INHERITANCE OF FENOXAPROP-P-ETHYL AND TRIFLURALIN RESISTANCE, AND GENETIC VARIABILITY AMONG GREEN FOXTAIL (*SETARIA VIRIDIS* (L.) BEAUV.) POPULATIONS

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Plant Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Abstract

Acetyl-CoA carboxylase (ACCase) inhibitor (Group 1 herbicide) and trifluralin (Group 3 herbicide) resistant green foxtail (*Setaria viridis* (L.) Beauv.) populations have been identified and now represent a serious weed management problem for farmers. Genetic control of herbicide resistance is a major factor influencing the rate of evolution of herbicide resistance. Understanding genetic variation among herbicide resistant and susceptible weed populations can assist in determining the origin and spread of herbicide resistance, and aid in the development of weed management strategies. The overall objectives of this thesis were: (i) to determine the mode of inheritance of fenoxaprop-p-ethyl (a common ACCase inhibitor herbicide) resistance in three green foxtail populations, UM8, UM131, and UM137, (ii) to determine the mode of inheritance of trifluralin resistance in the multiple resistant population, UM137, (iii) to determine if the genes for fenoxaprop-p-ethyl resistance and trifluralin resistance in the multiple resistant population, UM137, are linked, and (iv) to assess genetic variation among 30 herbicide resistant, and susceptible green foxtail populations. To study genetic control of herbicide resistance, crosses were made among plants from resistant and susceptible populations of green foxtail, followed by screening the F_2 and F_2-derived F_3 families for segregation. Fenoxaprop-p-ethyl resistance in green foxtail populations, UM8, UM131, and UM137 is controlled by single nuclear incompletely dominant genes, and

trifluralin resistance in UM137 is controlled by a single nuclear recessive gene. Furthermore, in the multiple resistant population UM137, the two resistance genes are not linked. Using a Random Amplified Polymorphic DNA (RAPD) survey, it was determined that there is very low genetic variation among green foxtail populations sampled in Manitoba. Herbicide resistant populations are likely the result of many independent mutations in susceptible populations leading to resistance, and followed by local seed spread. **Nomenclature:** Fenoxaprop-p-ethyl: Fenoxaprop-P[R-2-[4-[(6-chloro-2-benoxazolyl)oxy]phenoxy] propanoic acid, ethyl ester. Trifluralin: 2,6-dinitro-N,N-dipropyl-4-(trifluoro-methyl)-benzenamine.

ACKNOWLEDGMENTS

I would like to thank the following people for making the completion of this thesis possible,

Dr. Anita Brûlé-Babel, my advisor,

My advisory committee, consisting of Dr. Brian Fristensky, Dr. Ian Morrison, Dr. Terry Galloway, and Dr. Rene Van Acker,

Lyle Friesen and Bert Luit for their technical expertise,

The Plant Genetics Lab, Weeds Lab and Fristensky Lab, past and present,

My Family, and especially,

MY MOM, IRENE, for her support, and assistance.

FOREWORD

This thesis is written in manuscript style. It begins with a general abstract, introduction, and literature review, followed by the presentation of three chapters of experimental research, each representing a particular research theme. The format of each paper is as follows: abstract, introduction, materials and methods, and results and discussion. At the end of the thesis, there is a general discussion including conclusions and ideas for future research, followed by a list of references cited throughout the thesis. The thesis is written to conform with the requirements of the Canadian Journal of Plant Science.

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1. Introduction

Resistance to herbicides has evolved in many weed populations. Repeated use of the same herbicide or herbicide group has led to selection, multiplication and spread of initially rare resistant plants. Most of the earliest reported cases of herbicide resistance were triazine resistant weed species. Resistance to many other herbicide groups has since been reported (Heap 1997). To date, resistance to herbicides has been identified in over 180 weed species worldwide (Heap 1997).

Over the past 25 years, farmers on the Canadian prairies have depended on herbicides to control green foxtail, (*Setaria viridis* (L.) Beauv.), an important annual grass weed. Herbicide resistant green foxtail populations pose a serious threat to sustained productivity and profitability of western Canadian agriculture. Green foxtail populations with resistance to trifluralin, a dinitroaniline (Group 3) herbicide, were first confirmed in southwestern Manitoba in 1988 (Morrison et al. 1989). In 1991, green foxtail populations, UM8 and UM131, were identified as resistant to the ACCase inhibitor (Group 1) herbicides, which inhibit the enzyme, acetyl-CoA carboxylase (ACCase). Both UM8 and UM131 exhibited a similar level of resistance to fenoxaprop-p-ethyl, an ACCase inhibitor herbicide commonly used in canola, pea, and spring wheat (Heap and Morrison 1996). By 1994, hundreds of trifluralin resistant, and 20 ACCase inhibitor resistant green foxtail populations had been identified in fields across Western Canada (Friesen 1994). In addition, at least four green foxtail populations, including UM137, are

resistant to both trifluralin and the ACCase inhibitor herbicides (Heap 1994). As a result, western Canadian farmers are left with fewer herbicide options, TCA (trichloroacetic acid), propanil, or quinclorac, for selective control of green foxtail in cereal or oilseed crops (Morrison et al. 1995). Over the long term, farmers must adopt an integrated weed management approach, reducing their reliance on herbicides, to control both susceptible and herbicide resistant green foxtail in the field.

The mode of inheritance of herbicide resistance is one of the major factors that influences the rate of herbicide resistance evolution (Jasieniuk et al. 1996). The number of genes, and their allelic and genic interactions can influence the rate of spread of herbicide resistance within and among weed populations. Information about the inheritance of herbicide resistance can be useful in the development and verification of models which predict the evolution of herbicide resistance. Understanding genetic variation among herbicide resistant and susceptible weed populations can also assist in determining the origin and spread of herbicide resistance in agricultural fields. This information is essential for development of resistant weed management strategies.

The objectives of this thesis were: (i) to determine the mode of inheritance of fenoxaprop-p-ethyl resistance in green foxtail populations, UM8, UM131, and UM137, (ii) to determine the mode of inheritance of trifluralin resistance in the multiple resistant population, UM137, (iii) to determine if the genes for fenoxaprop-p-ethyl resistance and trifluralin resistance in the multiple resistant

population, UM137, are linked, and (iv) to assess genetic variation via Random Amplified Polymorphic DNA (RAPD) among 30 herbicide resistant and susceptible green foxtail populations.

2. Literature Review

2.1 Green Foxtail

Green foxtail (*Setaria viridis* (L.) Beauv.) is an important annual weed that originated in Eurasia and is now found throughout temperate regions of the world (Douglas et al. 1985; Wang et al. 1995). It is a C_4 grass which is most competitive under high temperature and light conditions. Green foxtail is a diploid species with 18 chromosomes (2N=18). As a prolific seed producer it may produce 5000-12000 seeds per plant (Douglas et al. 1985). Although it is highly self pollinating, green foxtail is morphologically variable. Since its introduction to North America, green foxtail has increased its range and population density, and new phenotypes have appeared (Wang et al. 1995).

Green foxtail was first identified in Manitoba, in 1888 at Emerson (Douglas et al. 1985). It was still a relatively insignificant weed in western Canada in 1931, but by 1948 it was widespread across the Prairies. It is now found throughout southern Manitoba and is the most abundant weed species in Saskatchewan and Manitoba (Douglas et al. 1985; Morrison and Devine 1994).

2.1.1 Herbicide Resistant Green Foxtail in Manitoba

Over the past 25 years, Manitoba farmers have depended on herbicides to control green foxtail, but now herbicide resistant green foxtail populations pose a serious problem. Green foxtail populations with resistance to trifluralin, a

dinitroaniline (Group 3) herbicide, were first identified in southwestern Manitoba in 1988 (Morrison et al. 1989). Resistance was recognized after fields had been treated with trifluralin eight to 12 times. In 1991, green foxtail populations with resistance to the ACCase inhibitor (Group 1) herbicides were identified. These populations had been treated with diclofop-methyl or sethoxydim for over 10 years (Heap and Morrison 1996). Furthermore, in 1992, the first multiple resistant (i.e. both ACCase inhibitor and trifluralin resistant) green foxtail population in western Canada was confirmed (Heap 1994). The number of fields identified with resistant weeds, as well as the size of infestations, will likely continue to increase unless alternative weed control practices are adopted by farmers.

2.2 The Acetyl-CoA Carboxylase Inhibitor Herbicides

The acetyl-CoA carboxylase inhibitor herbicides consist of two chemical families, the aryloxyphenoxypropionates (APPs) and cyclohexanediones (CHDs). These postemergent herbicides are used to control annual grasses such as green foxtail in crops. Diclofop-methyl, an APP, was first commercialized in 1976 for use in cereal crops in western Canada. Sethoxydim, a CHD, was introduced in 1983, for use in canola and flax (Morrison and Bourgeois 1995). Fenoxaprop-p-ethyl is another common APP herbicide, commercialized in 1989, that can be used in canola, pea, and in wheat (with a safener). In 1996, Manitoba farmers had the choice of eight ACCase inhibitor

active ingredients, available in 14 different commercial formulations (Bourgeois 1997).

In Manitoba, use of ACCase inhibitor herbicides increased from 15% to 50% of sprayed fields between 1981 and 1993 (Bourgeois 1997). Resistance is a major concern since 60% or more of the sprayed cereals, over 90% of flax, and about 50% of canola are treated with these herbicides annually in Manitoba (Goodwin 1992). It is now estimated that nearly half of the cultivated land in Manitoba is at risk of developing resistance to the ACCase inhibitor herbicides (Bourgeois 1997).

In grasses susceptible to ACCase inhibitor herbicides, growth of the meristems is inhibited after herbicide contact, and chlorosis of emerging leaves is observed within days of herbicide application. Plant death occurs a few weeks after treatment (Devine and Shimabukuro 1994). Resistant plants do not exhibit any severe herbicide injury symptoms. However, the levels and patterns of resistance to ACCase inhibitor herbicides vary widely among different resistant populations (Morrison and Devine 1994). This is seen in both field and laboratory dose response experiments (Heap and Morrison 1996).

2.2.1 The Acetyl-CoA Carboxylase Enzyme

Both families of graminicides, APPs and CHDs, inhibit acetyl-coenzyme A (CoA) carboxylase (ACCase), a key enzyme in fatty acid biosynthesis (Figure 2.1). ACCase, a high molecular mass multifunctional protein, catalyzes the

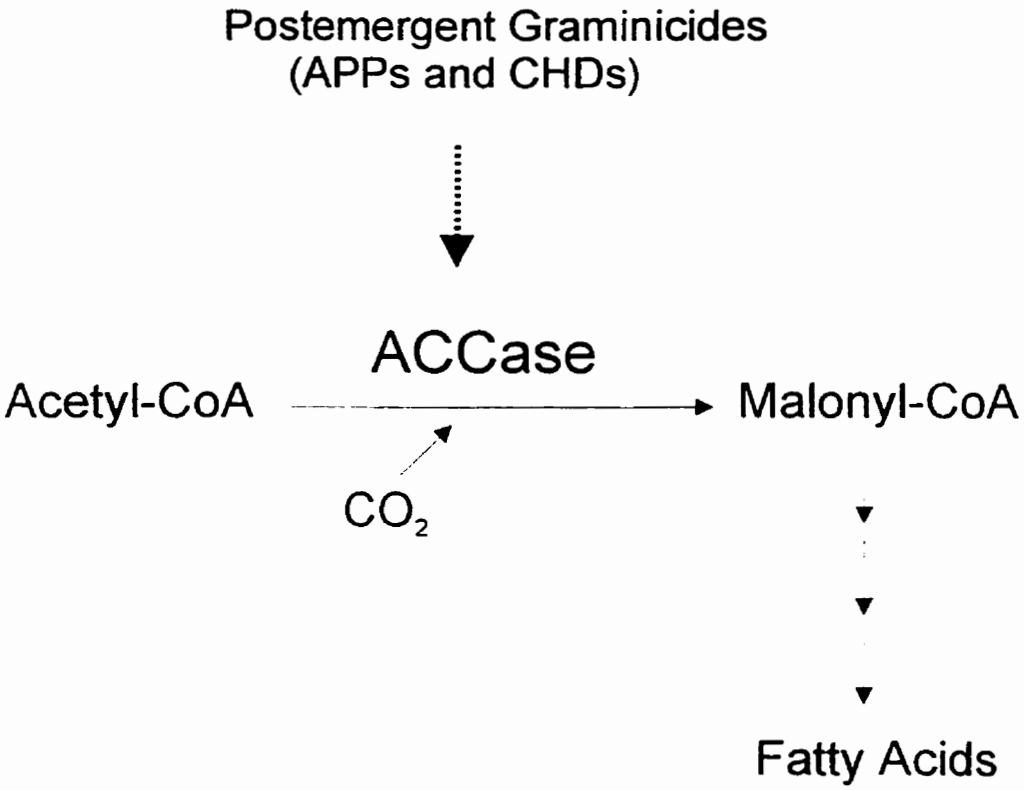

Figure 2.1. The interaction of graminicides with Acetyl-CoA carboxylase (ACCase) within the fatty acid biosynthesis pathway. Site of inhibition by APPs and CHDs is indicated by dashed arrow.

ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA. This process occurs in the chloroplasts, and plastids of non-green tissue (Harwood 1989). The enzyme reaction involves biotin, an intermediate carboxyl carrier. A carboxylated biotin serves as the donor to acetyl-CoA and it is this transcarboxylation which is specific to ACCase (Harwood 1989).

Both the APP and the CHD herbicides interfere with the carboxyltransfer site rather than the biotin carboxylation site of the ACCase. These herbicides may bind to the same binding domain on the enzyme, but interact with different amino acids (Lichtenthaler et al. 1989). Because there is differential inhibition of different acetyl-CoA carboxylases by graminaceous herbicides, several structural variants of the enzyme must be present (Harwood 1989).

ACCase occurs in prokaryotic and eukaryotic forms in nature (Sasaki et al. 1995). Dicots contain both types of the enzyme, a eukaryotic form in the cytosol and a prokaryotic form in the plastids. Grasses have the eukaryotic type of the enzyme in both parts of the cell (Sasaki et al. 1995). The presence of the eukaryotic form of ACCase, but not the prokaryotic form in the plastids of grasses, may in part explain the susceptibility of many grasses towards APPs and CHDs (Konishi and Sasaki 1994; Sasaki et al. 1995).

2.2.2 Mechanisms of Resistance to ACCase Inhibitor Herbicides

Resistance to APP and CHD herbicides in dicots is due to the presence of a herbicide insensitive prokaryotic form of ACCase. Some grasses such as

wheat, which has a sensitive enzyme, are tolerant to some of these herbicides because they can metabolize them. Other grasses are resistant because they contain a resistant eukaryotic form of ACCase.

Resistance to the ACCase inhibitor herbicides in green foxtail is due to reduced sensitivity of ACCase. Marles et al. (1993) reported that ACCase from a green foxtail resistant biotype, UM8, was much less sensitive to the ACCase inhibitor herbicides than the enzyme from a susceptible biotype, UM7. This conferred broad cross resistance to all ACCase inhibitors (Heap and Morrison 1996).

In corn (*Zea mays* L.) lines selected for resistance to sethoxydim and haloxyfop in cell culture, resistance was correlated with the presence of a resistant form of ACCase (Parker et al. 1990). The resistance conferred by the altered enzyme in these corn lines was encoded by a single, incompletely dominant nuclear gene (Parker et al. 1990; Marshall et al. 1992). Gronwald et al. (1992) found that resistance to diclofop in Italian ryegrass, *Lolium multiflorum* Lam., was due to the presence of a resistant form of ACCase, which was also encoded by a single, incompletely dominant nuclear gene (Betts et al. 1992). Finally, Egli et al. (1993) found that maize has at least two isozymes of the eukaryotic form of ACCase. From these studies of ACCase mutants of maize with different sensitivities to herbicides, three, or possibly five, alleles of maize ACCase structural genes may be present (Marshall et al. 1992).

Knowledge of the detailed structure of the ACCases in monocots,

including green foxtail, is needed to explain the differences in sensitivity between susceptible and resistant, or different resistant biotypes. At present, it is not known which sites on the enzyme are responsible for the sensitivity to herbicides. Comparison of the amino acid sequences deduced from various cDNAs of ACCase with different herbicide sensitivity could lead to the identification of the structural requirements for resistance (Sasaki et al. 1995).

2.3 Trifluralin, a Dinitroaniline Herbicide

Trifluralin, a dinitroaniline herbicide, was first introduced into western Canada in the early 1970s. It is the active ingredient in a variety of commercial herbicide products. Trifluralin is used to control green foxtail when applied either as a preplant incorporated treatment in oilseed crops, or as a preemergence incorporated treatment in wheat and barley. In grasses susceptible to trifluralin, injury symptoms include root pruning, swelling at the base of the shoot, and stunted growth (Morrison et al. 1989). Dinitroaniline herbicides were heavily used in southwestern Manitoba from 1982 to 1988. However, the use of these herbicides has decreased since the discovery of Group 3 resistant green foxtail throughout the region.

2.3.1 Mechanisms of Trifluralin Resistance

Trifluralin and other dinitroaniline herbicides inhibit cell division by binding to beta-tubulin and interfering with tubulin polymerization (Devine et al.

1993). In dinitroaniline resistant goosegrass (*Eleusine indica* (L.) Gaertn.), resistance is conferred by an alteration in the beta-tubulin (Vaughn and Vaughan 1990). Trifluralin resistance in green foxtail has been linked to an alteration in a microtubule-associated protein (MAP) (Smeda et al. 1992). The role that MAPs play in trifluralin action and resistance in green foxtail is unknown (Morrison and Devine 1994).

2.4 Multiple Herbicide Resistance in Weed Populations

To aid farmers in developing weed control strategies that reduce the risk of herbicide resistance, herbicides have been classified into herbicide groups (Groups 1 to 10, 25, and 26 in western Canada) (Appendix). Herbicides are included in a group if they have the same site and/or mechanism of action (Groups 1 to 7), or if resistance to one herbicide also confers resistance to another (Group 8) (Morrison and Devine 1994). Cross resistance can be defined as varying levels of resistance to herbicides within the same herbicide group. Meanwhile, multiple herbicide resistance refers to resistance to herbicides with different modes of action (i.e. resistance to herbicides from two or more herbicide groups) (Manitoba Agriculture 1996).

Annual ryegrass, *Lolium rigidum* Gaud., in Australia, and blackgrass, *Alopecurus myosuroides* Huds., in Europe, were the first grass species shown to exhibit both herbicide cross and multiple resistance (Powles and Preston 1995). Varying patterns of multiple resistance can be found in different biotypes of

annual ryegrass, *L. rigidum*, from Australia (Powles and Matthews 1991). Ryegrass has evolved unique mechanisms of resistance for each herbicide group. The genetic diversity in ryegrass and the frequent exposure of large numbers of plants to various herbicides has resulted in the selection of one to many different resistance genes (Powles and Matthews 1991).

In Manitoba, populations of wild oat (*Avena fatua* L.) resistant to three different herbicide groups, imazamethabenz (Group 2), flamprop-methyl (Group 25), and fenoxaprop-p-ethyl (Group 1) have been reported (Morrison et al. 1995). Furthermore, populations of green foxtail in Manitoba have been confirmed to be resistant to ACCase inhibitor (Group 1) herbicides as well as to trifluralin (Group 3) (Heap 1994). Indeed, multiple herbicide resistance will likely be discovered in more weed species around the world, and will seriously limit chemical weed control options.

2.5 Genetic Variation and the Origin of Herbicide Resistance in Weeds

Genetic variation for resistance is required for evolution of herbicide resistance to occur (Jasieniuk et al. 1996). Genetic variation in a specific population may occur through gene mutation or gene flow through pollen or seed (Jones and Luchsinger 1986). The relative importance of gene mutation versus gene flow as a source of resistance genes in susceptible weed populations is unknown (Jasieniuk et al. 1996). However, it is generally thought that mutation is likely to result in the initial appearance of herbicide resistance in a particular

geographic area, whereas gene flow is more likely to cause its spread among populations in a region.

A limited number of studies have been conducted to examine the source of genetic variability in herbicide resistant and susceptible weed populations. In most studies, only isozyme electrophoretic variation in triazine resistant populations have been assessed. Gasquez and Compoint (1981) and Warwick and Marriage (1982) observed distinct isozyme phenotypes in triazine resistant lamb's quarters (*Chenopodium album* L.) populations from various regions, but the same isozyme phenotype was found in populations from a given area. Mutation for resistance in the lamb's quarters' populations occurred independently in different geographic regions, but spread of herbicide resistance within an area was likely due to gene flow.

It is generally considered that there is less genetic variation in herbicide resistant populations than in susceptible ones. This would occur because of the founder effect, where the resistant population would spread from a single or only a few initial resistant plants (Warwick and Black 1986). The mating system of the weed species will also affect the degree or pattern of genetic variation within and among populations (Warwick 1990; Warwick and Black 1993). In predominantly selfing weed species, populations tend to be more genetically uniform within a particular location, with much more differentiation existing among populations. Also, in a highly selfed weed species, such as green foxtail, founder effects should be quite apparent compared to outbreeding species

(Warwick 1990; Warwick and Black 1993).

Warwick and Black (1986) studied isozyme electrophoretic variation in triazine resistant and susceptible populations of *Amaranthus retroflexus* L., redroot pigweed. Their results agreed with the founder effect hypothesis, as the resistant population had less genetic variation than the susceptible. Other isozyme studies by Gasquez and Compoint (1981), and Al Mouemar and Gasquez (1983) on lamb's quarters showed reduced genetic variability both within and among resistant populations from the same area. Warwick and Black (1993) also found that genetic variation for allogamous triazine resistant Polish canola, *Brassica rapa* L., populations was not as reduced as that previously reported for autogamous triazine resistant plant species.

The founder effect has not been confirmed in all studies. In studies of triazine resistant populations of *Poa annua* L. (Darmency and Gasquez 1981, 1983), and common groundsel, *Senecio vulgaris* L., (Putwain et al. 1983), resistant populations had as much isozyme polymorphism as susceptible ones. Dyer et al. (1993) investigated genetic variation within and among sulfonylurea resistant and susceptible kochia, *Kochia scoparia* L. Schrad. populations. Using Random Amplified Polymorphic DNA (RAPD) data, they indicated that the degree of genetic variability within kochia populations was equivalent to variability among populations, and suggested that even relatively low outcrossing rates of about 4% in kochia are sufficient to create heterogeneous populations (Dyer et al. 1993).

Chauvel and Gasquez (1994) examined the relationships between genetic polymorphism and herbicide resistance within allogamous blackgrass, *Alopecurus myosuroides* Huds., in an isozyme electrophoretic survey of 19 resistant and susceptible populations collected from different countries. Blackgrass had a high level of genetic polymorphism but there was little genetic differentiation between susceptible and triazine resistant populations (Chauvel and Gasquez 1994). Moodie et al. (1997) assessed genetic variation in populations of outcrossing wild mustard (*Sinapsis arvensis* L.) using RAPD analysis, and found that the range of genetic variation was as high in the herbicide treated populations as in those which were untreated.

2.5.1 Modes of Inheritance of Herbicide Resistance

The mode of inheritance of herbicide resistance is one of the major factors that influences evolution of herbicide resistance (Gressel and Segal 1978, 1982; Maxwell et al. 1990). The number of genes, and their allelic and genic interactions can influence the rate of spread of herbicide resistance within and among weed populations (Jasieniuk et al. 1994; Maxwell et al. 1990). Cytoplasmic, polygenic, and monogenic control are all possible modes of inheritance for herbicide resistance.

Cytoplasmic inheritance has been reported in many studies of triazine resistant weeds (Jasieniuk et al. 1996), including triazine resistant green foxtail, *Setaria viridis* (L.) Beauv. (Darmency and Pernes 1985). With cytoplasmic

inheritance, all progeny of the resistant female plant will be resistant (Scott and Putwain 1981). Cytoplasmic inheritance will not contribute to the spread of resistance among populations through pollen dispersal but will through seed dispersal.

There are only a few known cases of polygenic control of herbicide resistance. Siduron tolerance in foxtail barley, *Hordeum jubatum* L., was controlled by three dominant genes (Schooler et al. 1972). Faulkner (1974) found a quantitative pattern of variation for paraquat resistance in *Lolium perenne* L. More recently, Chauvel (1991) reported that polygenic inheritance of herbicide resistance occurred in blackgrass, *Alopecurus myosuroides* Huds. The fact that there are not many cases of polygenic control is not surprising. Where many genes confer resistance in a quantitatively inherited manner, the rate of evolution and spread of resistance would be slower than for a single nuclear encoded gene (Mortimer 1992). The probability of a plant having all the required resistance genes would be extremely low.

The mode of inheritance of ACCase inhibitor resistance in weed species has been examined in a limited number of studies. Diclofop-methyl resistance in Italian ryegrass, *Lolium multiflorum* Lam. (Betts et al. 1992), APP resistance in winter wild oat, *Avena sterilis* spp. *ludoviciana* (Barr et al. 1992), and ACCase inhibitor resistance in wild oat, *Avena fatua* L. (Murray et al. 1995) are controlled by single incompletely dominant nuclear genes.

In a weed population, a mutation for herbicide resistance will be rare and

initially appear in the heterozygous state (Jasieniuk et al. 1994;1996). If a mutation is dominant, the resistant trait will be expressed immediately in the progeny and resistance will spread. However, with a recessive mutation, resistance will be more difficult to achieve since the heterozygotes will also be susceptible and die when sprayed with herbicide (Jasieniuk et al. 1996). Resistance, controlled by recessive alleles in an outcrossing weed species, is much less likely to evolve because it takes longer for the recessive alleles to reach an appreciable frequency, especially under herbicide treatment (Mortimer 1992). Therefore, assuming diploidy and random mating, at low gene frequencies, a dominant allele will cause a faster spread of resistance than a recessive allele (Mortimer 1992). However, this would not be the case in self pollinating plant species (Jasieniuk et al. 1996).

Recessive control of herbicide resistance has only been found in one weed species. Jasieniuk et al. (1994) reported that trifluralin resistance in green foxtail (*Setaria viridis* (L.) Beauv.) was controlled by a single, nuclear recessive gene. Green foxtail's predominantly selfing nature, and its prolific seed production may have facilitated this type of genetic control of herbicide resistance. First, the rate of evolution of a favorable recessive mutation in a plant population is greatly increased by selfing. Selfing reduces the chance of loss of a newly arisen recessive mutation by rapidly increasing the frequency of the homozygotes (Jasieniuk et al. 1994;1996). Secondly, the prolific seed production of green foxtail would quickly increase the frequency of resistant

individuals in the population (Jasieniuk et al. 1994;1996).

2.6 Detection of Genetic Variation in Weed Populations - Random Amplified Polymorphic DNA (RAPD)

In the past, methods such as isozyme electrophoresis and restriction fragment length polymorphism (RFLP) analyses were used to assess genetic variability of weed populations. More recently, identification and evaluation of Random Amplified Polymorphic DNA (RAPD) (Welsh and McClelland 1990; Williams et al. 1990) has been used to detect genetic variation among plant populations. The utility of RAPDs in weed population genetics has been demonstrated by several workers (Bowditch et al. 1993; Dyer et al. 1993; Huff et al. 1993; Colosi and Schaal 1997; Moodie et al. 1997).

Both isozyme electrophoresis and RFLP techniques can provide a considerable amount of genetic information but they are also very slow and expensive, and are not suitable for large scale studies (Moodie et al. 1997). Isozyme studies require large amounts of plant tissue for analysis and only assess variability at a narrow range of loci corresponding to expressed alleles. RFLP assays require large amounts of pure DNA and usually a radioactive detection method. RAPD assays have the advantage of requiring only small quantities of DNA. They are used to assess total genomic DNA, require no prior knowledge of the plant species' genetics and are technically simple, swift, low in cost, and radioisotope free. The only major disadvantage with the RAPD

technique is its inability to identify heterozygous genotypes, so there is less genetic information for analysis. Isozyme electrophoretic studies on highly selfing species, such as green foxtail, have indicated limited polymorphism (Wang et al. 1995). A RAPD assay will provide an opportunity to assess more genetic loci.

Identification of genetic variation via RAPD is based on DNA amplification by the Polymerase Chain Reaction (PCR) using a single primer of arbitrary nucleotide sequence (Bowditch et al. 1993). Nucleotide sequence polymorphisms between individuals can be detected with RAPD. The RAPD assay involves extracting DNA from young leaf tissue of a plant. The DNA is then added to a reaction mixture that consists of a primer, nucleotides, DNA polymerase, magnesium chloride, and buffer (Williams et al. 1993). DNA strands are amplified via thermal cycling. In one reaction, during the heating stage of a temperature cycle, the double stranded DNA is denatured into two single strands. At a lower temperature, the primer, which is an arbitrary nucleotide sequence 10 bases long, anneals to complementary sites on the opposite strands of the now single stranded DNA. During the last step in the temperature cycle, a new DNA fragment is produced as complementary nucleotides are added onto the primer chain in each direction. Further amplification of the DNA fragments occurs by repeated temperature cycling, which is controlled by an automated thermocycler (Williams et al. 1993). The amplified DNA can be separated by agarose gel electrophoresis, visualized by

staining with ethidium bromide, and viewed under ultraviolet light.

DNA samples from different sources can be assessed with several different primers, under conditions that result in several amplified bands from each primer (Williams et al. 1993). RAPD patterns obtained are dependent on both the genomic DNA template and the specific primer used. Fragment sizes range from 0.1 kb to 3.0 kb, and fragment numbers per primer range from 0 to 10 (Yu et al. 1993). Polymorphisms for RAPDs may be due to single base pair changes or substitutions, deletions of primer sites, insertions that increase the distance between the priming sites giving fragments so large that amplification is inefficient, and small insertions or deletions that result in changes in the size of the RAPD product (Yu et al. 1993). Because of these differences in the nucleotide sequences of different individuals, not all individuals will have the same primer binding sites. As a result, in one individual, a DNA fragment may be present, whereas in another it is absent. These RAPD band patterns from different individuals or populations can then be assembled into a presence/absence or binary data matrix for subsequent phylogenetic analyses.

2.6.1 Phylogenetic Analysis of RAPD Data

Phylogenetic analysis of RAPD data can be used to identify individuals or populations which may have arisen from a common origin, and those which are most closely related. These relationships are displayed in the form of a radial phenogram or tree. With RAPD data, the systematic characters are defined as

the RAPD fragments of certain molecular weight or size, while the character states are the presence (1) or absence (0) of that band. It must be noted that the absence of a band can represent many different alleles at a RAPD locus, while the presence of a band demonstrates an amplifiable sequence of a specific length (Williams et al. 1993). Therefore the character state 0 may in reality encompass many different character states, such as point mutations or inversions. Therefore, the likelihood of band loss through mutation will generally be greater than the likelihood of regaining the same band; the transition probabilities between character states are asymmetrical (Williams et al. 1993). This situation is analogous to when restriction site data are used for phylogenetic analysis. One can not be sure that the comigrating RAPD bands are homologous in every sample analysed. However, the inference of homology is strong when total sequence divergence between the taxa is low and many RAPD bands are shared (Williams et al. 1993).

A variety of methods are available for the phylogenetic analysis of restriction site or RAPD data (Swofford and Olsen 1990; Holsinger and Jansen 1993). Each phylogenetic method has its own set of assumptions, strengths and weaknesses. No single ideal method exists. Distance, parsimony, and maximum likelihood methods are three general approaches to phylogenetic analyses. With distance methods, the character state data are summarized in a distance coefficient matrix which relates all pairs of taxa (Holsinger and Jansen 1993). There are a number of distance methods, including UPGMA (unweighted

pair group method using arithmetic averages) cluster analysis, the Fitch-Margoliash , and neighbour-joining (Holsinger and Jansen 1993). UPGMA has been chosen in the majority of plant genetic studies with RAPD data (Vierling and Nguyen 1992; Williams and St. Clair 1993; Mailer et al. 1994; Moodie et al. 1997). However, it appears to work well only if the rate of substitutions is constant and the distances among the taxa are large. These assumptions are not reasonable for many data sets (Holsinger and Jansen 1993).

In contrast to distance methods, parsimony methods are used to select the best tree or trees, which requires the minimum amount of evolutionary change from a set of all possible trees. Wagner, Dollo, and weighted (generalized) parsimony each have their own way in which evolutionary change is calculated, i.e. minimizing the length of the tree (Holsinger and Jansen 1993). The Dollo parsimony method (Farris 1977) assumes that the gain of the character state 1 is so unlikely relative to its loss that any taxa sharing that particular site must have inherited it from a common ancestor. So, character state 1 can be gained only once, but it may be lost many times. The Dollo criterion for minimizing the length of the tree corresponds to minimizing the total number of losses while allowing each site to be gained only once (Holsinger and Jansen 1993).

The maximum likelihood model is another promising approach for the phylogenetic analysis of restriction site data (Holsinger and Jansen 1993) or RAPD data. Smouse and Li (1987) showed how this model is used with only

four taxa, and Felsenstein (1992) extended this model to an arbitrary number of species. The assumptions of this method are that : i) each site evolves independently, ii) different lineages evolve independently, iii) each site undergoes substitution at an expected rate which can be specified, and iv) substitutions consist of replacement of a nucleotide by one of the other three nucleotides, chosen at random (Felsenstein 1993).

The maximum likelihood method has one great advantage over other phylogenetic methods in that its statistical properties are well understood (Holsinger and Jansen 1993). However, the major difficulty with the maximum likelihood method is that it is computationally inefficient, so it can not generally be used on large data sets (i.e. more than 20 taxa) (Felsenstein 1993). Yet, this method can still be useful as a means of evaluating trees of large data sets found using one of the other phylogenetic methods (Holsinger and Jansen 1993). For example, the Dollo parsimony and maximum likelihood models can be used in succession on a large data set to produce a phylogenetic tree with branch lengths.

Finally, bootstrapping can be used to assess the significance of the groupings within the phylogenetic tree that is produced by a chosen phylogenetic method (Holsinger and Jansen 1993). Bootstrapping samples from the original binary data set, drawing characters with replacement, to construct a series of 100 new character state data matrices (Felsenstein 1985,1993). Each of these data sets is then analysed (by a distance, parsimony, or maximum

likelihood method) and a record is kept of all groups of populations that form monophyletic subsets. There is significant evidence that a group of populations is monophyletic if it occurs in at least 95% of the bootstrap estimates (Felsenstein 1993).

3. Characterization of Fenoxaprop-p-ethyl Resistance in Green Foxtail Populations Used in the Inheritance Study

3.1 Abstract

Fenoxaprop-p-ethyl resistance levels in three parental resistant (R) green foxtail populations, UM8, UM131, and UM137, as compared with susceptible (S) green foxtail populations, UM7 and 439-86, were determined in growth room dose response experiments. The resistant green foxtail populations were 12 to 29 times more resistant to fenoxaprop-p-ethyl than the susceptible populations based on their R/S ratios.

3.2 Introduction

Herbicide resistant weeds have been identified in most major agricultural regions of the world (Heap 1997). Herbicide dose response experiments under controlled conditions are often conducted to confirm and characterize suspected herbicide resistant (R) weed populations. These dose response experiments also provide the basic information required for evaluation of populations in herbicide resistance inheritance studies. Field and growth room ACCase inhibitor (Group 1) dose response experiments have been conducted on various green foxtail populations, including UM8 and UM131 (Heap and Morrison 1996). In field experiments, Heap and Morrison (1996) confirmed that UM8 was resistant to diclofop-methyl, fenoxaprop-p-ethyl, sethoxydim, and tralkoxydim at

dosages up to 4 times the recommended rate. Under growth room conditions, UM131 and UM8 generally exhibited similar patterns and levels of resistance to these herbicides, with the exception of sethoxydim. UM131 was at least 75 times more resistant to sethoxydim than UM8 and the other populations tested. UM8 and UM131 exhibited a similar level of resistance to fenoxaprop-p-ethyl, with resistant/susceptible (R/S) ratios of 5 and 11, respectively (Heap and Morrison 1996).

The objective of this research was to quantify the level of resistance to fenoxaprop-p-ethyl in the parental R populations, UM8, UM131, and UM137, as compared to the susceptible (S) green foxtail populations, UM7 and 439-86.

3.3 Materials and Methods

3.3.1 Parental Populations Used in the Inheritance Study

The R populations, UM8 and UM131, were first identified in the autumn of 1990 and 1991, respectively. Population UM8 was collected from Thornhill, Manitoba, and UM131 was collected from Gilbert Plains, Manitoba (Heap and Morrison 1996). S population UM7 and R population UM137 (Heap 1994) were collected near Portage la Prairie, Manitoba. S population 439-86 originated from China. One characteristic of population 439-86 is a dominant genetic marker for anthocyanin, which is useful for identification of hybrids in crossing experiments (Jasieniuk et al. 1994). A detailed field history for UM8 is not known but the population was repeatedly exposed to diclofop-methyl and sethoxydim for 12

years (Heap and Morrison 1996). The field histories for populations UM131 (Heap and Morrison 1996) and UM137 are listed in Table 3.1.

3.3.2 Growth Room Dose Response Experiments

Green foxtail seed was sown 1 cm deep in 9 cm diameter plastic pots (volume 500 ml) filled with a 1 cm layer of peat on the bottom and then a clay loam/sand/peat potting mixture in a 2:1:1 (by volume) ratio. Seed of 439-86 was sown five days earlier than the other populations to compensate for its prolonged emergence period when compared to the other populations. About 16 seeds were sown into each pot. UM137 seeds were sown at a higher density than the other populations, about 50 seeds per pot, due to poor germination. After emergence, the green foxtail seedlings were thinned to 8 seedlings per pot. The plants were grown in a growth room at 22/16°C, 16/8 hour day/night regime at an average light intensity of 500 $\mu E\ m^{-2}\ s^{-1}$. Plants were watered daily and a water-soluble complete (20-20-20) fertilizer[1] was used as a dilute solution (2.4 g product L^{-1}) weekly.

When the green foxtail plants were at the 3- to 4- leaf stage, fenoxaprop-p-ethyl was applied in a cabinet sprayer equipped with a flat-fan nozzle[2] that delivered 117 L ha^{-1} of spray solution in a single pass over the foliage at a spray pressure of 310 kPa. The following rates of fenoxaprop-p-ethyl were applied: 0,

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

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

Table 3.1. Field histories for the herbicide resistant green foxtail populations, UM131 (from Heap and Morrison 1996) and UM137.

Year	UM131		UM137	
	Crop	Herbicide Treatment	Crop	Herbicide Treatment
1976	-	-	Barley	Difenzoquat
1977	-	-	Mustard	Diclofop-methyl
1978	-	-	Wheat	-
1979	-	-	Lentil	Trifluralin and Diclofop-methyl
1980	-	-	Lentil	Trifluralin
1981	-	-	Wheat	Diclofop-methyl
1982	-	-	Lentil	Trifluralin
1983	-	-	Wheat	-
1984	-	-	Flax	Sethoxydim
1985	Wheat	Diclofop-methyl	Wheat	-
1986	Wheat	Diclofop-methyl	Canola	Trifluralin and Sethoxydim
1987	Fababean	Sethoxydim	Wheat	-
1988	Wheat	Diclofop-methyl	Lentil	Trifluralin and Sethoxydim
1989	Barley	Diclofop-methyl	Bean	Sethoxydim[z]
1990	Mustard	Ethalfluralin	Wheat	-
1991	Wheat	Fenoxaprop-p-ethyl	Lentil	Trifluralin and Sethoxydim
1992	-	-	Bean	Sethoxydim

[z]Applied twice.

0.25, 0.5, 1, 2, 4, 8, and 16 g ai (active ingredient) ha^{-1} for the two S populations, and 0, 4, 8, 16, 40, 80, 160, and 320 g ai ha^{-1} for the three R populations. With the exception of untreated controls, four pots were used per treatment, in a randomized complete block design. For the untreated controls, 6 pots per population were harvested at the time of herbicide application, and when the experiment was terminated (i.e. 21 days after herbicide treatment (DAT)). Shoots were harvested 21 DAT, oven dried for 48 hours at 80°C, and then weighed to determine shoot dry weights. The mean shoot dry weight at the time of spraying was subtracted from total shoot dry weight of controls, and the results of the treated samples were expressed as a percentage of untreated controls.

The growth room dose response experiment was repeated once following the same methodology except that rates of fenoxaprop-p-ethyl were modified to provide better resolution of the dose response curves. For the R populations, the 4, 40, and 320 g ai ha^{-1} rates were dropped, and 30 and 60 g ai ha^{-1} were included. The 30 and 60 g ai ha^{-1} rates were included to provide additional data in this region of the dose response curves.

3.3.3 Statistical Analysis

A test for homogeneity of variances was done to determine if the results from the two dose response experiments could be combined (Gomez and Gomez 1984). Variances for shoot dry weight percent of control were homogeneous so the data were pooled.

The data were subjected to nonlinear regression analysis[3] on treatment means as recommended by Gomez and Gomez (1984). The model (Brain and Cousens 1989) fitted was:

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

where y= shoot dry matter expressed as a percentage of untreated controls, x= the herbicide dosage (g ai ha^{-1}), d= the lower asymptote, k+d= the upper asymptote, e= the base of the natural logarithm, and b and g determine the slope of the inflection region of the curve. GR_{50} values are calculated as the antilog of -g (i.e. $GR_{50}=e^{(-g)}$). This model was chosen because it was developed specifically for use with herbicide dose response data.

3.4 Results and Discussion

The sigmoidal dose response function generally fit the data well with high R^2 values (Figure 3.1; Table 3.2). Data points (means) generally fell close to the appropriate regression line, with the exception of UM137. For UM137, shoot growth at 30 g ai ha^{-1} (*ln* 3.4) was inhibited to a greater extent than at 40 g ai ha^{-1} (*ln* 3.7) (Figure 3.1). Only 4 replicates were included at the 30 and 40 g ai ha^{-1} rates, one in each of the dose response experiments. This is reflected in increased variability of the means in this region of the curve, particularly for population UM137. Also, most of the populations exhibited somewhat increased shoot biomass at very low dosages, as compared to the untreated control. This phenomenon is commonly observed in dose response studies (Brain and

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

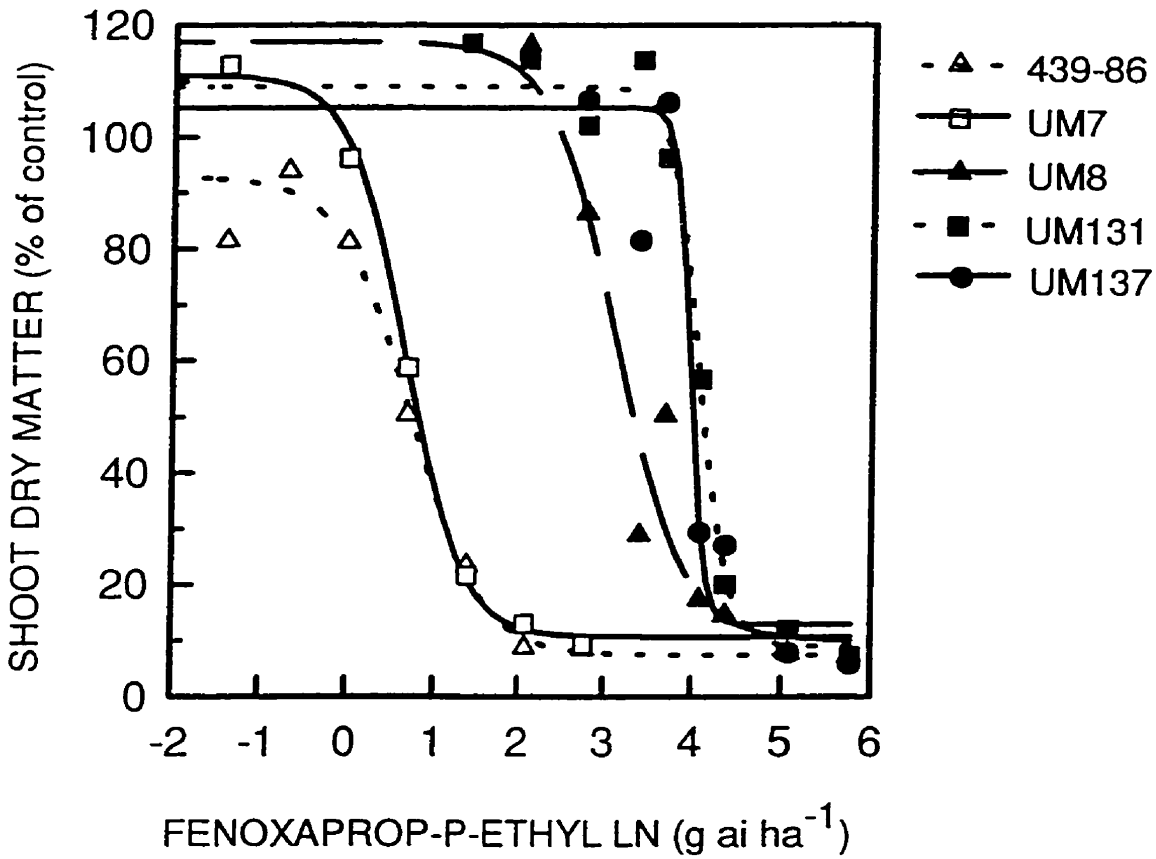

Figure 3.1. Response of five green foxtail populations to increasing dosages of fenoxaprop-p-ethyl. Refer to Table 3.2 for parameter estimates, GR_{50} values, and R/S ratios.

Table 3.2. Parameter estimates[z] used to describe the response of susceptible (UM7 and 439-86) and resistant (UM8, UM131, and UM137) green foxtail populations to fenoxaprop-p-ethyl.

Population	g	b	d	k	R^2	GR_{50}[y]	R/S[x]
UM7	-0.66	3.01	10.64	100.44	0.98	2	NA[w]
439-86	-0.72	2.43	7.37	85.58	0.98	2	NA
UM8	-3.12	2.57	9.98	107.09	0.94	23	12
UM131	-4.07	6.09	8.95	100.07	0.98	58	29
UM137	-3.97	12.16	12.91	92.37	0.94	53	27

[z]Parameter estimates are for shoot dry weight expressed as a percentage of untreated controls. The model fitted was $y=(k/(1+e^{bg}x^{b}))+d$ (d=lower asymptote, k+d=yield of untreated controls). R^2=coefficient of determination.

[y]GR_{50} values are the dosages in g ha^{-1} of fenoxaprop-p-ethyl that reduced shoot dry weights by 50% relative to the untreated control 21 days after spraying. Mean shoot dry weights for untreated controls of UM7, 439-86, UM8, UM131, and UM137 were 0.72, 0.76, 0.72, 0.73, and 0.66 g pot^{-1}.

[x]R/S is the ratio of the GR_{50} of the resistant population to the GR_{50} of the susceptible population, 439-86.

[w]NA=Not Applicable.

Cousens 1989).

Both S populations, UM7 and 439-86, exhibited similar responses to increasing dosages of fenoxaprop-p-ethyl (Figure 3.1). They also had the same GR_{50} value of 2 g ai ha^{-1}. Heap and Morrison (1996) reported a similar GR_{50} value of 3 g ai ha^{-1} of fenoxaprop-p-ethyl for UM7. The 439-86 population, which originated from China, was used as the male parent in genetic crosses to determine the inheritance of fenoxaprop-p-ethyl resistance in populations of green foxtail from Manitoba.

The R green foxtail populations were 12 to 29 times more resistant to fenoxaprop-p-ethyl than the S populations based on the R/S ratios (Table 3.2). Of the R populations, UM8 had the lowest level of resistance to fenoxaprop-p-ethyl with a GR_{50} of 23 g ai ha^{-1}. UM131 had the highest level of resistance with a GR_{50} of 58 g ai ha^{-1}. The GR_{50} values for UM8 and UM131 obtained from these dose response experiments are 1.5 to 1.7 times the values determined by Heap and Morrison (1996). This difference can be attributed to the difference in the calculated GR_{50} values of the S populations used, and possibly slight differences in the experimental conditions, such as temperature, levels of fertilizer, and/or seed vigor. The UM137 population had a level of resistance similar to UM131, with a GR_{50} of 53 g ai ha^{-1} (Table 3.2).

4. Inheritance of Fenoxaprop-p-ethyl and Trifluralin Resistance in Green Foxtail (*Setaria viridis* (L.) Beauv.).

4.1 Abstract

The inheritance of fenoxaprop-p-ethyl resistance was examined in three green foxtail (*Setaria viridis* (L.) Beauv.) populations, UM8, UM131, and UM137 from Manitoba. The inheritance of trifluralin resistance in the multiple resistant (R) population, UM137, was also determined. Since green foxtail is highly autogamous and has small reproductive structures, plants from the three R green foxtail populations were crossed with plants from a susceptible (S) Chinese population, 439-86, that has a dominant marker for anthocyanin pigmentation. F_1 hybrids were distinguished from selfed progeny by the purple pigmentation of the coleoptile, shoot, first leaf, and setae. F_1 hybrids were selfed and F_2 plants were sprayed at different rates of fenoxaprop-p-ethyl, and then visually scored as resistant (R), injured (I) or susceptible (S). UM137/439-86 F_2 seedlings were screened for trifluralin resistance or susceptibility at different trifluralin concentrations using a petri dish bioassay. Fenoxaprop-p-ethyl resistance in green foxtail populations, UM8, UM131, and UM137 is controlled by single nuclear incompletely dominant genes, and trifluralin resistance in UM137 is controlled by a single nuclear recessive gene. The genes for fenoxaprop-p-ethyl and trifluralin resistance in the multiple R population, UM137, are not linked. Green foxtail, with its highly selfing nature

and prolific seed production, allows both types of genetic control of herbicide resistance.

4.2 Introduction

Herbicide resistant (R) weeds pose a serious threat to sustained productivity and profitability of western Canadian agriculture. Trifluralin R green foxtail (*Setaria viridis* (L.) Beauv.) populations were the first herbicide R weeds confirmed in Manitoba (Morrison et al. 1989). Since then, green foxtail populations have been identified with resistance to the acetyl-CoA carboxylase (ACCase) inhibitor herbicides (Heap and Morrison 1996). By 1994, hundreds of trifluralin R, and 20 ACCase inhibitor R green foxtail populations had been reported in western Canada (Friesen 1994). At least four populations have been identified with resistance to both trifluralin and fenoxaprop-p-ethyl, a common ACCase inhibitor herbicide.

Seeds from suspected herbicide resistant populations of green foxtail were collected from fields in Manitoba in the fall of 1990 and 1991. One population from Thornhill (UM8) and one from Gilbert Plains (UM131) were identified as resistant to the ACCase inhibitor herbicides in growth room and field dose response experiments (Heap and Morrison 1996). Under field conditions, UM8 was resistant to diclofop-methyl, fenoxaprop-p-ethyl, sethoxydim, and tralkoxydim at up to 4 times the recommended rate. Under growth room conditions, UM131 generally had herbicide resistance responses

similar to UM8, but it was greater than 75 times more resistant to sethoxydim than UM8 and the other populations tested. In subsequent studies, a population from Portage la Prairie (UM137) was identified with resistance to both trifluralin and the ACCase inhibitor herbicides (Heap 1994).

The mode of inheritance of herbicide resistance is one of the major factors that influences the evolution of herbicide resistance (Gressel and Segal 1978,1982; Maxwell et al. 1990; Jasieniuk et al. 1996). Cytoplasmic inheritance has been reported in many studies on triazine R weeds (Jasieniuk et al. 1996). Generally, weed species with resistance to herbicides other than triazine have a single, nuclear, dominant or incompletely dominant gene controlling resistance (Jasieniuk et al. 1996). Only one instance of a single nuclear recessive resistance gene has been reported (Jasieniuk et al. 1994).

A limited number of researchers have investigated the mode of inheritance of ACCase inhibitor resistance in weed species. Diclofop-methyl resistance in Italian ryegrass (*Lolium multiflorum* Lam.) (Betts et al. 1992), APP resistance in *Avena sterilis* spp. *ludoviciana* (Barr et al. 1992), and ACCase inhibitor resistance in wild oat, *(Avena fatua* L.) (Murray et al. 1995) are all controlled by single, nuclear, incompletely dominant genes.

Green foxtail is a highly self pollinating weed species with numerous small reproductive structures and spikelets (Douglas et al. 1985). Because it is not possible to hand emasculate and cross pollinate, it is necessary to have a method to discriminate F_1 hybrids from selfed individuals in any crossing

attempts. Use of a dominant nuclear marker carried by the male parent can assist with identification of F_1 hybrids (Jasieniuk et al. 1994). Jasieniuk et al. (1994) made crosses by bagging panicles from green R plants to panicles of a S line from China, 439-86, that carried a dominant marker for anthocyanic pigmentation of the coleoptile, shoot, first leaf, and setae (Li et al. 1945; Cherisey et al. 1985; Darmency and Pernes 1985). F_1 hybrids could be identified by their purple pigmentation.

The objectives of this study were to: (i) determine the genetic control of fenoxaprop-p-ethyl resistance in green foxtail populations, UM8, UM131, and UM137, (ii) determine the inheritance of trifluralin resistance in the multiple herbicide R population, UM137, and (iii) determine if the genes for fenoxaprop-p-ethyl resistance and trifluralin resistance in the multiple R population, UM137, are linked.

4.3 Materials and Methods

4.3.1 General Plant Growth Conditions

All plant material was grown in 13 cm pots or flats, containing a mixture of clay loam, peat, and sand (2:1:1 by vol.). Each flat consisted of 50, 5 cm Jiffy peat pots[4]. For F_2 population screenings, five S 439-86 parental, five R parental, and 40 F_2 seeds were planted per flat. For the F_2-derived F_3 population screenings, 20 seeds from two families plus five S 439-86 parental, and five R

[4]Jiffy Products (N.B.) Ltd. Shippegan, Canada.

parental seeds were planted per flat. Plants were placed in a growth room with a 16 hour photoperiod and a 22/16°C day/night temperature. Plants were watered daily and fertilized once a week with a solution of water-soluble complete fertilizer[5] (20-20-20) at a concentration of 2.4 g L^{-1}.

4.3.2 Parental Populations

The field histories for populations UM131, and UM137 are listed in Table 3.1 (Chapter 3). A detailed field history for UM8 is not known but the population was repeatedly exposed to diclofop-methyl and sethoxydim over a period of 12 years (Heap and Morrison 1996). Fenoxaprop-p-ethyl was the ACCase inhibitor herbicide chosen for this study since both UM8 and UM131 exhibited a similar level of fenoxaprop-p-ethyl resistance (Morrison and Heap 1996). For the trifluralin bioassay, a trifluralin R population, BDay, from Deloraine (Jasieniuk et al. 1994) and a S green foxtail population from Portage la Prairie, UM7, (Heap and Morrison 1996) were included as check populations.

4.3.3 Genetic Crosses

Three herbicide R populations, UM8, UM131 and UM137 were crossed to the S Chinese population, 439-86. Crosses were made by using a wire to tie a panicle from a R plant to one or two panicles from a S plant and enclosing them in a glassine bag. To enhance pollen dispersal between panicles, the bags were tapped periodically in the morning when pollen dehiscence was at its peak.

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

The S plants, homozygous for the dominant anthocyanin pigmentation, were used as the male parent and the R plants, homozygous for the recessive green pigmentation, as the female. Seed was collected from only the R female plants. Seed was stored at room temperature for three months to overcome dormancy.

4.3.4 Screening for F_1 Hybrids

The F_1 hybrids could be distinguished from selfed progeny by their purple pigmentation (Jasieniuk et al. 1994). Seeds from each R plant were placed in petri dishes which were lined with moist filter paper. The petri dishes were placed in a germination cabinet in the dark at 28°C for 36 hrs to initiate germination, then transferred into a growth chamber under continuous fluorescent light. A high light intensity of 700-900 μE $m^{-2}s^{-1}$ at the level of the petri dishes was used to enhance the expression of the purple pigmentation. After screening over 10 000 seedlings, one UM8/439-86 hybrid, one UM137/439-86 hybrid and two UM131/439-86 hybrids were identified. The hybrids were planted in pots, and then self fertilized by enclosing each panicle in a glassine bag. F_2 seed was collected and stored at room temperature for about three months to overcome dormancy.

4.3.5 Screening F_2 Seedlings for Segregation of Resistance

Fenoxaprop-p-ethyl was applied to seedlings at the 3-4 leaf stage in a cabinet track sprayer equipped with a flat-fan nozzle[6] that delivered 117 L ha^{-1} of spray solution at a spray pressure of 310 kPa in a single pass over the foliage.

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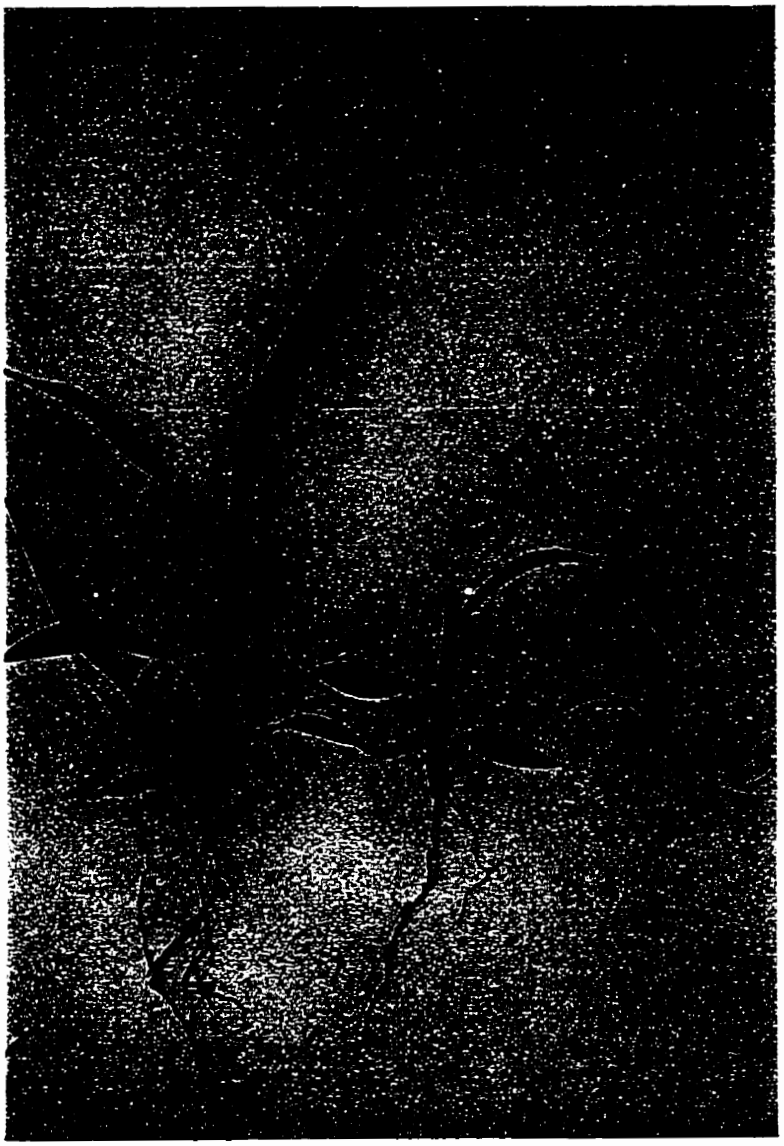
[6]TeeJet SS80015 flat fan nozzle tip, Spraying Systems Co., Wheaton, IL 60188.

The rates of fenoxaprop-p-ethyl used were: 7.5, 10, and 12.5 g ai (active ingredient) ha^{-1} for the UM8/439-86 F_2 seedlings, and 12.5, 15, and 20 g ai ha^{-1} for the UM137/439-86 and UM131/439-86 F_2 seedlings. Herbicide rates were chosen on the basis of the dose response curve (Chapter 3) of the respective parental populations to provide the maximum differentiation between R and S genotypes. Use of a range of herbicide rates for each population ensured accurate classification of response to fenoxaprop-p-ethyl.

The F_2 plants and parental check for each population were scored 21 days after treatment (DAT) based on a visual classification system. Individual plants were classified as either resistant (R), injured (I), or susceptible (S) (Figure 4.1). Resistant (R) plants continued normal leaf development and growth after spraying, similar to the R parental plants. Injured (I) plants were obviously stunted and chlorotic, compared to the R parentals, yet the deformed emerging leaf remained green. Susceptible (S) plants were dead; no new leaves were initiated during the 21 DAT and/or the whole plant was necrotic like the S parental plants.

4.3.6 Confirmation of Mode of Inheritance - F_2-derived F_3 Families

Some F_2 plants from each cross, classified as R or I, at the various herbicide rates, were tranplanted and grown to maturity to produce F_2 derived-F_3 families. To confirm these F_2 ratings, 20 plants of the F_3 families, and appropriate checks were screened at 10 g ai ha^{-1} fenoxaprop-p-ethyl. To confirm the overall F_2 results, at least 90 F_3 families derived from untreated F_2



R I S

Figure 4.1. F_2 plant response types (21 DAT) with 12.5 g ha^{-1} fenoxaprop-p-ethyl. R = resistant, I = injured, and S = susceptible. Refer to Materials and Methods for a description of the three response types.

plants from each cross, UM8/439-86, UM137/439-86, and UM131/439-86, were treated with 10 g ai ha^{-1} fenoxaprop-p-ethyl. The rate of 10 g ai ha^{-1} fenoxaprop-p-ethyl was an intermediate rate that easily distinguished between homozygous resistant (HR), segregating, and homozygous susceptible (HS) families for all the populations studied. At least 20 plants from each family were screened, and any poorly germinating families with unclear results were retested. Families were scored 14 DAT, when herbicide injury symptoms were apparent.

4.3.7 Inheritance of Trifluralin Resistance in UM137

UM137/439-86 F_2 seed, as well as seed from the trifluralin R population, BDay, and the S populations, UM7 and 439-86, were screened for resistance or susceptibility at four trifluralin concentrations, 0, 0.9, 1.2, and 1.5 µM, using a modified petri dish bioassay (Beckie et al. 1990; Jasieniuk et al. 1994). Approximately 20 seeds were placed in each 9 cm diameter petri dish, lined with two Whatman #1 filter papers moistened with 4 ml of trifluralin emulsion (0.9, 1.2, 1.5 µM), or distilled water (0 µM). The petri dishes were placed in the dark in a germination cabinet for three days at 28°C. Most seeds had germinated after this period of time. The germinated seedlings were removed from the germinator and then visually assessed as being either R or S (Figure 4.2). The S seedlings could be easily identified by their swollen stunted shoots and roots, whereas R seedlings were not stunted, similar to the R parental controls. The 1.2 µM concentration was the best for distinguishing between the S and R trifluralin parentals and the check populations. 112 F_2-derived F_3 families were

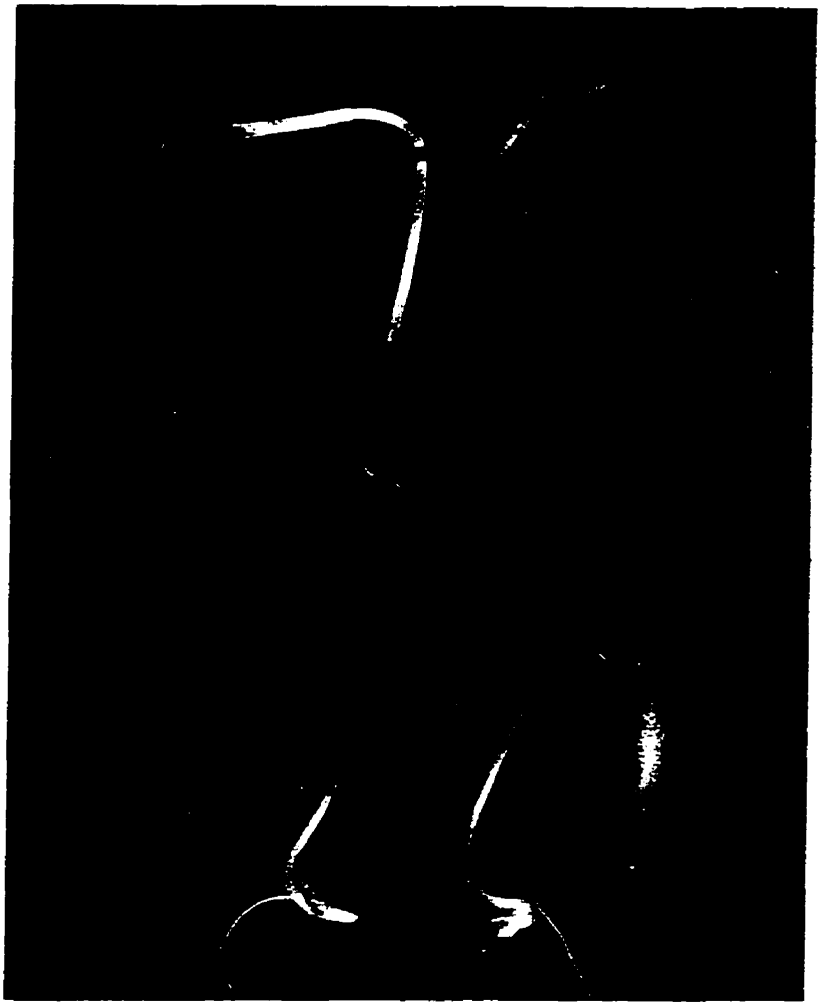


Figure 4.2. UM137/439-86 F_3 seedling bioassay response types (3 DAT) with 1.2 µM trifluralin. RC = resistant UM137 parental control, R = resistant, and S = susceptible. Refer to Materials and Methods for a description of the response types.

then screened at this concentration to confirm the mode of inheritance of trifluralin resistance in population UM137.

4.3.8 Statistical Analyses

Chi-square tests were used to determine goodness-of-fit of the data to expected ratios for different modes of inheritance of resistance. Tests for dominant, incompletely dominant, and recessive single gene control of herbicide resistance were done at each herbicide rate. Yates' correction factor was used when there was one degree of freedom or if the total number of individuals was less than 200 (Strickberger 1985). In tests for dominance, the I class was pooled with the R class. Chi-square tests for homogeneity were conducted to determine whether data across replications, rates, and populations could be pooled, and where possible pooled data are presented in the Results and Discussion.

4.4 Results and Discussion

4.4.1 Confirmation of F_2 Phenotypes

All F_3 families derived from treated R and I F_2 plants from any cross were either HR or segregating for resistance (data not shown). None of these families were HS so there was no misclassification of F_2 individuals. Therefore, individual plants classified as R or I were indeed either homozygous or heterozygous R, not S. This justifies the pooling of I plants with the R class for statistical analyses.

4.4.2 Population UM8/439-86

At the 7.5 and 10 g ai ha^{-1} rates of fenoxaprop-p-ethyl, the F_2 segregation ratios fit a 3:1 Resistant to Susceptible (R:S) ratio (Table 4.1). Results from higher herbicide rates are not included because there was no clear distinction between R, I or S genotypes. None of the herbicide rates used was the ideal rate to discriminate between classes or to indicate clearly an incompletely dominant trait.

The 90 UM8/439-86 F_3 families, derived from untreated F_2 plants, fit an expected ratio of 1 HR: 2 segregating: 1HS family (Table 4.2). Therefore, fenoxaprop-p-ethyl resistance in population UM8 is controlled by a single nuclear encoded gene. These results, with the UM8/439-86 F_2 data, indicate that fenoxaprop-p-ethyl resistance in population UM8 is controlled by a single, nuclear, incompletely dominant gene.

4.4.3 Population UM131/439-86

The UM131/439-86 F_2 data from the two UM131/439-86 F_1 hybrids were similar and pooled based on a chi-square test for homogeneity (individual data not shown). At the 12.5, 15, and 20 g ai ha^{-1} rates of fenoxaprop-p-ethyl, the observed numbers fit a 3:1 (R:S) segregation ratio (Table 4.1). The 162 UM131/439-86 F_3 families, derived from untreated F_2 plants, fit an expected ratio of 1 HR: 2 segregating: 1 HS family (Table 4.2). This suggests that fenoxaprop-p-ethyl resistance in population UM131 is controlled by a single nuclear gene. These results along with the UM131/439-86 F_2 results indicate that a single,

Table 4.1. Chi-square tests for segregation of fenoxaprop-p-ethyl resistance in UM8/439-86, UM131/439-86, and UM137/439-86 F_2 green foxtail populations, treated at different rates of fenoxaprop-p-ethyl.

		No. of Plants			
Population	Rate (g ai ha^{-1})	R	S	X^2	P^z
UM8/439-86	7.5	279	86	0.33	0.57
	10	179	64	0.17	0.69
	Total	458	150	0.04	0.84
	Homogeneity			0.46	0.50
UM131/439-86	12.5	665	211	0.34	0.55
	15	648	217	0.00	0.98
	20	698	232	0.00	1.00
	Total	2011	660	0.12	0.73
	Homogeneity			0.22	0.90
UM137/439-86	12.5	489	191	3.29	0.07
	15	165	67	1.65	0.20
	20	153	64	2.09	0.15
	Total	807	322	7.46	0.01
	Homogeneity			-0.43	—

[z]Probability values greater than 0.05 indicate that the data do not differ significantly from an expected 3:1 (R:S) ratio.

Table 4.2. Chi-square tests for fenoxaprop-p-ethyl resistance in the F_2-derived F_3 generation of green foxtail populations, UM8/439-86, UM137/439-86, and UM131/439-86.

		F_2-derived F_3 Families				
Population	Total	Resistant	Segregating	Susceptible	X^2	P^z
UM8/439-86	90	19	46	25	0.59	0.75
UM131/439-86	162	39	84	39	0.22	0.90
UM137/439-86	112	19	62	31	3.38	0.18
Total	364	77	192	95	2.88	0.24
Homogeneity					3.30	0.77

[z]Probability values greater than 0.05 indicate that the data do not differ significantly from an expected 1:2:1 segregation ratio.

nuclear, incompletely dominant gene controls fenoxaprop-p-ethyl resistance in green foxtail population, UM131.

4.4.4 Population UM137/439-86

The UM137/439-86 F_2 data fit a 3:1 (R:S) segregation ratio at the 12.5, 15 and 20 g ai ha^{-1} fenoxaprop-p-ethyl (Table 4.1). However, the F_2 data were not poolable across these rates. These results still suggest that the mode of inheritance of fenoxaprop-p-ethyl resistance in population UM137 is incomplete dominance. Again none of the herbicide rates used was the ideal rate to discriminate between the three phenotypic classes and clearly identify an incompletely dominant trait.

The 112 UM137/439-86 F_3 families, derived from untreated F_2 plants, fit an expected segregation ratio of 1 HR: 2 segregating: 1 HS family. Therefore, fenoxaprop-p-ethyl resistance in population UM137 is controlled by a single nuclear gene (Table 4.2). From the UM137/439-86 F_2 and F_2-derived F_3 families' results, fenoxaprop-p-ethyl resistance in green foxtail population UM137 is controlled by an incompletely dominant, single, nuclear encoded gene.

Fenoxaprop-p-ethyl resistance levels differ among the three green foxtail populations, UM8, UM131, and UM137 based on comparisons of the results from the three crosses. These differences correspond to the different levels of resistance among the R parentals, especially between UM8 and the other two populations (Chapter 3). Therefore, each R parental population may represent a different mutation for fenoxaprop-p-ethyl resistance. Further research is

required to determine if the same alleles or the same gene loci confer herbicide resistance in these R green foxtail populations. Murray (1996) crossed two ACCase inhibitor R wild oat (*Avena fatua* L.) populations, UM33 and UM1, that had different cross resistance patterns. Resistance in both populations was encoded at the same gene loci, but with different resistance alleles. Another method of detecting F_1 hybrids from selfed individuals in green foxtail must be established before crosses between different R green foxtail populations can be made.

4.4.5 Inheritance of Trifluralin Resistance in UM137

Trifluralin S seedlings could be visually distinguished from R seedlings by their swollen stunted shoots at various trifluralin concentrations. However, the 1.2 µM trifluralin concentration was the most consistent for distinguishing between the R and S phenotypes of parentals and the check populations, with the F_2 data fitting a 1:3 (R:S) segregation ratio (Table 4.3). The 112 UM137/439-86 F_2-derived F_3 families' data fit an expected ratio of 1 HR: 2 segregating: 1 HS family (Table 4.4). Therefore trifluralin resistance in population UM137 is controlled by a single nuclear recessive gene. Jasieniuk et al. (1994) reported that the inheritance of trifluralin resistance in other green foxtail populations from Manitoba was also controlled by a single nuclear recessive gene.

Table 4.3. Chi-square tests of segregation for trifluralin resistance in the F_2 generation for population UM137/439-86, at three trifluralin bioassay concentrations.

	No. of Seedlings			
Rate (μM)	R	S	X^2	P[z]
0.9	36	165	5.05	0.03
1.2	61	189	0.02	0.88
1.5	47	197	4.01	0.05
Total	144	551	6.79	0.01
Homogeneity			2.29	0.32

[z]Probability values greater than 0.05 indicate that the data do not differ significantly from an expected 1:3 (R:S) ratio.

Table 4.4. Chi-square test for trifluralin resistance in the F_2-derived F_3 generation of population UM137/439-86.

	F_2-derived F_3 Families				
Total	Resistant	Segregating	Susceptible	X^2	P^z
112	24	49	39	5.77	0.06

[z]Probability values greater than 0.05 indicate that the data do not differ significantly from an expected 1:2:1 segregation ratio.

4.4.6 Testing for Independent Assortment of Fenoxaprop-p-ethyl and Trifluralin Resistance Genes in Population UM137

A chi-square test was performed to determine if the incompletely dominant gene for fenoxaprop-p-ethyl resistance and the recessive gene for trifluralin resistance in the multiple herbicide R population UM137 were linked (i.e. located on the same chromosome). Each of the 112 UM137/439-86 F_2-derived F_3 families were placed in one of nine phenotypic classes (Table 4.5). The number of observed families in each class were compared to numbers that would be expected based on independent assortment of the genes. With a probability value of 0.07, the two genes are not linked (Table 4.5). This conclusion is further supported by the results of a chi-square test of the phenotypic classes of the F_2 plants (Table 4.6).

That the inheritance of fenoxaprop-p-ethyl resistance is incompletely dominant and the inheritance of trifluralin resistance is recessive is an indication that there is no way to predict the mode of inheritance of herbicide resistance prior to conducting controlled genetic studies. With a highly self pollinated species such as green foxtail, recessive and dominant mutations have similar rates of evolution (Jasieniuk et al. 1996). This is not the case with highly cross pollinated weed species. Green foxtail, with its highly self pollinating nature and prolific seed production, permits both types of genetic control of herbicide resistance. More research on the genetics of multiple herbicide resistance in other weed species and its associated biochemical and physiological

Table 4.5. Chi-square test for independent assortment of fenoxaprop-p-ethyl and trifluralin resistance genes in population UM137/439-86, based on phenotypic classifications of 112 F_2-derived F_3 families.

Family Class[z]	Observed No.	Expected No.[y]	X^2
HR_aHR_t	5	7	0.321
HR_aSeg_t	11	14	0.446
HR_aHS_t	3	7	1.750
Seg_aHR_t	17	21	0.583
Seg_aSeg_t	24	28	0.438
Seg_aHS_t	21	21	0.012
HS_aHR_t	2	7	2.893
HS_aSeg_t	14	14	0.018
HS_aHS_t	15	7	8.036
Total	112	112	14.497 (0.07)[x]

[z]Family Classes:
HR_aHR_t = Homozygous Fenoxaprop Resistant, Homozygous Trifluralin Resistant
HR_aSeg_t = Homozygous Fenoxaprop Resistant, Segregating for Trifluralin
HR_aHS_t = Homozygous Fenoxaprop Resistant, Homozygous Trifluralin Susceptible
Seg_aHR_t = Segregating for Fenoxaprop, Homozygous Trifluralin Resistant
Seg_aSeg_t = Segregating for Fenoxaprop and Trifluralin
Seg_aHS_t = Segregating for Fenoxaprop, Homozygous Trifluralin Susceptible
Hs_aHR_t = Homozygous Fenoxaprop Susceptible, Homozygous Trifluralin Resistant
HS_aSeg_t = Homozygous Fenoxaprop Susceptible, Segregating for Trifluralin
HS_aHS_t = Homozygous Fenoxaprop Susceptible, Homozygous Trifluralin Susceptible

[y]Expected Number is based on the following ratio:
1 HR_aHR_t : 2 HR_aSeg_t : 1 HR_aHS_t : 2 Seg_aHR_t : 4 Seg_aSeg_t : 2 Seg_aHS_t : 1 HS_aHR_t : 2 HS_aSeg_t : 1 HS_aHS_t

[x]Probability values greater than 0.05 indicate that the data do not differ significantly from the above expected ratio.

Table 4.6. Chi-square test for independent assortment of fenoxaprop-p-ethyl and trifluralin resistance genes in population UM137/439-86, based on F_2 phenotypes of 112 F_2-derived F_3 families.

Phenotype[z]	Observed No.	Expected No.[y]	X^2
R_aR_t	22	21	0.012
R_aS_t	59	63	0.194
S_aR_t	2	7	2.893
S_aS_t	29	21	2.679
Total	112	112	5.778 (0.12)[x]

[z]F_2 Phenotypes:
R_aR_t=Fenoxaprop-p-ethyl Resistant, Trifluralin Resistant
R_aS_t=Fenoxaprop-p-ethyl Resistant, Trifluralin Susceptible
S_aR_t=Fenoxaprop-p-ethyl Susceptible, Trifluralin Resistant
S_aS_t=Fenoxaprop-p-ethyl Susceptible, Trifluralin Susceptible

[y]Expected Number is based on the following ratio:
9 R_aS_t : 3 R_aR_t : 3 S_aS_t : 1 S_aR_t

[x]Probability values greater than 0.05 indicate that the data do not differ significantly from the above expected ratio.

mechanisms is required.

The number of identified fields of multiple herbicide R green foxtail will likely continue to increase and create a serious weed management problem for western Canadian farmers. Farmers are left with fewer herbicide options, only trichloroacetic acid (TCA), propanil, or quinclorac for selective control of green foxtail (Morrison et al. 1995). Over the long term, an integrated weed management program, reducing herbicide use, must be adopted to control herbicide R and S green foxtail in the field.

5. Genetic Variation among Herbicide Resistant and Susceptible Green Foxtail (*Setaria viridis* (L.) Beauv.) Populations

5.1 Abstract

Understanding genetic variation among herbicide resistant (R), and susceptible (S) weed populations can assist in determining the origin and spread of herbicide resistance. Genetic variation among 30 herbicide R and S green foxtail (*Setaria viridis* (L.) Beauv.) populations was assessed via Random Amplified Polymorphic DNA (RAPD). A total of 42 polymorphic bands produced by 16 primers were analysed phylogenetically using the maximum likelihood model. There is very low genetic variation among green foxtail populations from Manitoba. This is not entirely surprising since green foxtail is a highly self pollinating weed species. Herbicide R populations are likely the result of many independent mutations in S populations, followed by selection for resistance, and local seed spread. Farmers should not depend solely on the use of herbicides for control of green foxtail, but must adopt integrated weed management strategies to prevent the spread of herbicide resistance.

5.2 Introduction

Green foxtail (*Setaria viridis* (L) Beauv.) is an important annual self pollinating weed that originated in Eurasia and is found throughout temperate regions of the world (Douglas et al. 1985; Wang et al. 1995). It is a diploid

species, and a prolific seed producer (Douglas et al. 1985). Since its introduction to North America, likely through contaminated crop seed or the ballasts of ships, green foxtail has increased its range and population density, and new morphological variants have appeared (Douglas et al. 1985; Wang et al. 1995). Green foxtail was first identified in Manitoba in 1888, at Emerson, and now it is an abundant weed in the southern part of the province (Douglas et al. 1985).

For the past 25 years, farmers in Manitoba have depended on herbicides to control green foxtail. Trifluralin (Group 3) R green foxtail was first confirmed in southwestern Manitoba in 1988 (Morrison et al. 1989). In 1991, green foxtail populations were identified with resistance to the Acetyl-CoA carboxylase (ACCase) inhibitor (Group 1) herbicides (Heap and Morrison 1996). By 1994 hundreds of trifluralin R and 20 ACCase inhibitor R green foxtail sites had been detected in western Canada (Friesen 1994). At least four populations are resistant to both trifluralin and the ACCase inhibitor herbicides. This presents a serious problem for management of green foxtail in the field.

Genetic variation for herbicide resistance must be present in a S weed population for the evolution of herbicide resistance to occur (Jasieniuk et al. 1996). Gene mutation and gene flow through pollen or seed are the two major sources of genetic variation in a population (Jones and Luchsinger 1986). Spontaneous gene mutations may occur at gene loci, and may confer herbicide resistance (Jasieniuk et al. 1996). Gene flow, through pollen or seed, from a

herbicide R population to an adjacent S population may also provide an initial source of resistance genes within a field. Although pollen dispersal has been assumed to be the major mechanism of gene flow between plant populations, seed dispersal may play a much greater role in highly self pollinating weed species, such as green foxtail (Jasieniuk et al. 1996).

Phylogenetic analysis of Random Amplified Polymorphic DNA (RAPD) (Welsh and McClelland 1990; Williams et al. 1990) data can be used to identify individuals or populations which may have arisen from a common origin and which are the most closely related. Relationships among weed populations, with herbicide resistance and geographical location information, can provide insights into the origin and spread of herbicide resistance. RAPD assays have a number of advantages over other molecular genetic techniques, such as isozyme electrophoresis and restriction fragment length polymorphism (RFLP). They require only small quantities of DNA, are technically efficient, low in cost, and therefore suitable for large plant population genetic studies (Moodie et al. 1997).

The objective of this study was to assess genetic variation among green foxtail populations via RAPD, and to determine any relationships among these herbicide R and S populations.

5.3 Materials and Methods

5.3.1 Seed Collection

Eighteen green foxtail populations from within the township of Treherne,

Manitoba (Figure 5.1), 11 populations from elsewhere in southern Manitoba (Figure 5.2), and one population from China were assessed in this study (Table 5.1). The Treherne township is 6 miles by 6 miles, consisting of 36 sections, and has a 10 to 12 year history of ACCase inhibitor herbicide use (Bouregois 1997). Random samples of seeds from 5 to 10 plants from various fields within the township of Treherne were collected in August 1993, and 1994. Bioassays conducted in petri dishes were used to test for resistance to fenoxaprop-p-ethyl, sethoxydim (Group 1), and trifluralin at rates of 2 μM, 5 μM, and 0.9 μM, respectively (L. Bouregois, unpublished data). Seven S, six ACCase inhibitor R, four trifluralin R green foxtail populations and one multiple R population were identified in fields throughout the township (Table 5.1). Seed from the other green foxtail populations used in this study had been collected and characterized as R or S to the ACCase inhibitor and/or trifluralin herbicides in previous studies (L. Friesen, unpublished data; Morrison et al. 1989; Jasieniuk et al. 1994; Heap 1994, Heap and Morrison 1996) (Table 5.1).

5.3.2 DNA Extraction

Seed from each population was sown in flats containing a mixture of clay loam, peat, and sand (2:1:1 by vol.). The flats were placed in a growth chamber with a 16 hour photoperiod at an average light intensity of 500 $\mu E\ m^{-2}s^{-1}$ and 22/16°C day/night temperature. Plants were watered daily and fertilized once a

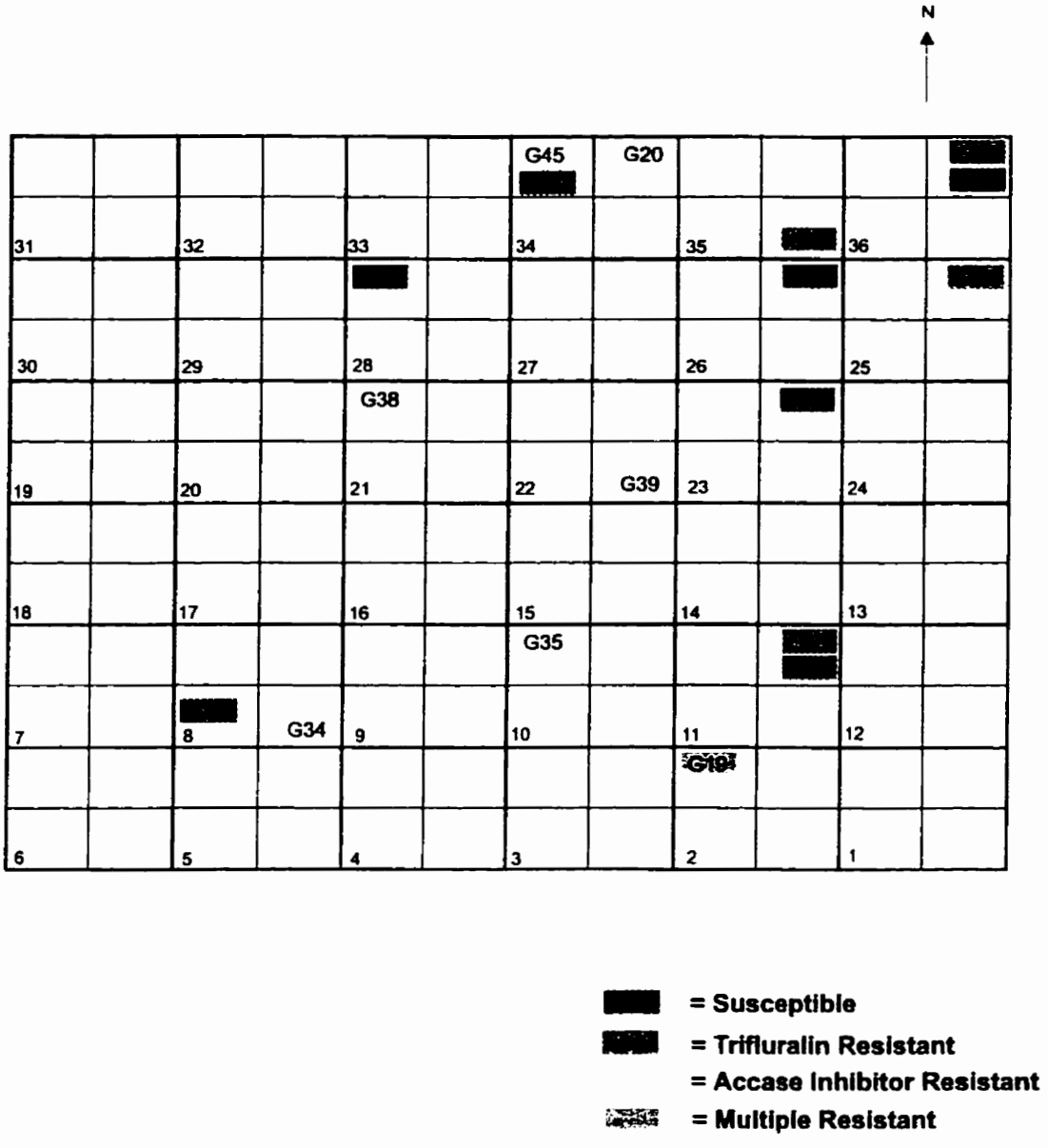


Figure 5.1. Distribution of 18 green foxtail populations collected within the township of Treherne.

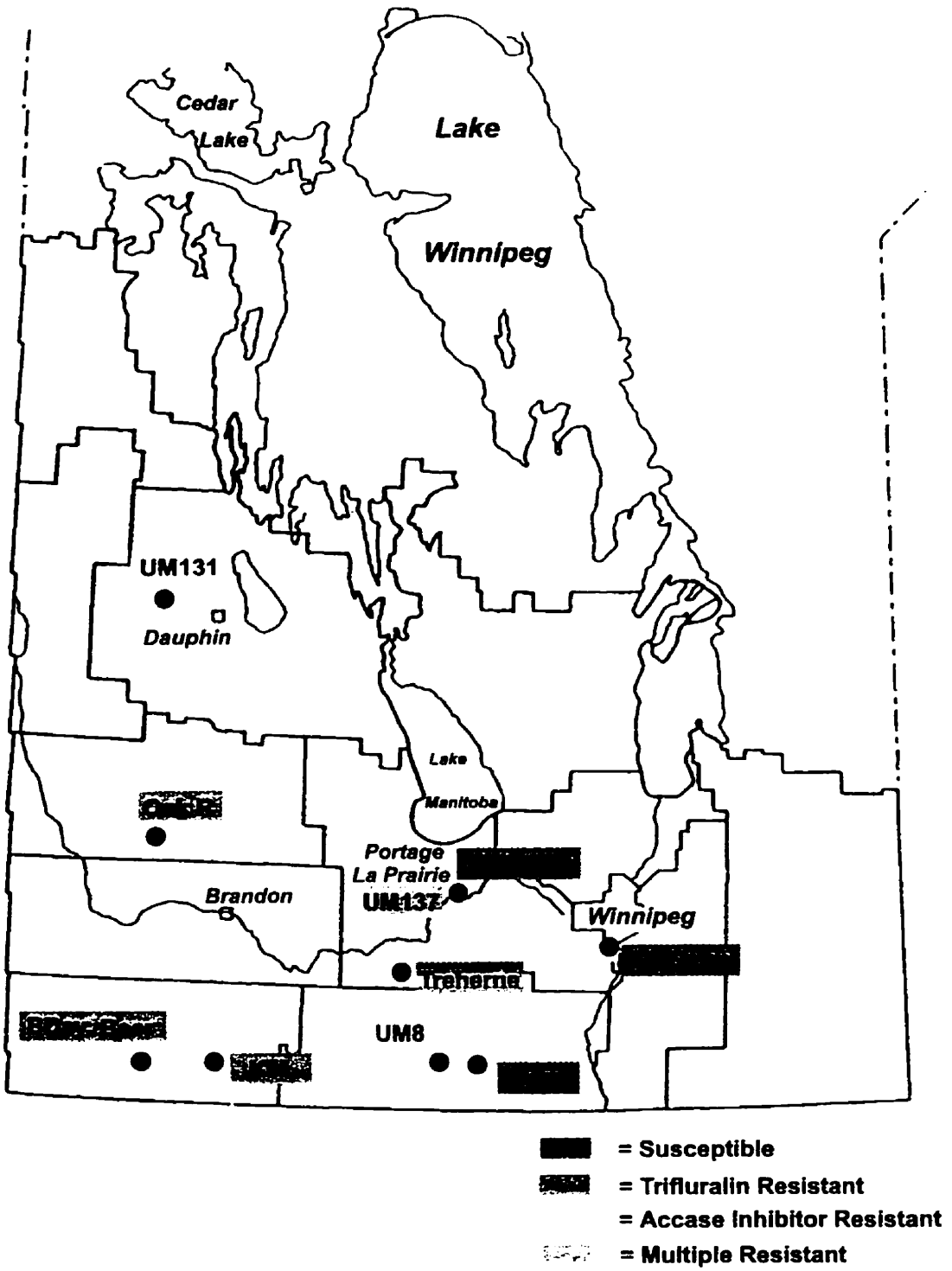


Figure 5.2. Locations of Manitoba green foxtail populations.

Table 5.1. Green foxtail populations included in study of genetic variation.

Population	Phenotype	Origin
439-86	S[z]	China
OakR	TR[y]	Oak River
Kill	TR	Killarney
Beer	TR	Deloraine
BDay	TR	Deloraine
Ciba	S	Portage la Prairie
Mord	S	Morden
St. Germ	S	St. Germaine
UM7	S	Portage la Prairie
UM8	AR[x]	Thornhill
UM131	AR	Gilbert Plains
UM137	MR[w]	Portage la Prairie
Treherne populations		**Quarter**
G13	S	NE 36
G14	S	SW 8
G15	S	NE 23
G16	TR	SE 35
G17	TR	NE 36
G18	TR	NE 11
G19	MR	NW 2
G20	AR	NE 34
G21	S	NE 11
G22	S	NW 28
G25	S	NE26
G34	AR	SE 8
G35	AR	NW 10
G38	AR	NW 21
G39	AR	SE 22
G44	TR	NE 25
G45	AR	NW 34
G47	S	NW 34

[z]S= Susceptible to trifluralin and ACCase inhibitor herbicides
[y]TR= Trifluralin resistant
[x]AR= ACCase inhibitor resistant
[w]MR= Multiple resistant (i.e. Trifluralin and ACCase inhibitor resistant)

week with a solution of water-soluble complete 20-20-20 fertilizer[7] at 2.4 g L^{-1}. When the plants were at the 5 to 6 leaf stage, leaf tissue (0.25 cm^2) from each of 10 plants per population was combined and used for DNA extraction. The leaf tissue was frozen in liquid nitrogen until extraction. Whole leaves were also frozen with liquid nitrogen and then stored at -20°C for later use.

A modified cetyltrimethyl ammonium bromide (CTAB) DNA extraction protocol was used (Procunier et al. 1990; R. Kutcher, personal communication). The pooled leaf tissue was ground in a 1.5 ml microcentrifuge tube, using a plastic pestle attached to a homogenizer. Then 0.5 ml 65°C 2% CTAB was added to each sample, which was then vortexed for about 20 seconds. Each tube was placed in a 65°C waterbath for at least 5 minutes, and then 0.5 ml of chloroform/isoamyl alcohol (24:1) was added. Each sample was microcentrifuged at 13000 rpm for 10 minutes. The supernatant (about 300 μl) was placed into a new tube, about 30 μl (1/10 volume) of 10% CTAB was added and the sample was mixed. One volume of chloroform/isoamyl alcohol (24:1) was again added and each sample was then microcentrifuged at 13000 rpm for 10 minutes. The supernatant (about 300 μl) was transferred into a new microcentrifuge tube with 2 volumes (600 μl) of cold 95% ethanol and placed on ice for at least 5 minutes. The tubes were microcentrifuged again at 13000 rpm for 5 minutes, the alcohol was poured off and 500 μl of 70% ethanol was added.

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

Again the samples were mixed and microcentrifuged at 13000 rpm for 5 minutes. The alcohol was poured off and the samples were dried in a dessicator for about 30 minutes. The small DNA pellets were then rehydrated in 100 μl of Tris (10 mM TrisCl, pH 8.0) -EDTA (1 mM) buffer. The DNA samples were stored at -20°C.

5.3.3 DNA Amplification and Detection

A set of 100 10-mer primers (#301-400), obtained from J. Carlson at the University of British Columbia, was used. A single primer was used in each reaction. The concentration of primer, DNA, and Taq polymerase (Gibco BRL) was optimized to give maximum band intensity and reproducibility. A 25 μl reaction volume was used. A reaction mixture included 1x PCR buffer (Gibco BRL), 2 mM $MgCl_2$ (Gibco BRL), 0.1 mM dNTPs (deoxyribonucleoside 5'-phosphates, Promega), 0.4 μM primer, approximately 50 ng of genomic DNA, and 1 unit of Taq DNA polymerase (Gibco BRL) made to a final volume of 25 μl with HPLC grade water. 20 μl of light mineral oil was added as an overlay to prevent evaporation of the sample during thermocycling. Amplification of the DNA was carried out in 0.6 ml microcentrifuge tubes using a MJ Research, Inc. PTC-100-60™ programmable thermal controlled cycler. The amplification program was 1 minute at 94°C followed by 45 cycles of [1 minute at 94°C (denaturation), 2 minutes at 36°C (annealing), and 1 minute at 72°C (elongation)], and a final stage of 10 minutes at 72°C. Samples were then held at 12°C, or frozen at -20°C if gel electrophoresis was not immediately

performed.

The reaction products (14 μl) plus 2 μl of 5x-Electophoretic Mix (stop solution / 0.5% Bromophenol blue, Xylene cyanole FF / glycerol: 200/400/200) were separated by gel electrophoresis using 1.2% agarose in 1x TAE (Tris/sodium acetate/glacial acetic acid/EDTA) buffer. A negative (no DNA), and a positive (one DNA/primer combination: 439-86/#352) sample were also included in each gel. A λDNA-Hind III/ ϕX-174 RF DNA-Hinc II digest (500 μg ml^{-1}) was included as a size marker. The bands were detected with ethidium bromide [15 μl (10 mg/ml)/250 ml] added to a gel, and viewed under ultraviolet light.

5.3.4 RAPD Data Analyses

Only polymorphic bands were used in the phylogenetic analyses. Phylogenetic analyses were done using PHYLIP (Phylogeny Inference Package) Version 3.5 c. by J. Felsenstein (1993). Two data sets, one consisting of only the 18 Treherne populations and the Chinese population, 439-86, and the other consisting of all 30 populations, were analysed.

To produce the Dollo parsimony-maximum likelihood consensus tree for the entire data set, one hundred bootstrapped data sets were generated from the initial binary data set using SEQBOOT. Parsimony trees were then generated using DOLLOP, and a consensus tree was derived using CONSENSE. Branch lengths and confidence limits for the consensus tree were generated using RESTML. The maximum likelihood consensus tree for the

Treherne and Chinese populations was generated using only RESTML. Input options for RESTML included 100 bootstraps, global rearrangements, an extrapolation factor of 100, and population 439-86 as the outgroup.

5.4 Results and Discussion

Of the 100 primers tested, 94 produced amplification products. Of those 94 primers, 45 produced low numbers of bands with poor resolution or reproducibility. Of the 49 primers that produced bands with good resolution, 20 produced only monomorphic bands, 13 showed differences unique to population 439-86, and 16 showed evidence of polymorphisms among the Manitoba and Chinese populations. A total of 97 bands were scored for differences among all populations. Of these bands, 55 were monomorphic and 42 were polymorphic among the 30 green foxtail populations (Table 5.2). The Chinese population, 439-86, was distinct from all the Manitoba populations (Figure 5.3).

It was apparent from this RAPD survey that there is very low genetic variation among green foxtail populations from Manitoba. These results are not entirely surprising due to the highly selfing nature of the species, and are also comparable to past isozyme studies on green foxtail populations (Nguyen Van and Pernes 1984; Wang et al. 1995).

Two pairs of populations, G34 and G35, and G38 and G39, found within the township of Treherne, had identical band patterns. These populations are also ACCase inhibitor R, and were located 1 to 3 miles from each other. Based

Table 5.2. RAPD band data for green foxtail populations.

Primer No.	No. Of Bands	No. Of Polymorphic Bands
303	7	4
304	10	8[z]
313	5	1
318	5	1
328	9	6[y]
336	7	4
349	6	2[y]
352	7	1
374	5	2
375	8	3
376	5	3
379	5	1
380	5	2
382	3	1
388	5	2
398	5	1
Total: 16	97	42

[z]6 among 439-86 and Manitoba populations; 2 among Manitoba populations
[y]1 among 439-86 and Manitoba populations

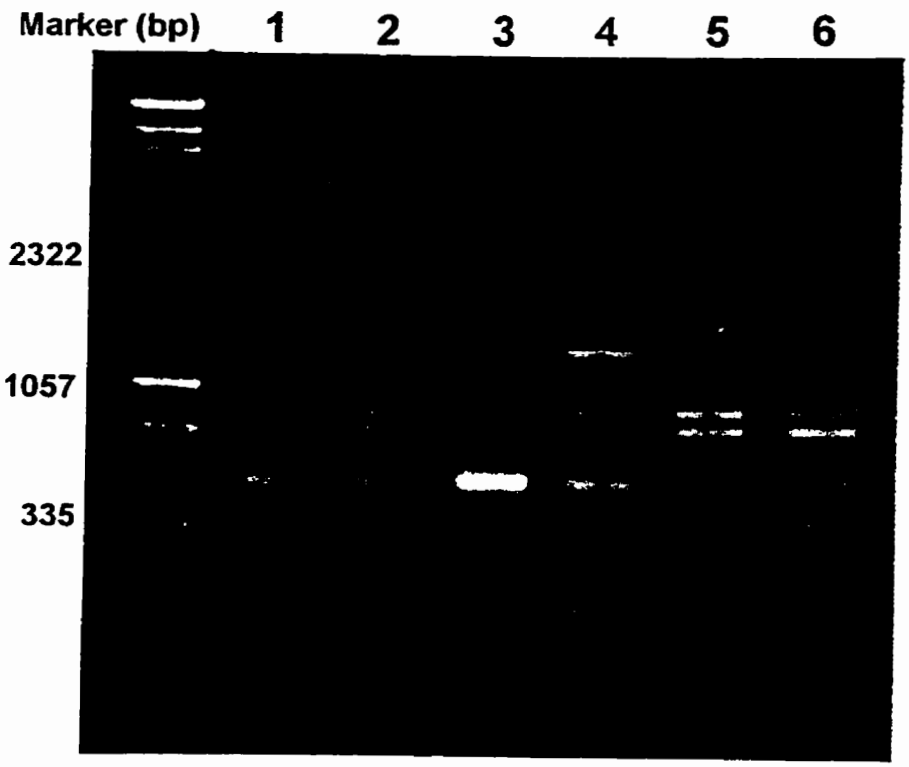

Figure 5.3. Agarose gel of primer #328 RAPD bands of green foxtail populations, G19 (Lane 1), UM137 (2), 439-86 (3), G13 (4), G14 (5), and G15 (6).

on this data set, one might consider each pair of populations as one large population. One mutation for ACCase inhibitor resistance could have occurred, followed by local spread of R seed. Herbicide dose response data from growth room experiments on these green foxtail populations would be helpful in determining if each population arose from the same mutation.

The maximum likelihood consensus tree for the Treherne and Chinese populations is illustrated in Figure 5.4. There is only one major significant monophyletic group found within the township of Treherne, the five ACCase inhibitor R populations, G34, G35, G38, G39, and G45. There is an overall low level of RAPD variation in the Treherne data set, so it is not surprising that there is little confidence in other groupings within the tree. However, it is also possible that there was only one or two initial introductions of green foxtail into the Treherne township, that then spread throughout the township. This would explain the uniformity among populations as well.

The Dollo parsimony-maximum likelihood consensus tree for the entire RAPD data set of 30 green foxtail populations is illustrated in Figure 5.5. No significant monophyletic groups could be distinguished. This result is likely to have arisen due to the high degree of similarity in the band patterns among the 29 green foxtail populations from Manitoba. Certainly, the green foxtail populations found within the township of Treherne do not appear as a distinct group from the rest of the Manitoba populations in the tree (Figure 5.5). Many of the groups shown in the consensus tree contain a mixture of Treherne and

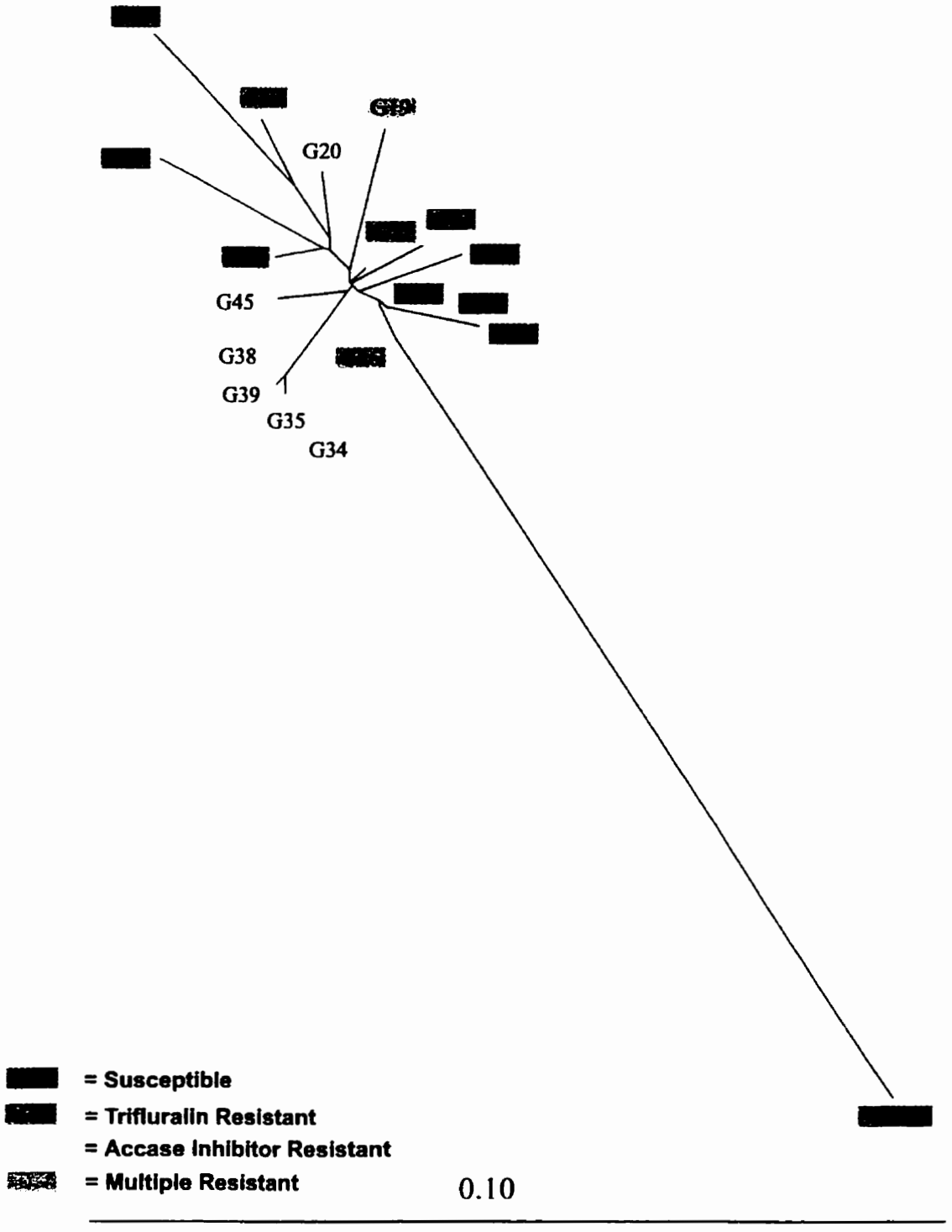

Figure 5.4. The maximum likelihood consensus tree for the Treherne and Chinese green foxtail populations generated using RESTML program of PHYLIP.

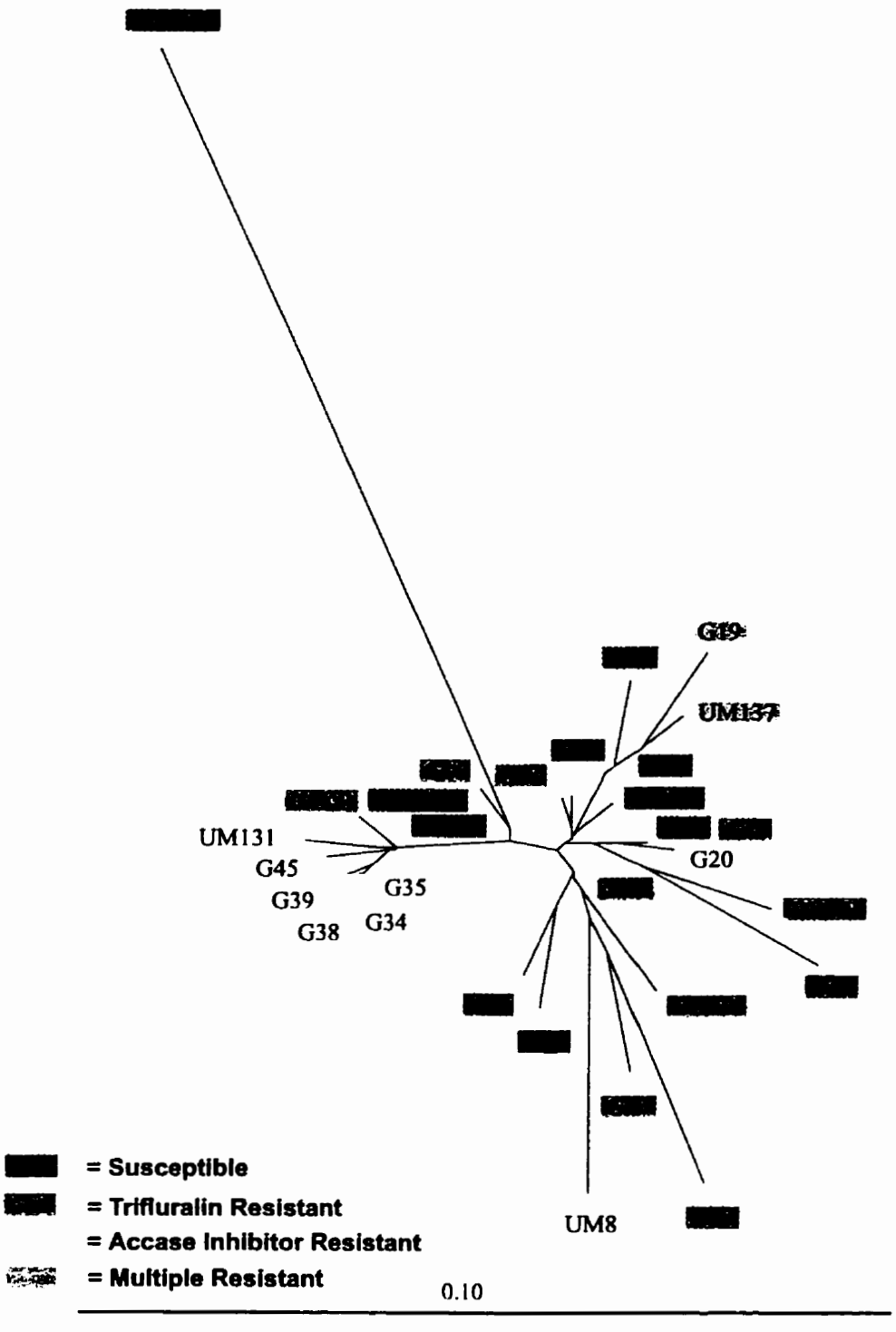

Figure 5.5. The Dollo parsimony-maximum likelihood concensus tree for the 30 green foxtail populations generated using DOLLOP and RESTML programs of PHYLIP.

southern Manitoba populations. These data suggest that there is as much RAPD variation within the township of Treherne as across the rest of Manitoba. A similar result was found in an isozyme variation study of green foxtail populations from the state of Iowa and other parts of the world (Wang et al. 1995).

The maximum likelihood consensus tree for the Treherne and Chinese populations is similar to the Dollo parsimony-maximum likelihood tree for all 30 populations. Both trees provide little significant evidence of strong group structure. Yet the Treherne ACCase R populations, G34, G35, G38, G39, and G45 are found as a monophyletic group in both trees (Figures 5.4 and 5.5). The ACCase R, G20, and the multiple R population, G19, are the only other ACCase R populations from Treherne which do not fall into this group. Another example is the pairing of populations, G13 and G17, in both trees (Figures 5.4 and 5.5). Although G13 is a S population and G17 is trifluralin R, they are found in very close proximity within the township (Figure 5.1).

Based on this RAPD survey of Manitoba green foxtail populations, herbicide R green foxtail populations in Manitoba are likely the result of independent mutations for resistance in S populations, followed by selection and local seed spread. This has important implications for herbicide R weed management strategies. Since independent mutations are likely the main source of the spread of herbicide R weeds, farmers must not continue to depend solely on the use of herbicides for weed control. By decreasing herbicide use,

selection pressure for resistance to a particular herbicide is reduced, thereby delaying herbicide resistance development. Rotating among herbicides with different modes of action will also delay the onset of any one type of resistance. Ultimately, farmers must adopt an integrated weed management system (Derksen 1990). Alternative weed control options such as rotation of different herbicide groups, crop rotations, the use of forage crops in rotations, tillage, and any agronomic practices which will increase the competitiveness of the crop, will reduce both S and herbicide R weed densities. Finally for local control of R seed dispersal, cleaning of harvesting equipment, and the use of clean crop seed, should be stressed to the farmer (Stephenson et al. 1990).

6. General Discussion and Conclusions

In past approaches to modeling the evolution of herbicide resistance, it was assumed that resistance was controlled by a single nuclear completely dominant gene (Gressel and Segal 1978;1982). However, Maxwell et al. (1990) allowed for different inheritance patterns and incorporated other important factors, such as mating system and gene flow, into their model. Information about the genetic control of herbicide resistance can be useful in the development and verification of models which are used to predict the evolution of herbicide resistance.

The number of genes, and their allelic and genic interactions can influence the rate of spread of herbicide resistance within and among weed populations (Jasieniuk et al. 1996). This research determined that fenoxaprop-p-ethyl resistance in green foxtail populations, UM8, UM131, and UM137 is controlled by single nuclear incompletely dominant genes. However, because of the differing levels of resistance among these green foxtail populations, different mutations conferring fenoxaprop-p-ethyl resistance may have occurred in each population. Further research is required to determine if different alleles or different gene loci confer fenoxaprop-p-ethyl resistance in these green foxtail populations.

Trifluralin resistance in the multiple herbicide R green foxtail population, UM137, is controlled by a single nuclear recessive gene. Jasieniuk et al. (1994)

also reported that trifluralin resistance in other green foxtail populations from Manitoba was controlled by a single nuclear recessive gene. Finally, it was also determined that the single fenoxaprop-p-ethyl resistance gene and the single trifluralin resistance gene in the multiple R population, UM137, are not linked. Green foxtail, with its highly selfing nature and prolific seed production, permits both types of genetic control of herbicide resistance.

A number of factors affect herbicide resistance evolution, including the type of mutation conferring herbicide resistance, the initial frequency of R alleles, and the weed's mating system. If a mutation is dominant, the R trait will be expressed immediately in the offspring. With a recessive mutation, the heterozygote will still be susceptible to herbicide and die if treated (Jasieniuk et al. 1996). Therefore, herbicide resistance will be established much faster with a dominant mutation. Under selection in random mating or highly outcrossing weed populations, dominant resistance alleles increase in frequency more rapidly than recessive alleles (Mortimer 1992). So herbicide resistance is unlikely to be under recessive genetic control in outcrossing species. Indeed, there have been no reports of herbicide resistance being controlled by a recessive gene in an allogamous weed species (Jasieniuk et al. 1996).

In highly self pollinating species, dominant and recessive resistance alleles increase in frequency at approximately the same rate (Jasieniuk et al. 1996). Selfing reduces the chance of loss of a recessive mutation by rapidly increasing the frequency of homozygotes. Since green foxtail is a highly selfing

species, the probability of occurrence of a recessive R mutant plant is similar to that of a R plant with a dominant allele (Jasieniuk et al. 1996). The self pollinating and prolific seed production characteristics of green foxtail facilitate the development of either recessive or incomplete dominant genetic control of herbicide resistance. Certainly, the number of fields identified with multiple herbicide R green foxtail will continue to increase. There is also no way of predicting the mode of inheritance of herbicide resistance prior to conducting controlled genetic studies.

The mechanisms of multiple herbicide resistance in green foxtail population, UM137, has not been examined. However, Marles et al. (1993) determined that resistance to the ACCase inhibitor herbicides in other green foxtail populations is due to the presence of a R form of the ACCase enzyme. Trifluralin resistance in other green foxtail populations has been associated with an alteration in a microtubule-associated protein (MAP) (Smeda et al. 1992). Further research on the genetics of multiple herbicide resistance and its associated biochemical and physiological mechanisms is required.

From the RAPD survey of 30 herbicide R, and S green foxtail populations, it is clear that there is very low genetic variation among green foxtail populations in Manitoba. This is not entirely surprising since green foxtail is a highly selfing weed species. It is also possible that there were only a few initial introductions of the weed into the province. Herbicide R populations are likely the result of independent mutations in S populations, selection for resistance, and followed

by local seed spread.

Typical mutation rates, for a single dominant allele, that have been assumed in models of herbicide resistance, are 1×10^{-5} and 1×10^{-6}, i.e. about one in a million (Jasieniuk et al. 1996). Despite these very low mutation rates, the probability of occurrence of at least one herbicide R mutant plant in heavily weed infested fields can be high. For example, there is a very high chance that a mutant plant will be found in a 30 ha field with a weed density of 5 plants m^{-2} or greater, regardless of the weed mating system (Jasieniuk et al. 1996). It is therefore not surprising that herbicide R plants have been identified in a weed species such as green foxtail. Green foxtail is a prolific seed producer and the most abundant weed in Manitoba, with densities ranging from about 1 to greater than 750 plants m^{-2}. A weed such as green foxtail is more likely to be treated with herbicides because of its abundance. Seed dispersal would also be an important mechanism of gene flow in green foxtail.

Selection by herbicides is the most important factor influencing the rate of development of herbicide resistance (Gressel and Segel 1978; Maxwell et al. 1990). The selection pressure imposed by herbicides is very intense. It is a function of the herbicide's high efficacy and the frequency of herbicide use. Today's herbicides often kill 95 to 99% of the S plants. Herbicide R weed populations have been detected where the same herbicide group has been used repeatedly for several years on the same fields. Herbicide resistance can develop within a field in three to 25 years (Morrison and Devine 1994).

Dependence on trifluralin and ACCase inhibitor herbicides for green foxtail control has and will result in the development of herbicide R green foxtail populations. Only a limited number of other chemical weed control options, trichloroacetic acid (TCA), propanil, or quinclorac exist (Morrison et al. 1995), and selection for new resistances is likely. The underlying principle of any strategy to reduce the occurrence of herbicide R weeds is to lower the selection pressure on weed populations. Only reducing overall herbicide use will be effective in avoiding herbicide resistance.

In the short term, herbicide rotation, that involves rotating herbicides with different modes of action, may be one of the most practical and immediate methods for the farmer to delay the onset of herbicide resistance. For example, Manitoba Agriculture (1996) recommends that ACCase inhibitor (Group 1) products should not be used more frequently than one year in three on the same field, and recommends the same for other herbicide groups. However, herbicide rotation between different herbicide groups will only slow down the appearance of herbicide R weeds. Farmers can not depend solely on the use of herbicides for control of weeds, such as green foxtail. Obviously, it is much easier to employ good agronomic practices to prevent or delay the appearance of R weed populations than to control them after they infest an area (Beckie 1992). Crop rotations, agronomic practices which increase the competitiveness of the crop, cleaning of harvesting equipment, and the use of clean crop seed can reduce both S and R weed densities in the field.

Further research emphasis should be placed on assessing weed population dynamics. Long term research and monitoring needs to be conducted on herbicide R populations in the field. Studies are needed to determine how various agronomic practices impact the occurrence and spread of herbicide resistance. The future of western Canadian agriculture is dependent on the adoption of an integrated weed management system.

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8. Appendix

Herbicide groups.

Herbicide Group[z]	Herbicides
Group 1 (ACCase Inhibitors)	fenoxaprop-p-ethyl
	diclofop-methyl
	sethoxydim
Group 2 (ALS/AHAS Inhibitors)	chlorosulforon
	imazamethabenz
Group 3 (Dinitroanilines)	trifluralin
	ethalfluralin
Group 4 (Growth Regulators)	quinclorac
	2,4-D
Group 5 (Triazines)	atrazine
Group 6 (Photosynthetic Inhibitors)	bromoxynil
Group 7 (Photosynthetic Inhibitors)	linuron
Group 8 (thiocarbamates)	difenzoquat
	triallate
Group 9 (EPSP Synthase Inhibitor)	glyphosate
Group 10 (Glutamine Synthetase Inhibitor)	glufosinate
Group 25	flamprop-methyl
Group 26 (TCA)	trichloroacetic acid
Other Herbicides	propanil

[z]Modified from Manitoba Agriculture (1996).

IMAGE EVALUATION
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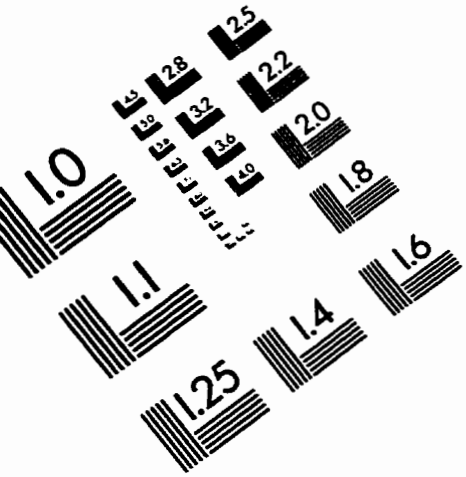

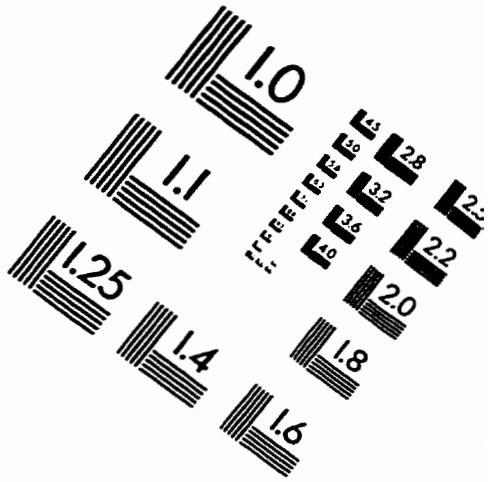

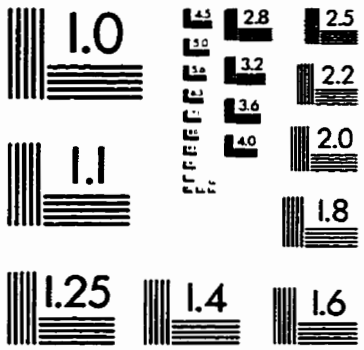

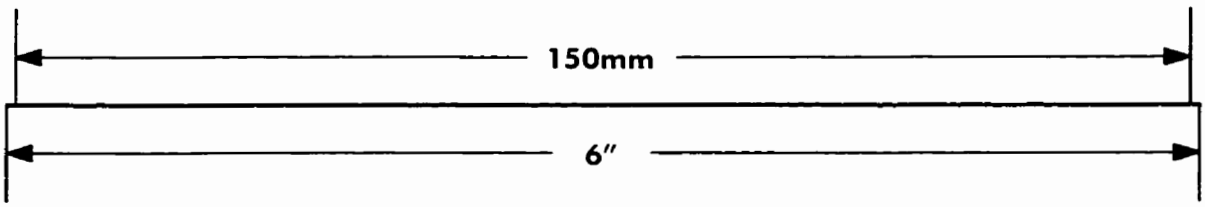

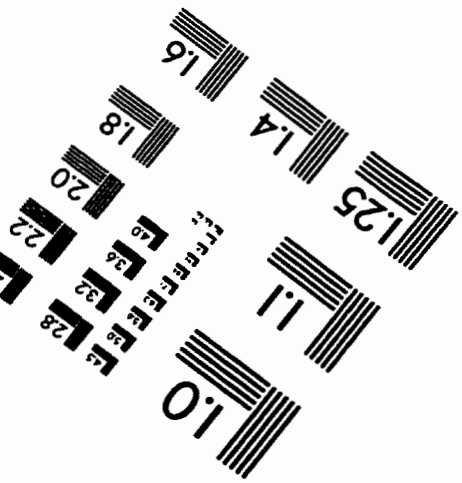

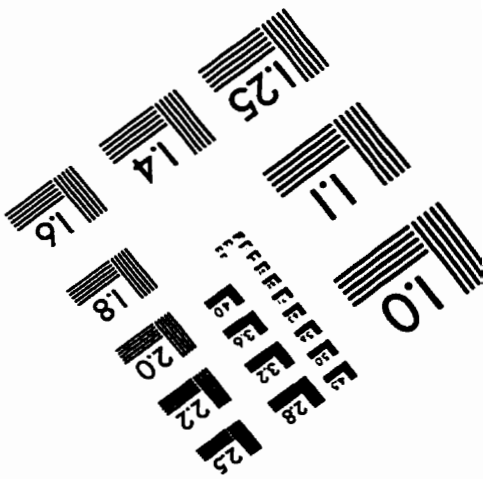

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