	Phenotype				
	R	I	S	X^2	P
UM33 / UM5	—	no. of plants	—		
Step 1, Flat 1[a]	17	49	22	1.70	0.43
Step 2, Flat 1	23	36	27	2.65	0.27
Step 2, Flat 2	23	41	21	0.20	0.91
Step 2, Flat 3	19	37	26	1.98	0.37
Total	82	163	96	1.81	0.41
				4.72	0.58
Homogeneity					
UM5 / UM33					
Step 1, Flat 1	20	50	20	1.11	0.57
Step 2, Flat 1	21	45	23	0.10	0.95
Step 2, Flat 2	25	45	18	1.16	0.56
Step 2, Flat 3	20	46	20	0.42	0.81
Total	86	186	81	1.16	0.56
				1.63	0.95
Homogeneity					
Total (overall)	168	349	177	0.26	0.88
				9.01	0.83
Homogeneity					

[a]Step no., Flat no. = F_2 experimental step and flat number.

response experiment, and further indicate that resistance to fenoxaprop-P is controlled by one gene.

F_3 family segregation experiment. F_3 families were also treated with a discriminatory dosage of 1200 g ha^{-1} fenoxaprop-P. Segregation patterns based on family responses were determined by visual assessment of the 18 progeny in each family. F_3 families originating from both crosses segregated in a 1:2:1 (homozygous R:segregating R,I, and S:homozygous S) pattern (Table 4-3). Results were tested for homogeneity using a chi-square test, and the data were pooled over crosses. Analysis of the pooled data (195 F_3 families) yielded a chi-square value of 0.63 and an associated probability of 0.73 for a 1:2:1 segregation pattern (Table 4-3). These results supported the conclusions drawn from F_1 dose-response and F_2 segregation experiments, and provided conclusive evidence that resistance in UM33 is governed by a single, partially dominant, nuclear gene.

Field F_2 segregation experiment. Although the above studies indicated that resistance was governed by a single, partially dominant, nuclear gene, it did not follow that expression would be the same in the field. The recommended field application dose for fenoxaprop-P ranges from 90 to 100 g ha^{-1} [14] depending on the commercial product. This dosage is much lower than that required to visually distinguish heterozygotes from homozygous resistant genotypes (Figure 4-1).

When treated with the recommended dosage of fenoxaprop-P (100 g ha^{-1}), the

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[14]Guide to Chemical Weed Control, Manitoba Agriculture, Box 1149, Carman Manitoba, R0G- 0J0.

Table 4-3. Segregation for ACCase inhibitor resistance in the F_3 generation treated with 1200 g ha^{-1} fenoxaprop-P. Chi-square values (X^2) and associated probabilities (P) are the result of tests for goodness of fit to a 1:2:1 (homozygous R:segregating R,I,S:homozygous S) segregation ratio. Homozygous R = all F_3 progeny in family display R response; segregating = F_3 progeny display all three response types; homozygous S = all F_3 progeny in family display S response.

Reciprocal	F_3 Family response			X^2	P
	Homozygous R	Segregating R,I,S	Homozygous S		
	—	no. of families	—		
UM5 / UM33	24	51	25	0.06	0.97
UM33 / UM5	20	49	26	0.85	0.65
Total	44	100	51	0.63	0.73
Homogeneity				0.28	0.87

UM5/UM33 F_2 population segregated in a 3:1 (R:S) segregation pattern (Table 4-4). At this dosage only two phenotypes (R & S) were evident. When treated at the discriminatory dosage (1200 g ha^{-1}), F_2 populations segregated in a 1:2:1 (R, I, S) pattern. When treated with either dosage of fenoxaprop-P UM33 plants were unaffected and UM5 plants died.

The recommended dosage (100 g ha^{-1}) was not high enough to exert an adequate selection pressure on the heterozygous genotypes for visual separation from homozygous resistant genotypes. In this instance, heterozygous individuals appeared to be as fit as homozygous resistant individuals. Only when an excessive dosage (1200 g ai ha^{-1}) was applied was there an observable selective advantage for the homozygous R genotypes over the heterozygotes. Consequently, when fenoxaprop-P is applied to populations with resistant genotypes like UM33 in the field, resistance evolution would be rapid since heterozygous genotypes will respond as 'functionally' dominant individuals (Georghiou and Taylor 1977; Maxwell et el. 1990; Jasieniuk et al. 1996).

4.3.2 UM1/UM33 F_2 segregation analysis

In order to ensure identification of potential homozygous susceptible progeny, a dosage of 150 g ha^{-1} fenoxaprop-P was used in the UM1/UM33 F_2 segregation analysis. Based on previous dose-response experiments, this dosage was sufficient to control only those genotypes that did not possess at least one resistance allele from either UM1 (15) or UM33 (Figure 4-1). According to standard genetic hypotheses, if resistance in the UM1 and UM33 populations is due to an alteration of the same gene

Table 4-4. Segregation for ACCase inhibitor resistance in the F_2 generation of field grown plants treated with either 100 or 1200 g ha^{-1} fenoxaprop-P. Chi-square values (X^2) and associated probabilities (P) are the result of tests for goodness of fit to a 3:1 (resistant:susceptible) segregation ratio for plants treated with 100 g ha^{-1} or a 1:2:1 (resistant:intermediate:susceptible) segregation ratio for plants treated with 1200 g ha^{-1} fenoxaprop-P . Resistant (R) = no injury symptoms; intermediate (I) = stunted plant with some chlorotic and necrotic foliage; susceptible (S) = dead, 100% necrosis.

Fenoxaprop-P Dosage	Phenotype				
	R	I	S	X^2	P
	—	no. of plants	—		
100 g ha^{-1}					
UM5 / UM33	104	*na*[a]	30	0.43	0.51
1200 g ha^{-1}					
UM5 / UM33	33	72	31	0.53	0.77

[a]not applicable.

locus, then treatment of the F_2 populations with this dosage would produce only R responses (Figure 4-2). If resistance in the two populations is due to alterations at two independently segregating, partially dominant or dominant gene loci, then the treated F_2 populations would segregate in a 15:1 (R:S) pattern. In this case homozygous susceptible genotypes would be the only plants killed (Figure 4-2).

A total of 584 F_2 plants were screened, and in every instance only R responses were observed (Table 4-5). UM1 and UM33 parental plants were unaffected, whereas UM5 plants were killed. The fact that none of the F_2 plants treated with fenoxaprop-P died, provides strong evidence that resistance in both UM1 and UM33 result from alterations at the same gene locus. The probability of finding at least one susceptible recombinant out of 584 plants if the loci were further than 10 centimorgans apart is 97.4% (Strickberger 1976).

These results suggest that the distinct APP and CHD cross-resistance patterns observed in the UM1 and UM33 populations are due to different mutations at the same gene locus. APP and CHD resistance in UM1 is now believed to be due to a mutation in the target site, (i.e. ACCase) (Morrison and Devine 1994). Therefore APP resistance in UM33 is also probably due to an alteration in this target site enzyme, albeit from a different mutation. Gene sequencing would be required to confirm this.

4.3.3 Summary

The results indicate that development of ACCase inhibitor resistance may occur rapidly. With more than one functional mutation encoding for resistance, there will be an increase in the rate of resistance development. This is because the probability of

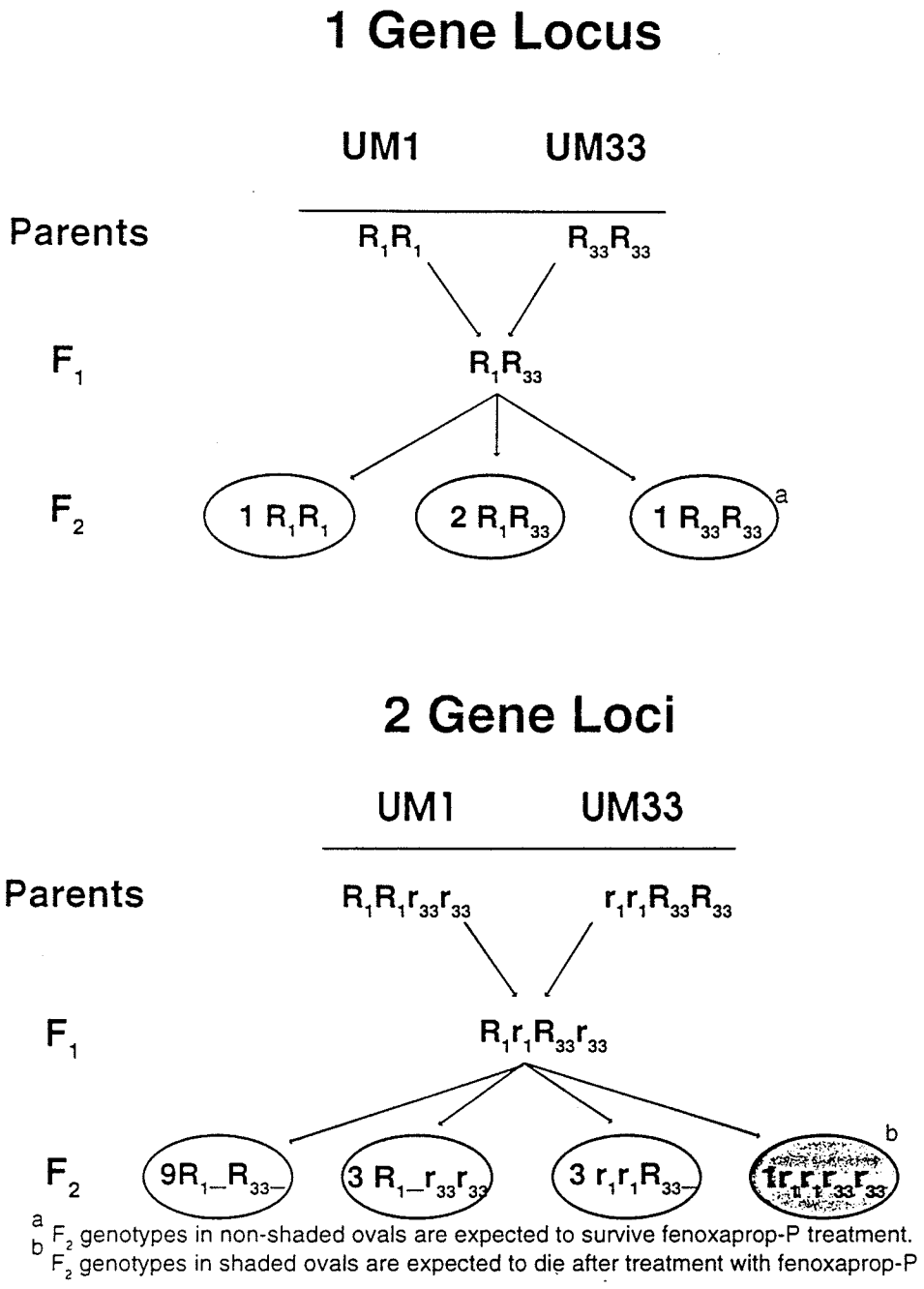

Figure 4-2. F_2 segregation patterns for one and two gene loci systems treated with 150 g ha^{-1} fenoxaprop-P. R = resistance allele, r = susceptible allele. Subscript numbers indicate origin of resistance.

Table 4-5. Segregation of ACCase inhibitor resistance in the F_2 generation treated with 150 g ha^{-1} fenoxaprop-P. Resistant (R) = no injury symptoms; susceptible (S) = dead, 100% necrosis.

F_2 population	Phenotype		
	R		S
UM1 / UM33	—	no. of plants	—
Flat 1	99		0
Flat 2	97		0
Flat 3	97		0
Total	293		0
UM33 / UM1			
Flat 1	98		0
Flat 2	97		0
Flat 3	96		0
Total	291		0
Total (overall)	584		0

finding an initial resistant mutant in an unselected population increases with an increase in the number of types of functional mutations (Jasieniuk et al. 1995). The fact that numerous cross-resistance patterns to APP and CHD herbicides have been identified in western Canadian wild oat populations suggests that many different resistance mutations exist (Morrison and Bourgeois 1995).

In addition, the rate of resistance development for weed populations possessing mutations similar to those of UM1 and UM33 should be rapid due to the fact that resistance is encoded by a single, functionally dominant, nuclear gene (Georghiou and Taylor 1977; Maxwell et al. 1990; Jasieniuk et al. 1996). With this type of inheritance, resistance can evolve rapidly because of the relative ease with which resistance alleles can become established within a population, and because resistance alleles are not lost in the heterozygous genotypes when the herbicide is applied at recommended field dosages.

5. SEED BIOASSAY TO IDENTIFY ACETYL-CoA CARBOXYLASE INHIBITOR RESISTANT WILD OAT (*Avena fatua*) POPULATIONS.[15]

Abstract. A seed bioassay was developed and tested for the rapid identification of aryloxyphenoxypropionate (APP) and cyclohexanedione (CHD) resistance in wild oat. Two susceptible (S) genotypes, UM5 and Dumont, were treated with fenoxaprop-P and sethoxydim over a range of dosages on filter paper and agar. The former is a wild oat line and the latter a tame oat cultivar. Within 5 days, shoot and root development of both genotypes were completely inhibited by 10 µM fenoxaprop-P and 5 µM sethoxydim. These dosages were then tested to determine if they were suitable for distinguishing between resistant (R) and susceptible (S) plants. Agar medium was preferred over filter paper because of the ease of preparation and maintenance. Four known R wild oat populations were included in the tests. Those with high levels of resistance produced significantly longer coleoptiles and roots than S genotypes, but those with moderate or low levels of resistance could not be statistically separated from S biotypes based on quantitative measurements. However, after exposing the germinating, treated seeds to light for 24 to 48 h, all the R populations produced green coleoptiles and initiated a first leaf, unlike the S genotypes which did not turn green or produce any new growth. This procedure proved useful in discriminating between R and S genotypes and in ranking populations in terms of relative levels of resistance.

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[15]This chapter was published as the following: Murray, B. G., L. F. Friesen, K. J. Beaulieu, and I. N. Morrison. 1996. A seed bioassay to identify acetyl-CoA carboxylase inhibitor resistant wild oat (*Avena fatua*) populations. Weed Technol. 10:85-89.

Nomenclature: Fenoxaprop-p-ethyl, (R)-2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid, ethyl ester; sethoxydim, 2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; wild oat, *Avena fatua* L. #[16] AVEFA; oat, *Avena sativa* L. # AVESA.

Additional index words: ACCase inhibitor, herbicide resistance, fenoxaprop-p-ethyl, sethoxydim, aryloxyphenoxypropionate, cyclohexanedione, AVEFA.

5.1 INTRODUCTION

Resistance to the aryloxyphenoxypropionate (APP)[17] and cyclohexanedione (CHD)[17] herbicides has now been reported in at least ten weed species (Heap and Morrison 1996). In western Canada both wild oat and green foxtail [*Setaria viridis* (L) Beauv.] populations have evolved resistance to these herbicides (Morrison and Bourgeois 1995). Initial whole plant, dose-response experiments conducted with wild oat in both the growth-room and the field indicated that R[17] populations were highly variable in levels of resistance and patterns of cross resistance to several APP and CHD herbicides (Heap et al. 1993; Morrison and Bourgeois 1995).

To date, the identification of resistance to APPs and CHDs, also referred to as

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[16]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 1508 W. University Ave., Champaign, IL 61821-3133.

[17]Abbreviations: ACCase, acetyl-CoA carboxylase; APP, aryloxyphenoxypropionate; CHD, cyclohexanedione; GR_{50}, herbicide dosage required to inhibit plant growth by 50% relative to untreated controls; R, resistant; R/S, resistant to susceptible ratio, based on GR_{50} values; S, susceptible.

acetyl-CoA carboxylase (ACCase)[17] inhibitors, in wild oat has involved applying post emergence applications of herbicides to plants growing in pots under controlled environment conditions. Although this method is reliable, it is expensive in terms of infra-structure costs and labour requirements. Furthermore it takes approximately 6 weeks to complete. Thus, there is a need for a rapid, inexpensive, accurate technique to identify resistance in suspect populations.

Seed bioassays are comparatively quick and inexpensive. Such bioassays are particularly useful for routine screening of large numbers of suspected resistant populations (Heap 1994). Beckie et al. (1990) described a petri dish bioassay to identify trifluralin (2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoro-methyl)benzenamine) resistant green foxtail based on initial differences in seedling growth between R and S populations. The objectives of this research were to develop a similar assay system for ACCase inhibitor resistant wild oat by characterizing the response of two standard S genotypes to increasing dosages of fenoxaprop-P and sethoxydim, and confirming the utility of the procedure by testing appropriate concentrations of each herbicide on several known R populations.

5.2 MATERIALS AND METHODS

5.2.1 Dose-response experiments.

Two genotypes were selected for the dose-response studies; UM5, a wild oat line and Dumont, a Canadian domesticated oat cultivar. Both UM5 and Dumont are susceptible to a wide range of ACCase inhibitors registered for wild oat control in

western Canada, including fenoxaprop-P and sethoxydim. The origin of UM5 was described by Heap et al. (1993).

Dose-response experiments were conducted using 13.5 by 12.5 by 3.5 cm deep, plexiglass germination boxes in a randomized design with two replications. An individual germination box containing 30 seeds was considered a treatment unit. Prior to placing the seeds in germination boxes, seeds were dehulled by hand and soaked in distilled water for 12-18 h in sealed glass vials at 5° C in the dark. Dehulling and soaking the seed resulted in more uniform germination, particularly for wild oat (data not shown).

Filter paper procedure. In separate experiments, 8-ml aliquots of aqueous emulsions of commercially formulated fenoxaprop-P (an APP herbicide) and sethoxydim (a CHD herbicide) were applied over a range of dosages to two sheets of filter paper[18] lining the bottoms of the germination boxes. The dosages used were 0, 0.031, 0.063, 0.125, 0.25, 0.5, 1, 2, 3, 5, 10, 20, and 40 µM. Imbibed seeds were then placed on the paper. Up to 2 ml of additional herbicide emulsion was added daily to maintain adequate moisture levels. The filter paper remained moist, but with little free liquid. The lids on the germination boxes were not sealed to allow for gas exchange to avoid an anaerobic environment.

Germination boxes were placed in the dark in a germination cabinet set at a constant 28° C. Humidity levels in the cabinet were not controlled; however, open basins of water were placed in the bottom of the cabinet to maintain a high humidity.

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[18]Whatman #1, Whatman Int. Ltd., Maidstone, U.K.

After five days, the coleoptiles and total root length (seminal and radicle roots) per seed were measured. Dose-response experiments were carried out twice for each herbicide.

Agar procedure. Experiments similar to those described above, were also conducted using agar media. Procedures, herbicide treatment, and germination conditions were the same as described, with the following modifications. Medium was prepared by heating distilled water to 70 C, and then adding agar[19]. Agar was mixed at a concentration of 8 g/L of distilled water. The agar suspension was further heated to boiling and then removed from the heat. The herbicides were mixed into the viscous agar solution after it had cooled to a temperature of 45 C, and a layer 1.5 to 2 cm deep was immediately poured into the bottom of the germination boxes. The agar was allowed to solidify at room temperature. Imbibed seeds were placed on top of the agar gel. During the 5-day germination period, no additional herbicide or water was added to the media.

5.2.2 Verification of bioassay.

Four R wild oat populations (UM1, UM2, UM3, and UM33) that had been characterized previously in dose-response experiments conducted in a growth-room (Heap et al. 1993) were selected to verify the utility of the bioassay procedure. The two susceptible genotypes, UM5 and Dumont, also were included. Based on the results of the dose-response experiments, a single discriminating dosage of each herbicide that inhibited both coleoptile and root development of the susceptible lines was selected

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[19]Gum agar. Cat. no. 7002, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO.

(10 µM for fenoxaprop-P and 5 µM for sethoxydim). Also based on the results of the dose-response experiments and ease of maintenance, only agar medium was used. Ten seeds of each biotype were placed into three separate germination boxes (untreated control, 10 µM fenoxaprop-P, and 5 µM sethoxydim). Treatments were replicated four times. Other conditions and procedures were the same as those described previously for the dose-response experiments.

After the coleoptiles and roots were measured, the germination boxes were transferred to an east facing window ledge at room temperature (18-22 C) for 2 days. Seedlings were then visually assessed by three different individuals and classified as alive or dead based on the presence or absence of a green first leaf.

5.2.3 Statistical procedures.

A sigmoidal model (Brain and Cousens 1989) was fitted to the dose-response data using a derivative-free nonlinear regression procedure (Freund and Littell 1986). The model fitted was

$$y = k/(1+e^{bg}x^{b})+d$$

where y is the dependent variable [mean coleoptile or mean root length (mm)], x is the herbicide dosage (µM), e is the base of the natural logarithm, k is the difference between the upper and lower asymptotes (i.e. $k + d$ is the upper asymptote), d is the lower asymptote, and b and g determine the shape of the curve. GR_{50}[3] values were calculated as the negative antilog of g [g = -log e (GR_{50})] . Parameter estimates were considered to be statistically significant at the 0.05 level where the standard error was less than half the numerical value of the estimate (Koutsoyiannis 1977). The standard

error of a parameter is a measure of confidence and if it is large the parameter is poorly estimated. Additionally, to test for significant differences between main effects of genotype, media, and dosage and their interactions the data were analyzed by analysis of variance (ANOVA) using SAS[20] .

Data from the verification experiment were analyzed by ANOVA and means were separated using Fisher's Protected LSD test at the 0.05 level of significance (Gomez and Gomez 1984).

5.3 RESULTS AND DISCUSSION

5.3.1 Dose-response studies.

The responses of the two susceptible genotypes, UM5 and Dumont, to increasing dosages of fenoxaprop-P and sethoxydim were comparable (Figure 5-1, Tables 5-1 and 5-2). The calculated GR_{50} values indicate that coleoptiles were less sensitive to either herbicide than the roots (Tables 5-1 and 5-2). GR_{50} values for the coleoptiles ranged from 13- to 250- fold higher than corresponding GR_{50} values for the roots.

In all instances, increasing dosages of fenoxaprop-P or sethoxydim significantly reduced coleoptile and root lengths for both UM5 and Dumont. The main effects of media and genotype were significant for both coleoptile and root lengths for fenoxaprop-P and for root length for sethoxydim. Coleoptile lengths did not differ significantly between genotypes when either genotype was treated with sethoxydim on

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[20]SAS Version 5. 1985. SAS Inst. Inc., Box 8000, Cary, NC 27511-8000.

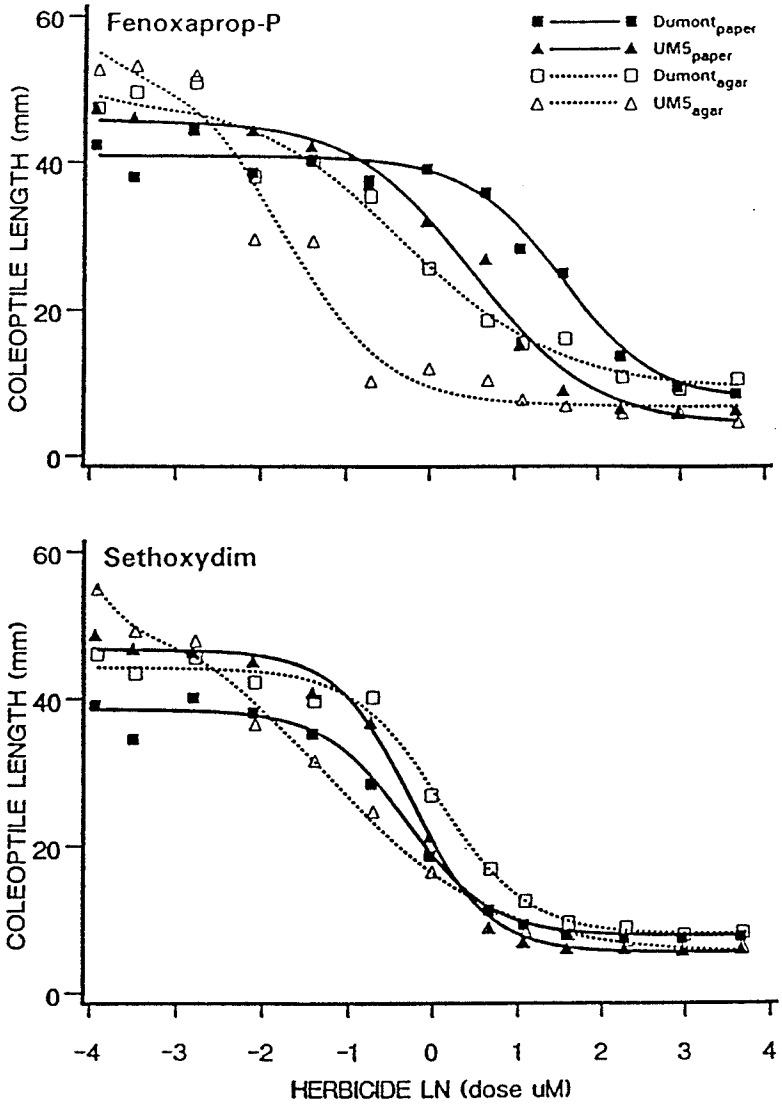

Figure 5-1. Coleoptile growth of Dumont tame oat and UM5 wild oat as influenced by herbicide concentration and media. Refer to Tables 5-1 and 5-2 for parameter estimates.

Table 5-1. Parameter estimates[a] (standard errors in parentheses) describing the growth of UM5 and Dumont seedlings on filter paper or agar media treated with fenoxaprop-P.

	Media	*g*	*b*	*d*	*k*	R^2	GR_{50}
Root length							(μM)
UM5	Paper	3.0	1.3	4.2	155.9	0.99	0.050
		(0.1)	(0.2)	(2.4)	(6.2)		
	Agar	6.7	0.4	0.8	125.9	0.99	0.001
		(0.7)	(0.1)	(3.1)	(3.8)		
Dumont	Paper	2.2	1.2	10.9	161.2	0.98	0.111
		(0.2)	(0.2)	(5.0)	(11.8)		
	Agar	5.7	0.3	-0.2	174.4	0.99	0.003
		(0.4)	(0.1)	(5.5)	(6.4)		
Coleoptile length							
UM5	Paper	-0.5	1.4	4.4	41.3	0.99	1.65
		(0.1)	(0.2)	(1.8)	(2.3)		
	Agar	1.8	1.6	6.8	48.2	0.97	0.17
		(0.2)	(0.4)	(1.8)	(4.2)		
Dumont	Paper	-1.6	1.8	7.7	33.2	0.98	4.95
		(0.2)	(0.4)	(2.4)	(2.7)		
	Agar	0.3	1.1	9.3	39.8	0.98	0.74
		(0.2)	(0.2)	(2.3)	(3.5)		

[a] Refer to Materials and Methods section for a description of model fitted.

Table 5-2. Parameter estimates[a] (standard errors in parentheses) describing the growth of UM5 and Dumont seedlings on filter paper or agar media treated with sethoxydim.

	Media	*g*	*b*	*d*	*k*	R^2	GR_{50}
Root length							(μM)
UM5	Paper	2.9	1.3	4.0	130.7	0.99	0.055
		(0.1)	(0.1)	(0.6)	(1.5)		
	Agar	4.0	1.1	5.6	125.3	0.99	0.018
		(0.1)	(0.1)	(0.6)	(1.5)		
Dumont	Paper	2.9	0.9	2.2	161.1	0.99	0.055
		(0.1)	(0.1)	(2.2)	(3.6)		
	Agar	4.2	0.7	3.7	165.5	0.99	0.015
		(0.1)	(0.1)	(1.2)	(2.4)		
Coleoptile length							
UM5	Paper	0.2	2.2	5.5	41.2	0.99	0.82
		(0.1)	(0.2)	(0.7)	(1.1)		
	Agar	1.3	1.0	5.3	49.7	0.99	0.27
		(0.1)	(0.1)	(0.9)	(1.8)		
Dumont	Paper	0.3	2.0	7.8	30.8	0.99	0.74
		(0.1)	(0.3)	(0.8)	(1.2)		
	Agar	-0.1	2.0	8.1	36.2	0.99	1.11
		(0.1)	(0.3)	(0.9)	(1.3)		

[a] Refer to Materials and Methods section for a description of model fitted.

filter paper or agar. In instances where the type of media significantly influenced growth, herbicide treatments were more inhibitory to both coleoptile and root growth when agar was used as compared to filter paper (Figure 5-1, Tables 5-1 and 5-2). The differences in response to media may have been due to better root contact with the agar. Dumont produced significantly longer coleoptiles and roots than UM5 where the main effect of genotype was significant. This may be related to the fact that Dumont seeds were larger (one-thousand caryopsis weight of 31.1 g vs. 12.5 g for UM5), and initial growth of Dumont was more vigorous than that of UM5.

Given the significant effects for dosage, medium, and genotype, careful consideration was given to the selection of a single dosage and media system that would reliably discriminate between vigorous S^{17} genotypes (the cultivated oat, Dumont was considered to be equivalent to a very vigorous S wild oat) and R biotypes with a low level of resistance (those populations with R/S^{17} resistance levels not much greater than 1). Cross-resistance patterns vary greatly between R wild oat populations, further complicating the issue (Heap et al. 1993). Based on the results of the dose-response experiments, 10 µM (ln 2.3) fenoxaprop-P and 5 µM (ln 1.6) sethoxydim were selected as the discriminating dosages. These dosages were considered the minimum required for complete inhibition and death of the coleoptiles and roots of both UM5 and Dumont (Figure 5-1, Tables 5-1 and 5-2).

Agar was selected as the germination medium because the response of the germinating seeds was visually more uniform from box to box and because it was not necessary to add additional moisture, thereby simplifying maintenance. Germination

boxes with filter paper dried unevenly depending on their position in the germination cabinet and herbicide treatment. There was an apparent higher moisture requirement in boxes where coleoptile and root growth was less inhibited. In contrast, the agar media did not dry differentially and therefore was a simpler system to manage.

5.3.2 Verification of bioassay.

The results of the verification experiments corresponded with GR_{50} values and R/S ratios determined from previous whole-plant experiments conducted in the growth-room and field with these genotypes (Heap et al. 1993). When treated with 10 µM fenoxaprop-P, the genotypes ranked from the most to least resistant as follows: UM33 > UM1 >= UM3 > UM2 >=Dumont >= UM5, based on coleoptile length (Table 5-3). The ranking based on root lengths was: UM1 = UM33 > UM3 >= UM2 >= Dumont >= UM5. Although genotypes with the highest levels of resistance to fenoxaprop-P (UM33 and UM1) were statistically different from the two susceptible genotypes (Dumont and UM5) based on root measurements, those genotypes with lower levels of resistance (UM2 and UM3) were not statistically different from Dumont. On the basis of coleoptile measurements, UM33, UM1, and UM3 differed significantly from Dumont while UM2 did not.

When treated with 5 µM of sethoxydim, the genotypes ranked: UM1 > UM3 >= UM2 = UM33 > Dumont >= UM5, based on coleoptile length (Table 5-3). For root length the rankings were: UM1 >> UM3 > UM2 = UM33 >= Dumont >= UM5. In interpreting the results it is important to remember that based on whole plant experiments UM33 is highly resistant to fenoxaprop-P, but susceptible to sethoxydim

Table 5-3. Mean coleoptile and root lengths (sum of all roots per seed) for six genotypes germinated on agar media with and without herbicide.

	Untreated Controls		Fenoxaprop-P 10 µM		Sethoxydim 5 µM	
Genotype	Shoot	Root	Shoot	Root	Shoot	Root
	mm	mm	mm	mm	mm	mm
UM1	42	172	27	27	46	124
UM2	41	112	14	9	16	10
UM3	37	148	22	10	20	21
UM5	46	85	7	5	8	6
UM33	53	154	44	27	16	9
Dumont	45	171	10	8	9	7
LSD (0.05)	5.0	34.7	5.1	3.8	5.1	9.9

(Heap et al. 1993). Root growth of those genotypes with high or moderate levels of resistance to sethoxydim (UM1 and UM3) was statistically different from the susceptible Dumont and UM5 genotypes, whereas the biotype with a lower level of resistance (UM2) was not. However, based on coleoptile measurements, all of the R biotypes (including UM33) were more resistant than Dumont and UM5. These results indicate that the seed bioassay system may be more sensitive than whole-plant assays for discriminating between low-level R genotypes and S genotypes.

Visual assessments of coleoptile and root growth and health separated low-level R biotypes from S biotypes where differences in quantitative measurements were not statistically significant. For both fenoxaprop-P and sethoxydim, all R genotypes including UM2 produced green coleoptiles and extruded a first leaf after 24 to 48 h on the window ledge. Susceptible genotypes (Dumont and UM5 when treated with fenoxaprop-P and Dumont, UM5, and UM33 when treated with sethoxydim) had hollow, often rotting coleoptiles which did not turn green in the light and did not initiate leaves. The combination of leaf extrusion and root and coleoptile growth allowed 3 independent observers to successfully distinguish resistant and susceptible genotypes as well as correctly rank genotypes in order of increasing resistance.

5.3.3 Summary

The seed bioassay technique described above is a simple, comparatively rapid, inexpensive and accurate methodology for identifying ACCase inhibitor resistant wild oat populations. To ensure accuracy, standard R and S genotypes should be included in the bioassay along with the unknown or suspected R populations. Details on the

collection and handling of seed samples for resistance testing have been described by Heap (1994).

If additional APP or CHD herbicides are used in the seed bioassay, dose-response curves for the standard R and S populations should be generated and results compared with whole-plant assay results. Given the diversity of cross-resistance patterns between various R wild oat populations, it is possible that a population may be susceptible to fenoxaprop-P and sethoxydim but resistant to certain other APP and CHD herbicides.

Slight changes in the methodology may significantly alter seed bioassay results, therefore, it is essential that procedures are consistent over time. The use of recently formulated herbicides is recommended as some APP and CHD herbicides degrade significantly over time (Anonymous, 1994). This may affect the results of the bioassay since such low herbicide concentrations are used.

The rapid and accurate identification of R weed populations through use of this seed bioassay system will assist in determining the nature and extent of the problem of ACCase inhibitor resistance on the North American Great Plains. Producers can collect seed samples from patches that are suspected to be resistant and subsequently be informed of a potential problem in a timely fashion. Alternative and effective weed management practices can then be implemented before the problem becomes unmanageable.

6. POLLEN-MEDIATED GENE FLOW IN WILD OAT (*Avena fatua*).[21]

Abstract. Separate field experiments were conducted to quantify the degree of out-crossing and pollen-mediated gene flow (PMGF) in wild oat. The purpose of the study was to determine the extent to which pollen movement could contribute to the spread of herbicide resistance in this species. In both experiments, a herbicide resistant genotype (UM1) was used as a pollen donor and a susceptible genotype (UM5) as the pollen receptor. Hybrid progeny resulting from a cross between UM1 and UM5 were identified using the herbicide resistance trait as a marker. In the out-crossing experiment, single UM5 plants were surrounded by 20 homozygous resistant UM1 plants in hills. By screening seed from the susceptible parent for resistance, out-crossing was determined to range from 0 to 12.3%, with a mean of 5.2% over 10 hills. In the PMGF experiment, single, homozygous resistant UM1 plants were surrounded by UM5 plants arranged in a hexagonal pattern at low and high densities (19 and 37 plants m^{-2}) over-seeded to wheat or flax. In wheat, mean out-crossing was 0.08 and 0.05% at low and high densities, respectively. In the less competitive flax crop, the corresponding values were 0.10 and 0.17%, respectively. By fitting the data from the high density planting in flax to a Weibull function, it was determined that 90% of successful out-crossing events would occur within a distance of 56 cm of the resistant pollen donor. Up to 85 resistant hybrid seeds were recovered from 6 m^2 in the PMGF

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[21]This chapter was submitted to Weed Science as the following: Murray, B. G. and I. N. Morrison. Pollen mediated gene flow in wild oat (*Avena fatua*).

experiments, indicating that PMGF contributes to resistance evolution. Despite this, the contribution of pollen movement to resistance evolution and the spread of resistance in a wild oat populations would be relatively small compared to resistant seed production and dispersal from a resistant parent. Nomenclature: Sethoxydim [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one]; wild oat, *Avena fatua* L. #[22] AVEFA; spring wheat, *Triticum aestivum* L. 'Roblin'; flax, *Linum usitatissimum* L. 'Norlin'.

Additional index words. Sethoxydim, cyclohexanedione, ACCase inhibitor, herbicide resistance, resistance evolution, AVEFA.

6.1 INTRODUCTION

Gene flow is a collective term that includes all mechanisms resulting in the movement of genes between populations (Slatkin 1985). For plants, gene flow can involve pollen transport. This type of gene flow is referred to as pollen-mediated gene flow (PMGF)[23]. PMGF can be described either as potential gene flow or as actual or realized gene flow. Potential PMGF is a measure of pollen grain movement and deposition, whereas actual PMGF is a measure of successful fertilizations often

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[22]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds Revised 1989. Available from WSSA, 309 W. Clark St.,Champaign, IL 61820.

[23]Abbreviations: ACCase, acetyl-CoA carboxylase; APP aryloxyphenoxypropionate; CHD, cyclohexanedione; R/S, resistant to susceptible ratios based on GR_{50} values; PMGF, pollen mediated gene flow; R, resistant, living with healthy root and coleoptile growth; S, susceptible, dead with stunted roots and coleoptile.

determined through progeny analysis. To date the characterization of PMGF has mostly been limited to measurements of potential PMGF (Levin and Kerster 1974).

The degree of PMGF between plants and plant populations is determined by a multitude of factors including population size and shape, plant height, wind velocity and direction, foraging behaviour of pollen vectors, the distance between populations or between plants within the same population, and the mating system of the plant (Levin and Kerster 1974; Handel 1983). The reproductive biology of the species heavily influences the degree of PMGF between and within populations.

Both the level of out-crossing and the degree of PMGF are factors that influence the rate of herbicide resistance evolution (Maxwell et al. 1990; Jasieniuk et al. 1996). With the occurrence of herbicide resistant weed populations and the production of transgenic crops, there is renewed interest in the reproductive biology of many of our weed species, as well as in the role pollen movement plays in carrying herbicide resistance and other introduced genes within and between populations and species (Manasse 1991; Manasse and Kareiva 1991). The objectives of this study were to determine the potential out-crossing between wild oat plants grown in the field, and to determine the degree of PMGF between wild oat plants grown at two planting densities in two crops with different growth forms and competitiveness.

6.2 MATERIALS AND METHODS

6.2.1 Plant material.

The two parental wild oat lines were UM1 and UM5. UM1 is resistant to a

number of aryloxyphenoxypropionate (APP)[23] and cyclohexanedione (CHD)[23] herbicides collectively referred to as the acetyl-CoA carboxylase (ACCase)[23] inhibitors, whereas UM5 is susceptible to these herbicides (Heap et al. 1993). The wild oat lines used in both experiments were selfed for three generations and sub-samples were screened with foliar sethoxydim treatments to ensure homozygosity for resistance and susceptibility, respectively. ACCase inhibitor resistance in UM1 is governed by a single, partially dominant, nuclear gene (Murray et al. 1994). This simple, dominant, nuclear mechanism of inheritance, coupled with its high level of expression (R/S^3 >152, for sethoxydim) (Heap et al. 1993), makes ACCase inhibitor resistance in UM1 an ideal genetic marker. In the out-crossing and pollen-mediated gene flow studies UM1 served as the resistant pollen donor and UM5 the pollen receptor.

6.2.2 Out-crossing experiments.

Hill plantings were established outdoors at the University of Manitoba on May 9, 1992. The soil was a Riverdale silty loam that had been fallowed the previous growing season. Ten individual hills were planted, each with 20 UM1 pollen donor plants surrounding a single UM5 pollen receptor plant (Figure 6-1). Hills were separated by 3 m isolation strips. No other wild or domesticated oats were allowed to grow within 3 m of the out-crossing experiment.

Pre-germinated wild oat seeds were hand planted at a depth of 3.75 cm. Hills

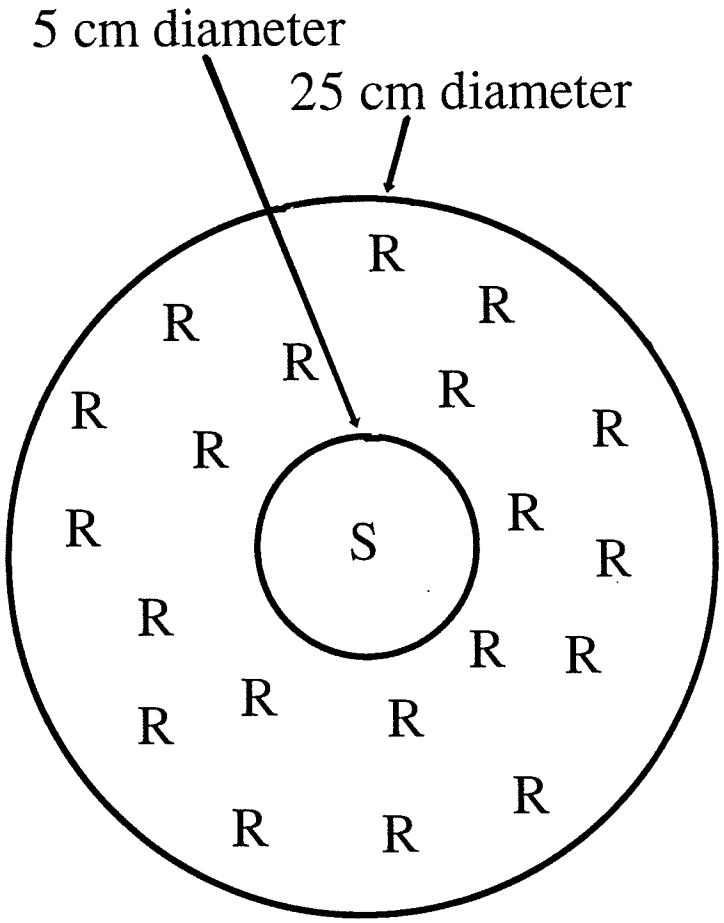

Figure 6-1. Diagrammatic representation of hill planting arrangement for out-crossing experiment. R represents UM1 pollen donor plant locations, S represents UM5 pollen receptor plant location.

were mulched with a 2.5 cm layer of a soilless mixture[24] to avoid crusting of the soil surface. Hills were watered every other day until the plants reached the 2-leaf stage of development.

Individual UM5 plants were hand harvested on August 21, 104 days after planting. Seed from individual hills was bagged and stored separately.

6.2.3 Pollen-mediated gene flow experiments.

Plots were planted on the same block of land as the out-crossing experiments, with a separation of 9 m between the two experiments and 6 m between plots. Planting date, seed preparation, and planting practices were identical to those described for the out-crossing experiments. Individual plots consisted of susceptible (UM5) plants surrounding a single resistant UM1 plant. Wild oat plants were spaced equidistantly in an hexagonal design covering an area of 1 m^2, similar to experiments described by Smyth and Hamrick (1987). UM5 plants were sown at two densities (18 and 36 plants m^{-2}) (Figure 6-2), in an area over-seeded to wheat and flax. Each combination of weed density and crop species was replicated six times.

Both the wheat and flax crops were seeded with a press drill immediately before planting the wild oat. 'Roblin' wheat was planted at 65 kg ha^{-1}, at a depth of 6.5 cm. 'Norlin' flax was sown at 35 kg ha^{-1}, at a depth of 4 cm.

Individual wild oat plants (UM1 and UM5) were hand harvested on August 21, 1992, the same day as the out-crossing experiments. Seed from individual hills was

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[24]Metro-Mix 200, W.R. Grace and Co., Ajax, Ontario.

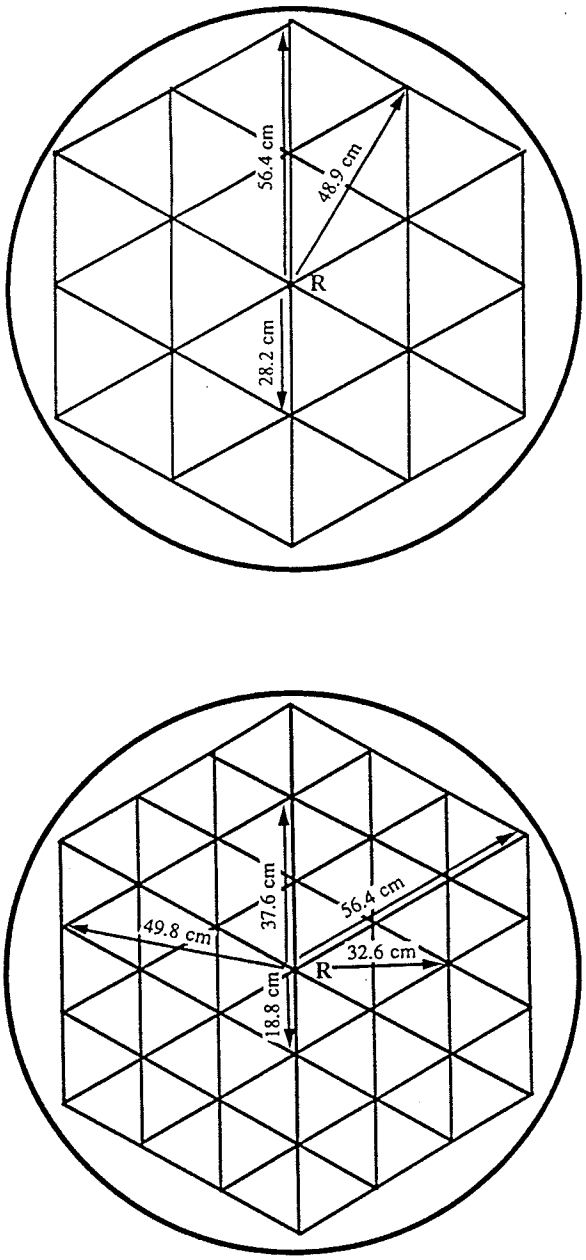

Figure 6-2. Diagrammatic representation of low and high density plantings for pollen mediated gene flow experiments. Line intersects represent susceptible UM5 plant locations, R represents UM1 pollen donor plant. The distances (cm) between the UM1 pollen donor plant and various UM5 pollen acceptor plants are indicated by the arrows. Area within the circles equals 1 m^{-2}.

bagged and stored separately.

6.2.4 Screening procedure.

Seed collected from both experiments was stored at room temperature in paper bags for approximately one year, after which it was dehulled by hand. Dehulled seed was then screened for ACCase inhibitor resistance using a seed bioassay procedure described by Murray et al. (1996). Seed was screened in plexiglass germination boxes (13.5 by 12.5 by 3.5 cm deep) on Whatman #1[25] filter paper using a 5 µM emulsion of sethoxydim, [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one]. One hundred seeds were screened in each germination box, with up to 7000 seeds (70 germination boxes) screened per 6 d cycle. One hundred UM1 and 100 UM5 seeds were also included in each cycle to ensure accuracy. Five days after initial treatment, germinated seeds were visually inspected and scored as being either resistant (R)[23] or susceptible (S)[23] on the basis of coleoptile and root growth and health. Germination was considered to have occurred when both the radicle and the coleoptile were visible.

6.2.5 Statistical procedures.

Pollen dispersal is likely to be well described by one of three probability density functions: gamma, Weibull or exponential (Kareiva et al. 1991; Kareiva et al. 1994). Using the data from the flax high density PMGF experiment, the proportion of hybrid seed versus planting distance from the pollen donor plant fit a Weibull probability density function best. Only the data from the flax high density plantings

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[25]Whatman #1, Whatman Int. Ltd. Maidstone, U.K.

was used due to the limited number of data points and hybrids produced in the other plantings. The Weibull function used was as follows:

$$y = e^{ax}b$$

where y is the dependent variable, proportion of total outcrossing events, x is the distance from the resistant pollen source, e is the base of the natural logarithm and a and b describe the shape of the curve (Kareiva et al. 1994).

6.3 RESULTS AND DISCUSSION

6.3.1 Seed bioassay confirmation.

Prior to screening seed collected from field experiments, the bioassay procedure was tested to ensure that heterozygous resistant genotypes could be identified. The procedure was tested in two separate experiments. The first involved screening a known F_2 population (UM5/UM1) with the seed bioassay procedure and observing segregation patterns; the second involved re-treating seedlings rescued from the seed bioassay with a foliar application of sethoxydim.

A total of 278 UM5/UM1 F_2 seeds were screened. Two seedling responses were observed: R and S. Results from the F_2 segregation experiment fit a 3:1 (R:S) pattern, ($X^2 = 0.17$, $P = 0.68$) with 205 seedlings displaying R and 73 S responses (data not shown). A 3:1 (R:S) segregation ratio indicated that the seed bioassay procedure was suitable for separating resistant heterozygotes from homozygous susceptible genotypes.

Additionally, 42 R-rated seedlings from the out-crossing and PMGF studies

were rescued immediately after assessment, and transplanted into wooden flats. The seedlings were treated at the 3 leaf stage with 200 g ha^{-1} sethoxydim . In all cases, rescued plants were able to withstand this treatment. UM1 control plants survived treatment whereas UM5 plants were dead 21 DAT.

These results coupled with those from the F_2 segregation analysis indicated that the seed bioassay procedure was suitable for the differentiation of R/S hybrids and that separation of R heterozygotes from S homozygotes could be performed solely on a visual basis.

6.3.2 Out-crossing experiments.

Both UM1 and UM5 plants emerged 9 days after planting. Both wild oat genotypes grew at approximately the same rate, and flowered synchronously. Anther dehiscence began on July 13, and continued for approximately two weeks. An average of 149 seeds per plant were harvested from the 10 UM5 pollen receptor plants (Table 6-1).

In total 78 resistant heterozygous seeds were identified from the out-crossing experiment. Percent out-crossing ranged from 0 to 12.3% among the UM5 plants, with an average of 5.24% (Table 6-1).

There has only been one other study that these authors are aware of that has reported an out-crossing rate for wild oat (*Avena fatua*) (Imam and Allard 1965). The levels of out-crossing estimated by indirect measurements ranged from 1.24 to 11.78%. There have been a number of studies reported that have measured the level of introgression between wild oat (*Avena fatua*) and cultivated oat (*Avena sativa*) using

Table 6-1. Number of seeds screened, number of resistant seeds identified and percent out-crossing for individual UM5 plants harvested from out-crossing study.

UM5 plant	Number of seeds screened	Number of R seeds	% out-crossing
1	20	0	0
2	187	23	12.30
3	409	15	3.67
4	136	3	2.21
5	36	3	8.33
6	169	8	4.73
7	99	3	3.03
8	78	6	7.69
9	217	7	3.23
10	139	10	7.19
Total	1490	78	
Average	149	7.8	5.24

direct measurements (Coffman and Wiebe 1930; Harrington 1932; Derrick 1933; Bickelman and Leist 1988). Introgression rates reported in these studies range from 0.1 to 9.82%. Out-crossing rates observed in our study falls close to the range previously reported by Imam and Allard (1965) and earlier introgression studies (Coffman and Wiebe 1930; Harrington 1932; Derrick 1933; Bickelman and Leist 1988). Prior to this study there has not been any reports where the level of out-crossing between wild oat plants (*Avena fatua*) was directly measured through the use of progeny analysis.

6.3.3 Pollen-mediated gene flow experiments.

Wheat, flax and wild oat plants (both UM1 and UM5) emerged 9 days after planting. The flax density was approximately 440 plants per m^2, and the wheat density was 140 plants per m^2. Both UM1 and UM5 plants developed at approximately the same rate, although some UM5 plants initiated flowering 1-2 days ahead of UM1. To ensure synchronous flowering, those panicles of UM5 that produced pollen ahead of UM1 were removed by hand. At maturity (104 days after planting), both UM1 and UM5 plants were hand harvested and bagged separately.

At the time of wild oat flowering, plant height of 100 randomly selected plants of each of the crop species and UM5, and 24 UM1 plants were measured and means calculated (Table 6-2). On average, the wild oat plants were over 60 cm taller than the flax and 40 cm taller than the wheat (Table 6-2).

In total 126,520 wild oat seeds were collected from the UM5 mother plants in the PMGF experiments and screened. At the low and high density plantings in flax

Table 6-2. Mean plant height for both crop species and both wild oat genotypes in pollen-mediated gene flow experiments. One hundred randomly selected flax, wheat and UM5 plants, and all UM1 plants (24 plants) were measured to calculate mean heights.

Plant	Mean Height (cm)
Wheat	111.4
Flax	86.4
UM5	154.5
UM1	153.8

Table 6-3. Mean number of wild oat seeds produced at low and high density plantings in flax and wheat. The number of plants missing from the hexagonal plantings, over 6 replicates, in brackets.

	Wild Oat Seeds			
	Flax		Wheat	
Wild Oat Density	Seeds / Plant	Seeds / m^2	Seeds / Plant	Seeds / m^2
Low (19 m^{-2})	463 (6)	7870	89 (15)	1585
High (37 m^{-2})	287 (24)	9196	82 (22)	2642

Table 6-4. Total number of resistant seeds and percent out-crossing at low and high density plantings in flax and wheat.

	Wild Oat Seeds			
	Flax		Wheat	
Wild Oat Density	R Seeds[a]	% Out-crossing	R Seeds	% Out-crossing
Low (19 m^{-2})	44	0.10	6	0.08
High (37 m^{-2})	85	0.17	7	0.05

[a] Sum of six replicates.

the mean number of wild oat seeds produced was 7870 and 9196 m^{-2} respectively (Table 6-3). In contrast, in wheat, a more competitive crop, the mean number of wild oat seeds produced was 1585 and 2642 m^{-2} at high and low densities respectively.

All the seed collected from the PMGF experiments was screened in the seed bioassay. Two seedling responses, R and S, were observed 5 days after initial treatment. A total of 85 resistant (R) seedlings were identified from the 48,952 seeds from all 6 replicates of the high density wild oat planting in flax (Table 6-4). This represents 0.17% out-crossing. At the low density wild oat planting in flax the level of out-crossing was 0.10%. Comparable values for low and high density plantings in wheat were 0.08 and 0.05%. These values are much lower than the average observed in the out-crossing experiments where the source of resistant marker pollen was much larger (20 UM1 plants per hill planting). In contrast, in the PMGF studies only 1 UM1 plant was available for each hexagonal planting, and, therefore, as compared to the previous experiment, marker pollen would be limited. The vast majority of the pollen available for out-crossing in the PMGF studies would be UM5 pollen and, therefore, the majority of out-crossing events would occur between UM5 plants.

The spacial distribution of the resistant hybrid seeds within the hexagonal arrangement for both crops and planting densities is diagrammed in Figure 6-3. In flax, where the majority of hybrids were identified, most of the hybrids were produced on susceptible plants adjacent to the resistant pollen donor. The number of resistant seeds recovered from these plants ranged from 1 to 12 at the low density and from 1 to 8 at the high density.

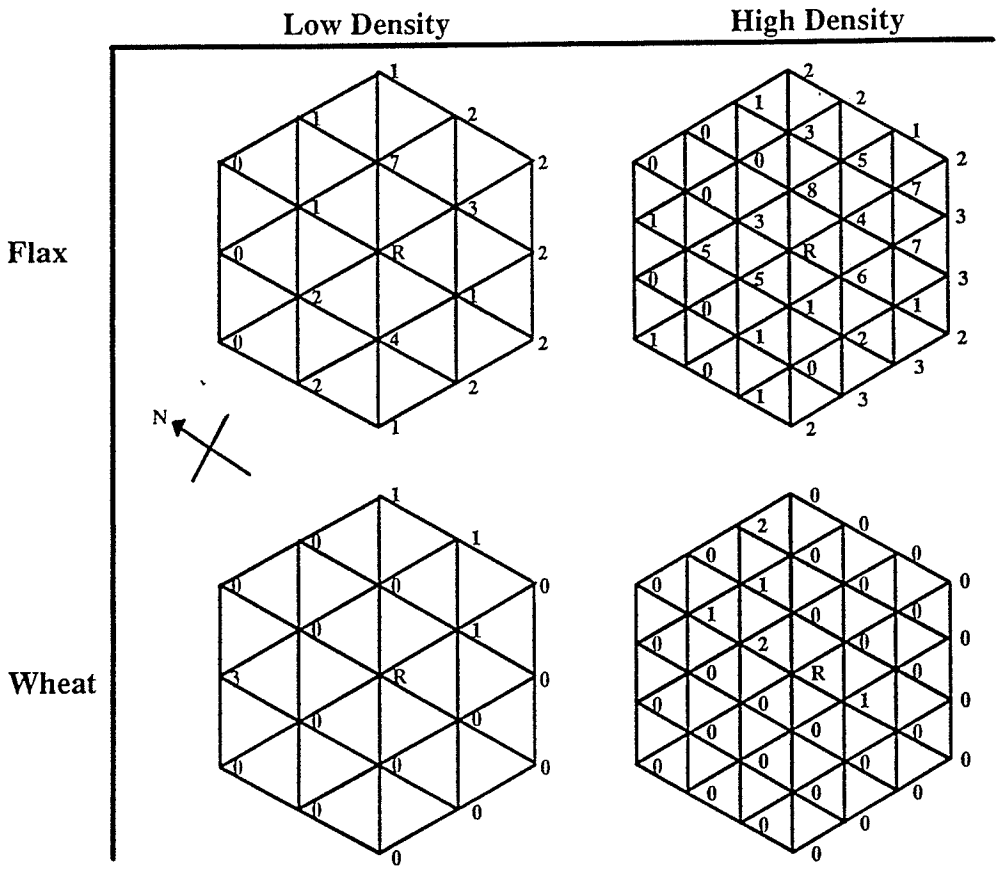

Figure 6-3. Diagrammatic representation of low and high density plantings in flax and wheat. The numbers to the left of line intersects indicate the number of hybrid seeds identified at each point within the design summed over six replicates. The R in the center of the hexagon represents the resistant pollen donor, UM1.

The prevailing wind at the time of wild oat flowering (July 13 to July 31) was north or northwest (data not shown). This is evident in the asymmetrical distribution of hybrid seeds for both flax plantings, where most of the hybrids occur along the south and east sides of the hexagonal plantings. The distribution of hybrids in the wheat plantings appears to be much more random. This may be an artifact due to the limited number of hybrid seeds produced in the wheat plantings.

In flax, the number of out-crossing events decreased as the distance from the central pollen donor to the susceptible parent increased. At the furthest points (56.4 cm) from the resistant parent no more than two hybrid seeds were identified at any individual point. Using the data from the flax high density plantings the proportion of hybrid seeds versus distance from the pollen donor plant was fitted to a Weibull probability density function (Figure 6-4). The resulting model indicated that 90% of the out-crossing events between the resistant pollen donor and the susceptible pollen receptor plants occurred within the limits of the design. By extrapolation less than 10% out-crossing would occur at distances greater than 60 cm. It was not possible to plot probability distributions for the remaining three weed density/crop combinations because of the limited size of the data sets.

The out-crossing rates from resistant to susceptible plants in the PMGF experiments would be indicative of those occurring early in the evolution of resistance in a field where there is a limited number of resistant individuals. Up to 85 resistant hybrid seeds were recovered from six m^2, indicating that PMGF contributes to resistance evolution in wild oat populations. Despite this the contribution of pollen

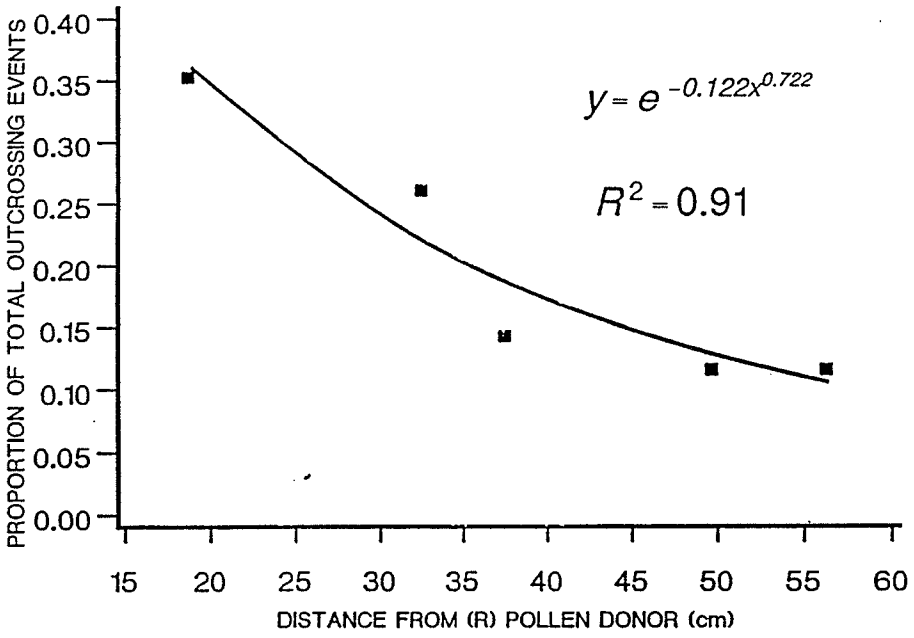

Figure 6-4. The relationship between the proportion of resistant hybrid seeds and distance from the pollen donor plant fitted to a Weibull probability density function for the high density plantings in flax.

flow to resistance evolution and the spread of resistance in wild oat populations would be small compared to resistant seed production and dispersal from a resistant parent. Seed can be carried many miles via seeding, tillage and harvesting equipment (Thill et al. 1994). For the most part, pollen transfer among wild oat is restricted to comparatively short distances, i.e. less than 60 cm, although the furthest extent of pollen transfer was not determined in this experiment.

Considering both seed production and pollen transfer, resistant wild oat populations would potentially increase more rapidly in flax than in wheat. The more competitive wheat crop drastically reduced both wild oat seed production and pollen transfer between R and S plants. Therefore, an effective strategy for reducing seed production and slowing resistance evolution would be to include competitive crop species in the crop rotation.

7.0 SUMMARY AND CONCLUSIONS

Pest resistance to toxicants is not a new phenomenon, although the appearance of herbicide resistant weed populations is relatively recent. In retrospect, the widespread occurrence of herbicide resistance should have been expected. The development of resistance is a consequence of basic evolutionary processes, in which resistant variants increase in frequency upon selection. Whenever intense selection is applied to large populations the probability of selecting for resistance is high (Jasieniuk et al. 1996).

Wild oat resistance to acetyl-CoA carboxylase (ACCase) inhibitors was first suspected in western Canada in the fall of 1990. Since then hundreds of populations have been verified to be resistant (Morrison and Bourgeois 1995). Most are from fields with a history of eight or more treatments with ACCase inhibitors. Following the introduction of diclofop-methyl in 1972, the ACCase inhibitors have become the preferred graminicides in western Canada. In Manitoba, ACCase inhibitor use increased from 15 to 50% of the sprayed area between 1981 and 1993 (Bourgeois and Morrison 1996).

Although a number of variables influence the rate of resistance evolution in weed populations, by far the most important is the level of selection imposed by the herbicide (Jasieniuk and Maxwell 1994). Generally the ACCase inhibitors are highly effective, often causing greater than 95% mortality. This high level of efficacy coupled with a heavy reliance on the ACCase inhibitors in western Canada were the

main driving forces behind the rapid development of ACCase inhibitor resistant populations.

Experiments conducted by Heap et al. (1993) on four populations indicated that they differed in both their levels of resistance and patterns of cross resistance to various aryloxyphenoxypropionate (APP) and cyclohexanedione (CHD) herbicides. Such variation indicates that these populations probably evolved independently, and that several different mutations for ACCase inhibitor resistance exist. The fact that many different mutations can confer resistance to wild oat will speed the rate of resistance evolution by increasing the probability of finding an initial mutant in a previously unselected population.

This situation is similar to that reported for ALS inhibitors. Various cross-resistance patterns have been identified in ALS inhibitor resistant weed populations (Saari et al. 1994; Guttieri et al. 1995). Molecular genetic analysis has indicated that numerous mutations to the ALS enzyme cause resistance, and therefore the likelihood of finding a initial resistant mutant in a population is increased (Guttieri et al. 1995). For example a 100-fold increase in the number of initial mutants would hasten the evolution of resistance by 1 to 3 years depending on the intensity of selection, the relative fitness of resistant and susceptible genotypes, and the mating system of the weed (Jasieniuk and Maxwell 1994).

The mode of inheritance of the resistance trait also influences the probability of occurrence and the subsequent survival of initial mutants, both before and after selection. The inheritance of ACCase inhibitor resistance in the two wild oat

populations, UM1 and UM33 was determined to be governed by single, partially dominant, nuclear genes. These results are consistent with previous reports on biological adaption to pollutants, heavy metals and other xenobiotics in which resistance is attributed to single, dominant, nuclear gene systems (MacNair 1991; Jasieniuk et al. 1996). Recent studies with ACCase inhibitor resistant green foxtail collected in Manitoba also indicated that resistance in this species is governed by a single, dominant, nuclear gene (G. Stilkowski, personal communication)[26].

F_2 segregation analysis of crosses between UM1 and UM33 determined that the distinct APP and CHD cross-resistance patterns observed in the UM1 and UM33 populations are allelic and are due to different mutations at the same gene locus. Subsequent studies have indicated that the mechanisms of resistance for UM1 and UM33 are both due to altered sites of action (ACCase) (Marles and Devine 1995; M. Devine, personal communication)[27], further substantiating the conclusions drawn from the inheritance studies.

The results from the two inheritance studies confirmed our initial hypothesis that more than one mutation could result in resistance in wild oat. Additionally, it was confirmed that resistance to the chemically unrelated herbicides fenoxaprop-P and sethoxydim in UM1 was encoded by the same gene alteration. The different levels of resistance observed to various ACCase inhibitors within populations may be due to a

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number of reasons. For those populations with altered sites of action like UM1 and UM33, the specific mutations may influence the ability of individual APP and CHD herbicides to bind to the target site. For example, UM1 is highly resistant to sethoxydim and moderately resistant to diclofop-methyl. For this particular genotype the alteration in the target site must interfere with the ability of sethoxydim to bind to ACCase to a much greater degree than it does for diclofop.

As mentioned previously, the mechanism of inheritance for resistance will influence the rate of resistance evolution. The fact that both wild oat populations possessed a single, partially dominant, nuclear gene alteration for resistance implies that resistance evolution will be rapid (Jasieniuk et al. 1996). The fact that resistance is encoded by a "dominant", nuclear gene also indicates that the trait can be transmitted via pollen and expressed in hybrid offspring. Therefore the subsequent spread of resistance can occur through pollen migration.

Gene flow is a potent evolutionary force that may serve to speed the rate of resistance evolution through introducing initial resistant individuals to new populations at much higher frequencies than natural mutation rates (Jasieniuk et al. 1996). Many factors will influence the rate of gene flow between populations including the reproductive biology of the species and the overall structure of the population.

The out-crossing rate between UM1 and UM5 was determined using ACCase inhibitor resistance as a gene marker. Out-crossing between UM1 and UM5 plants ranged from 0-12.3%, with a mean of 5.2%. To the author's knowledge this is the first time the level of out-crossing has been determined for wild oat using direct

measurements (progeny analysis). Previous reports have been limited to indirect measurements and estimates.

Pollen-mediate gene flow (PMGF) was also characterized using UM1 as the gene marker source. Wild oat plants were grown at two planting densities (19 & 37 plants m^2) and within two crop species, wheat and flax. Out-crossing and wild oat seed production was higher in the less competitive flax crop as compared to the wheat. Considering both seed production and pollen transfer, the evolution of herbicide resistance would potentially be more rapid in the less competitive flax, as compared to wheat.

Although gene flow via pollen did occur, the relative contribution of pollen flow to an increase in resistant plant numbers is small compared to seed production on the resistant parent. In flax, the number of out-crossing events decreased as the distance from the resistant pollen source increased. It was determined, using data from the high density planting in flax that 90% of the out-crossing events would have occurred within the confines of the experiment (56.4 cm). Therefore, it is not likely that PMGF will contribute a great deal to the long distance spread of herbicide resistance in wild oat.

In contrast, the movement of seed could contribute significantly to long distance migration. Movement of resistant seed via seeding, tillage and harvesting equipment and through the use of contaminated crop seed could significantly increase the number of resistant cases and drastically increase the spread of this problem (Thill et al. 1994). Producers would be well advised to clean equipment when moving from

field to field, and use only clean, certified crop seed free of wild oat.

All of the above factors, coupled with the extremely high selection pressures that the ACCase inhibitors impose on wild oat, suggest that resistance evolution will be rapid. Tardiff and Powles (1993) recently estimated that resistance to the ACCase inhibitors could evolve with as few as 3 to 5 applications. Western Canadian field histories to date have demonstrated that as few as 5 applications of these herbicides have resulted in detectable resistance (I. N. Morrison, personal communication)[3]. Additionally, the resistant populations studied to date all appear to be as fit as susceptible genotypes in the absence of selection. This then suggests that the frequency of these genotypes would not decline if Group 1 herbicide use was terminated. Therefore, if a producer was to select for detectable levels of resistance in a field, simply stopping Group 1 applications for an extended period of time would not alter the frequency of resistance alleles in that population significantly.

During the course of this study, the number of confirmed instances of ACCase inhibitor resistant wild oat populations in western Canada increased from 4 in 1990 to several hundreds in 1995 (Morrison and Bourgeois 1995). More recently, four populations of wild oat from Manitoba have been confirmed to be resistant to certain ACCase inhibitors as well as to ALS inhibitors and flamprop-methyl which has a unique mode of action (Morrison and Bourgeois 1995). The consequences of this multiple-resistance are far reaching. Depending on the mechanism of resistance and the mode of inheritance, the main strategy aimed at delaying the onset of resistance (rotation of herbicide groups) may be called into question. If these populations are

multiple resistant due to a single gene alteration, then the use of herbicide group rotations will be much less effective, or ineffective. Using a 1 in 3 rotation including Group 1, Group 2, and flamprop-methyl would not have slowed the development of these populations.

If producers are to delay ACCase inhibitor resistance on their farms they must act immediately. Practising proper herbicide rotations, coupled with integrating non-herbicide weed management practices and adequate sanitation practices will serve to delay the occurrence of resistance (Thill et al. 1994; Morrison and Bourgeois 1995). The question for producers is no longer whether herbicide resistance will occur, but rather when it will occur.

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