



Temporal and spatial dynamics of herbicide resistant weeds
by Robert Mayfield Davidson

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
Crop and Soil Science

Montana State University

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Abstract:

Evolution of herbicide resistance in agricultural weeds is one of the major problems of production for dryland and irrigated crops. Over 150 weed species have evolved resistance to one or more herbicides. Cross and multiple herbicide resistance in weeds, such as rigid ryegrass (*Lolium rigidum*), illustrates the need for alternative management strategies. Integrated weed management programs have been recommended to reduce the evolution and spread of resistant weeds. These recommendations, however, are mostly based on model predictions that have not been tested in the field. Experiments were established at Bozeman (greenhouse) and Fairfield (field), in Montana USA, to test a number of these recommendations and to identify processes influencing resistance evolution in the field. *Arabidopsis thaliana* was used in population studies to identify the influence of herbicide use on resistance evolution. The use of herbicide mixtures and rotations, using combinations of chlorsulfuron, 2,4-D and imazethapyr, significantly decreased resistance evolution compared to the population sprayed annually with chlorsulfuron alone. The addition of susceptible seed at each generation had little impact on the rate of resistance evolution. Increased herbicide efficacy resulted in an increased rate of resistance evolution. Triallate-resistant wild oat (*Avena fatua*) in field populations were monitored to determine spatial distribution of resistant and susceptible plants at regional, field and patch scales. Patterns of resistance were detected at each of the three scales examined. The frequency of fields in the Fairfield region containing triallate-resistant wild oat was monitored over four years. Resistance frequency declined when triallate was no longer used. In the absence of triallate, susceptible plants were identified to be less fit than resistant plants which contributed to the decline in resistance observed. Large changes in resistance frequency of wild oat between years, within fields, could be partially attributed to sampling error. Low sample number reduced the precision of resistance estimates. The herbicide use patterns, the spatial distribution of resistance, and the relative fitness of resistant plants, significantly influenced the dynamics of weed populations. Integrated weed management programs, therefore, need to incorporate these components of spatial and temporal dynamics of herbicide resistance to be successful.

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**MONTANA STATE UNIVERSITY-BOZEMAN
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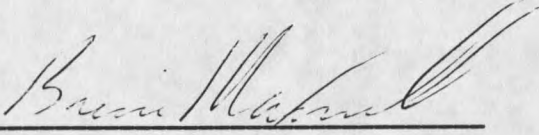
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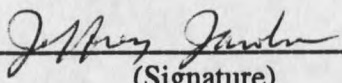
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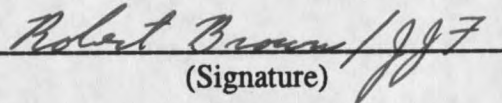
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ABSTRACT

Evolution of herbicide resistance in agricultural weeds is one of the major problems of production for dryland and irrigated crops. Over 150 weed species have evolved resistance to one or more herbicides. Cross and multiple herbicide resistance in weeds, such as rigid ryegrass (*Lolium rigidum*), illustrates the need for alternative management strategies. Integrated weed management programs have been recommended to reduce the evolution and spread of resistant weeds. These recommendations, however, are mostly based on model predictions that have not been tested in the field. Experiments were established at Bozeman (greenhouse) and Fairfield (field), in Montana USA, to test a number of these recommendations and to identify processes influencing resistance evolution in the field. *Arabidopsis thaliana* was used in population studies to identify the influence of herbicide use on resistance evolution. The use of herbicide mixtures and rotations, using combinations of chlorsulfuron, 2,4-D and imazethapyr, significantly decreased resistance evolution compared to the population sprayed annually with chlorsulfuron alone. The addition of susceptible seed at each generation had little impact on the rate of resistance evolution. Increased herbicide efficacy resulted in an increased rate of resistance evolution. Triallate-resistant wild oat (*Avena fatua*) in field populations were monitored to determine spatial distribution of resistant and susceptible plants at regional, field and patch scales. Patterns of resistance were detected at each of the three scales examined. The frequency of fields in the Fairfield region containing triallate-resistant wild oat was monitored over four years. Resistance frequency declined when triallate was no longer used. In the absence of triallate, susceptible plants were identified to be less fit than resistant plants which contributed to the decline in resistance observed. Large changes in resistance frequency of wild oat between years, within fields, could be partially attributed to sampling error. Low sample number reduced the precision of resistance estimates. The herbicide use patterns, the spatial distribution of resistance, and the relative fitness of resistant plants, significantly influenced the dynamics of weed populations. Integrated weed management programs, therefore, need to incorporate these components of spatial and temporal dynamics of herbicide resistance to be successful.

CHAPTER 1

INTRODUCTION

The dynamics of weed communities in agro-ecosystems is dependent on species composition, the environment and management practices. Traditional agricultural methods of the past 30 years, have relied heavily on herbicides for the management of major weed problems. A major goal of weed management practices has been to attain the lowest possible weed populations with the technology available in both crops and pastures. However, even with herbicides, which have been extremely effective, we have not been able to eliminate weed species from agricultural systems. Weed management practices have commonly resulted in a reduction in weed density and a change in the composition of the weed population, rather than the elimination of weeds. Herbicide resistance is one of the more serious changes in weed composition that has occurred. Herbicide-resistant weeds have evolved throughout the world, in over 150 species (Heap 1997). This has highlighted the need for a change in our approach to weed management. The recommended strategies for weed control now call for an integration of weed management techniques (Powles et al. 1997). Integrated weed management incorporates many weed control strategies and recommends that herbicides be applied only when weeds exceed a threshold level. The development of integrated approaches to weed management is complex and requires a detailed understanding of the spatial and temporal dynamics of

the weed community throughout the field. This information is not currently available for any agricultural weed communities.

Strategies to delay the development of herbicide resistance emphasize modification of herbicide use. It is recommended that the frequency of herbicide application be reduced in favor of non-chemical weed control techniques (Powles et al. 1997). It has also been recommended that herbicides with different modes of action be rotated and/or mixed to reduce the rate of resistance evolution (Powles et al. 1997). The recommendations for herbicide use are based on theoretical models that have not been fully validated in the field (Gressel and Segal 1990; Maxwell et al. 1990). Maxwell et al. (1990), by simulating resistance evolution, identified two important factors that determine the rate of resistance evolution, namely gene flow and fitness. Although experiments have been conducted to determine gene flow from resistant to susceptible plants, no information is currently available on the changes that occur in the field at different spatial scales and the processes that are driving the changes.

Metapopulation models are widely used to examine population dynamics where subpopulations exist due to restricted gene flow (Hanski and Simberloff 1997). Levins' (1969) classical metapopulation definition assumed a large network of small patches of similar sizes. The dynamics within a patch were assumed to be occurring at a much faster time scale than the dynamics between patches, simplifying the model structure. The term metapopulations is now also used to describe the gene flow occurring between patches of different sizes. The development of models in agricultural systems that incorporate the metapopulation concept will enable examination of more complex dynamics within a

field. The first step in this process is to determine if weed patches can collectively be described as metapopulations. Examination of population structure and gene flow is required to assess the suitability of this classification. The dynamics of the population within a patch must be determined to validate the assumptions of a metapopulation model. Weed dynamics within a field could then be simulated examining alternative management strategies, including the use of spatial fragmentation of crops to determine the most effective means to limit the spread of resistance genes within a field.

The dynamics of resistant weeds within a field are affected by fitness differences between susceptible and resistant biotypes. Fitness differences between biotypes have been identified predominantly in triazine-resistant weeds (Holt 1996). Differences in fitness in weeds resistant to other herbicides have been less common. The current methodology used to assess fitness differences, however, is flawed (Cousens et al. 1997). Fitness differences, unless large, can only be assessed using isogenic lines in the same environment that resistance evolved. Several biotypes should be assessed simultaneously as not all biotypes will have the same growth and development (Gill et al. 1996). Methods are required to rapidly assess fitness differences to aid in the development of timely management strategies.

The implementation of integrated weed management requires more than an understanding of population dynamics. For adoption of management strategies to occur, a method is also required to assess and regularly monitor whole field populations. Currently, the most common sampling strategy for assessment of weed populations is random sampling. A quadrat is randomly placed within a field and weed density is

determined. This is repeated until the sampler is satisfied with the mean value and variance. If weeds were distributed randomly over the field this technique would provide adequate results. However, weeds are often aggregated (Wiles et al. 1992). A number of other techniques can be used that are designed to improve the precision of the mean value for weed density or resistance by altering the pattern of coverage within the field. Sampling strategies include stratified sampling, transect sampling and the W-method. No evaluation of these techniques for sampling herbicide-resistant weeds has been published. The adoption of global positioning (GPS) technology in farm management increases the management tools available for weed control and the sampling strategies available. Creation of a map of weed location is often the aim when sampling using GPS technology (Colliver et al. 1996). Adaptive cluster sampling combined with geostatistical methods may be the most appropriate method to create a weed map.

This study of temporal and spatial dynamics of herbicide-resistant weeds aimed to provide information to improve our ability to manage weed populations. The first objective was to determine the impact of a range of currently recommended herbicide management strategies on the rate of evolution of chlorsulfuron resistance in *Arabidopsis thaliana*. The second objective was to determine the spatial genetic structure of triallate resistant proportions of wild oat (*Avena fatua*) populations and to determine the importance of spatial scale dependent processes based on patterns observed. On a smaller scale, the third objective aimed to determine if a genetic structure exists within a patch, and if so, how this changes over time. Accurate and valid methodology is essential to understanding weed problems, yet where weeds are not evenly distributed our past

techniques are inadequate. The fourth objective was to develop alternative methodology for the assessment of fitness differences between herbicide susceptible and herbicide-resistant weed biotypes within the field. The final objective was to evaluate the effectiveness of different sampling strategies in determining weed density and resistance levels, within a field, to assist in monitoring the effects of different herbicide resistance management strategies.

CHAPTER 2

EFFECT OF HERBICIDE USE ON THE RATE OF CHLORSULFURON RESISTANCE EVOLUTION IN *Arabidopsis thaliana*

Introduction

Intensive agriculture has maintained a high reliance on the use of selective herbicides since the introduction of 2,4-D in the late 1940's. The introduction of selective grass herbicides, when combined with the previously available broadleaf herbicides, made it possible to achieve satisfactory weed control in many crops without additional non-chemical weed management. The range of herbicides continued to expand in the 1980's with the introduction of acetolactate synthase (ALS) inhibiting herbicides. These herbicides are now widely used in most agricultural crops today (Powles et al. 1997). Crops that have been genetically engineered to be tolerant to the sulfonylurea and imidazolinone herbicides will further increase the exposure and selection intensity for weeds resistant to these herbicides. The introduction of these herbicides has resulted in a dramatic shift in farm management. Minimum tillage (or zero till) and crop residue retention programs have removed tillage and straw burning from the management options list. The reduction in tillage and burning of crop residue has had beneficial results, increasing soil organic matter and decreasing soil erosion in many systems. However, reduction in the use of non-chemical weed control methods has also increased reliance on herbicides for weed control. Herbicide-resistant weeds are now widespread in many

areas of the world with over 150 weed species reported in the most recent survey (Heap 1997). Many of the herbicide-resistant weeds have evolved in agricultural regions where monoculture agriculture is practiced. The continued effectiveness of herbicides will require a modification in their recommended use including integration with other weed management methods. Integrated weed management programs have been developed and are widely used for management of rigid ryegrass (*Lolium rigidum*) in Australia (Powles et al. 1997).

The most common recommended management strategies to delay herbicide resistance currently include rotation of herbicides from different herbicide families and/or the use of mixtures of herbicides with different modes of action. The aim of these strategies is to reduce the selection intensity of the herbicide and maintain a high proportion of wild type (susceptible) alleles in the weed population. Models have predicted that use of mixtures and rotation of herbicides can dramatically reduce the rate of evolution of resistance (Gressel and Segal 1990). However, the effect of rotation of herbicides representing different modes of action, and or the use of mixtures of herbicides with different modes of action, has not been tested experimentally. The rationale behind recommendations for herbicide use is that for each herbicide a gene for resistance is required for a plant to be resistant. If the frequency for each resistant gene in the population is 1×10^{-7} then the probability that one plant is resistant to both herbicides (contains both resistance genes) is 2×10^{-14} (Gressel and Segal 1990). There are a number of weed species where this may not apply due to the nature of the resistance mechanism. In non-target site mechanisms for resistance, the gene responsible for resistance to one

herbicide may also endow the species with cross-resistance to other herbicides. Mixtures and rotations of herbicides under these circumstances are unlikely to provide any benefit. It is unclear what effect herbicide management has on the type of herbicide resistance mechanisms selected within the population. However, there are examples where resistance has evolved simultaneously to both herbicides in a mixture (Wrubel and Gressel 1994).

Maintenance of susceptible alleles within the population is the basis for maintaining herbicide effectiveness. It has been suggested that reducing the selection intensity by reducing herbicide efficacy will also maintain a higher level of susceptible alleles in the population (Gressel and Segel 1990). Predictions by models based on Darwinian evolution indicate that increases in efficacy are associated with an increased rate of resistance evolution in weeds (Maxwell et al. 1990). The difference in time for a population to evolve resistance exposed to the same level of selection could be several years if resistance is also associated with reduced fitness. If a herbicide has a residual effect then the proportion of the total population controlled is greater, increasing its efficacy and selection intensity. Field observation of the rapid development of resistance to many of the ALS inhibiting herbicides, which generally have a high efficacy enhanced by residual activity, supports this theory. The consequences of reducing the herbicide rate on the evolution of resistance (if associated with a decrease in efficacy) have not been investigated. Observations from the field in India, where *Phalaris minor* evolved resistance to isoproturon, indicated that low dosing (assumed reduced efficacy) can lead to the development of resistance at a faster rate than at high doses (Gressel 1995). Low doses in this case resulted in polygenic resistance (Gressel 1995). It was suggested that

in regions receiving a high dose, herbicide resistance, when it appears, will be the result of a single dominant gene (Gressel 1995).

The objective of this study was to experimentally evaluate the effect of herbicide use patterns and initial gene frequency on the rate of evolution of resistance. *Arabidopsis thaliana* was chosen as the model plant species to evaluate resistance management due to the relatively short generation time and the suitability of this species to greenhouse studies.

Materials and Methods

Plant Material

Arabidopsis thaliana is a member of the Brassicaceae family that is native to Europe and is common in many areas of the northern temperate zones (Ratcliffe 1961). Its advantages as an organism to study the evolution of resistance relate to both its small size (height less than 10 cm in high density plantings), and to its relatively quick generation time of only a few months. *Arabidopsis thaliana* has been used extensively in the study of plant morphogenesis due to the following genetic advantages. It is a self pollinating species that has a small genome, estimated at 7×10^7 base-pairs (Leutwiler et al. 1984). Linkage maps have been constructed for the five chromosomes using over 80 morphological mutants (Koornneef et al. 1987). The size of the genome and the number of markers permit identification and cloning of genes where new mutations have occurred, using techniques as chromosome walking and DNA tagging. The availability of these techniques enables the detection and identification of additional resistant genes from a

mutation event. Seeds of both a chlorsulfuron resistant *A. thaliana* mutant GH50 (Haughn and Somerville 1986), and wild type var. Columbia were used for these experiments. The chlorsulfuron resistant mutant was the result of a single point mutation which caused a decreased inhibition of ALS by the chlorsulfuron herbicide. The mutation was previously mapped to the locus designated *csr-1* on chromosome 3 (Chaleff and Ray 1984, Haughn et al. 1988).

Husbandry

Each population of *A. thaliana* was grown in a flat (45 cm by 61 cm by 9 cm) containing 6 cm of greenhouse soil mix (silt loam, washed sand, and peat moss; 1:1:1 by vol). The flats were placed in a greenhouse receiving 16 hr of light (daylight plus supplemental light) at 25°C ±3°C and 16°C ±3°C for day and night temperatures, respectively. *Arabidopsis thaliana* is a long-day plant. Long days and high temperature promote a shorter period to flowering. The decrease in flowering time associated with the increases in temperature also results in reduced seed production due to the reduced number of siliqua formed (Chintraruck and Ketellapper 1969). A compromise between seed production and generation time was made in choosing temperature and lighting regimes. Flats were fertilized weekly using Petersons solution (20:10:20; N, P and K by weight) at a rate of 75 ppm. Each flat was sown with 100,000 seeds at the start of each generation. Seed from the previous generation was used and was supplemented with additional seed (resistant and susceptible), grown in a separate nursery, maintaining the chlorsulfuron resistance frequency.

Greenhouse bays were steam cleaned between generations to kill insects and increase the time to re-infestation by insects in subsequent generations of *A. thaliana*. Seed was sown into each flat by using a hand sprayer with a spray carrier of 1 gram of Captan per litre of water. The application of seed by sprayer was necessary to obtain an even distribution of seed on the soil surface. Captan was added to the seed mixture to reduce death of seedlings by fungal attack (damping off). The seed was immediately covered with clear plastic sheeting (6 mm thick) to maintain a high humidity for 7 to 10 days. Seedlings were watered by an automatic misting system, on a daily interval, following removal of the plastic covering.

Herbicide Application

Three herbicides were used in the experiments described (Table 2.1). Herbicide rates remained the same for applications involving a single herbicide or applications involving mixtures of herbicides. The appropriate herbicide rates were established by evaluating *A. thaliana* response in a dose response experiment using similar methodology and analysis as that described by Seefeldt et al. (1996). Differentiation between resistant and susceptible *A. thaliana* was not possible based on mortality alone as no level of chlorsulfuron application resulted in 100% mortality of the susceptible biotype and 0% mortality of the resistant biotype. Differences in the percent of flowering plants between the biotypes were identified and this was used to determine the appropriate application rate.

Table 2.1. Herbicide application rates used in all *A. thaliana* population experiments.

Herbicide	Application rate g ai ha ⁻¹	Adjuvants % of spray vol.
chlorsulfuron	3	0.1 ^a
2,4-D ester	54	0
imazethapyr	24	0.2 ^a

^aNon-ionic surfactant

Herbicides were applied using a spray table, TeeJet 8002E nozzle, at 220 kPa, output 135 L ha⁻¹. Herbicides were applied approximately two weeks following removal of the plastic covering. *Arabidopsis thaliana* at this stage had a minimum of two true leaves with some of the plants more advanced up to 6 leaf stage.

Initial Gene Frequency

The rate of increase in chlorsulfuron resistant mutants of *A. thaliana*, in response to a range of herbicide management strategies, was examined over three generations. The initial level of resistance was set at 0.1%, 1% and 10% for each group of herbicide treatments (Table 2.2). The factorial design consisted of 15 treatments (factor A: three initial resistant frequencies by factor B: five herbicide treatments) with five replications. A randomized complete block design was used for this experiment due to the size of the experiment and possible variation between greenhouses. The response to each herbicide treatment was monitored over three generations. Herbicide were applied to 95% of the population contained within each flat (95% efficacy). This was achieved by covering each flat with strips of cardboard that restricted the herbicide to 95% of the flat.

Table 2.2. Herbicide treatments applied to populations of *A. thaliana*. Initial resistance frequencies of the chlorsulfuron resistant mutant were 0.1%, 1.0% and 10%.

Herbicide	Chlorsulfuron application frequency
No herbicide	Never
Chlorsulfuron	Every generation
2,4-D ester	Never
Chlorsulfuron + 2,4-D (mixture)	Every generation
Chlorsulfuron + 2,4-D (rotation)	First and third generation

Herbicide Efficacy

The rate of increase in chlorsulfuron resistant mutants of *A. thaliana* was measured over three generations in response to treatment of chlorsulfuron with different levels of efficacy. Wild type seeds (100 seeds per 100,000) were also added each generation, to half of the treatments, to representing gene flow from refuges (Table 2.3). All populations had an initial resistance chlorsulfuron frequency of 1%. The seven treatments were replicated five times in a completely randomized design. Herbicide efficacy was controlled by placing cardboard strips over the flats during spraying. This restricted the area where herbicides were applied resulting in a range of efficacy values from 75% to 95%.

Table 2.3. Chlorsulfuron applied at 3 g ai. ha⁻¹ to each generation of *A. thaliana* at three levels of efficacy.

Herbicide	Efficacy	Susceptible seed added ^a
No herbicide		
Chlorsulfuron	95	No
Chlorsulfuron	95	Yes
Chlorsulfuron	85	No
Chlorsulfuron	85	Yes
Chlorsulfuron	75	No
Chlorsulfuron	75	Yes

^aadditional 100 wild type seed added each generation

Mixtures and Rotations

The rate of evolution of resistance to chlorsulfuron was examined under a range of herbicide treatment strategies (Table 2.4). Chlorsulfuron was applied alone, in mixture with 2,4-D, and rotated with 2,4-D plus imazethapyr. Herbicides were applied to achieve a 95% efficacy level with the initial frequency of chlorsulfuron resistance set at 1% of the population. The seven treatments were replicated five times in a completely randomized design.

Table 2.4. Herbicide treatments applied to each generation of *A. thaliana*.

Herbicide	Chlorsulfuron application frequency
No herbicide	Never
Chlorsulfuron	Every generation
2,4-D ester	Never
Imazethapyr	Never
Chlorsulfuron + 2,4-D (mixture)	Every generation
Chlorsulfuron + 2,4-D (rotation)	First and third generation
Chlorsulfuron + imazethapyr (rotation)	First and third generation

Resistance Testing

The change in the frequency of the chlorsulfuron resistant *A. thaliana* biotype was determined following harvest at each generation. Seed harvested at each generation from each flat was hand threshed and sieved using a 500 µm diameter mesh sieve. A petri dish test was used to determine the level of chlorsulfuron resistance. Blue filter paper was used in 15 mm by 100 mm diameter petri dishes. Seed was applied to eight petri dishes using a spray table, 8008 nozzle, 220 kPa. Four herbicide rates were applied with two replications (Table 2.5). A total volume of 6 ml of herbicide solution was applied to each petri dish. The petri dishes were stored in the dark at 5°C for 7-10 days before germination was recorded. The LD₅₀ was calculated using similar methodology to Seefeldt et al. (1996) and the percent resistance was determined by comparison with the susceptible and resistant biotype. Resistance to 2,4-D and imazethapyr was tested using the same methods.

Table 2.5. Herbicide concentrations used in the petri dish test to determine resistance frequency for herbicide resistance in *A. thaliana* to chlorsulfuron, 2,4-D and imazethapyr.

Herbicide		Concentration mg ai ml ⁻¹		
		0	0.023	0.09
chlorsulfuron	0	0.023	0.09	0.38
2,4-D ester	0	0	0.01	0.44
imazethapyr	0	0.01	0.014	0.24

Statistical analysis

All experiments were subject to analysis of variance. The frequency of chlorsulfuron resistance was the dependant variable. *Arabidopsis thaliana* response to herbicides varied between generations due to variation in the greenhouse environment. Analysis of change in resistance frequency, from one generation to the next, was therefore carried out to determine if significant difference between herbicide treatments were present in any single year. ANOVA was conducted using the Statistica statistical package (StatSoft 1996).

Results and Discussion

Initial Frequency Of Resistance

The initial frequency of chlorsulfuron resistant *A. thaliana* significantly affected the rate of resistance evolution (Figure 2.1). No increase in chlorsulfuron resistance in *A. thaliana* was detected over the three generations when the initial gene frequency was 0.1%. This is in contrast to the high rate of increase in resistance that was detected under

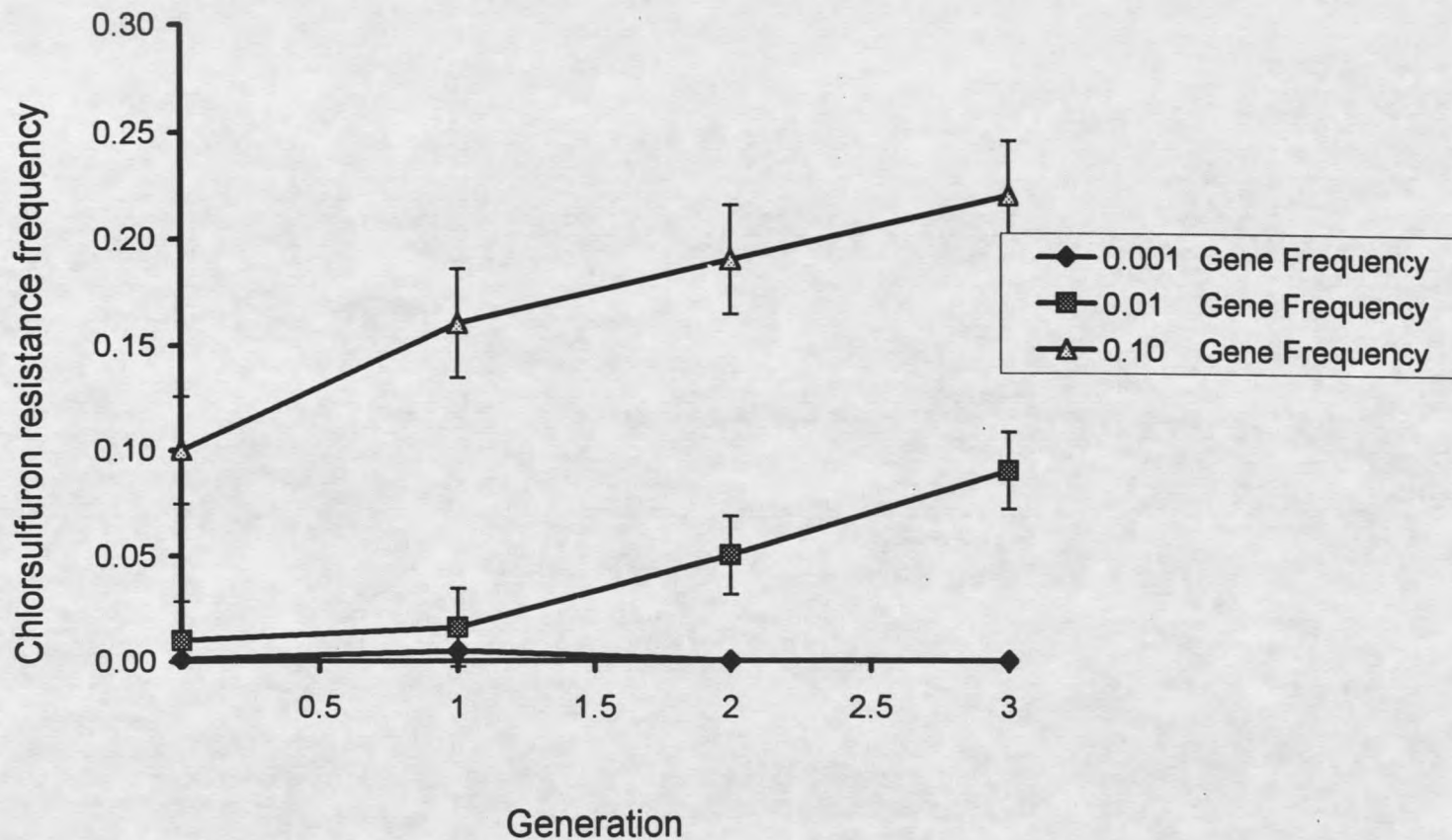


Figure 2.1. Frequency of chlorsulfuron resistant *A. thaliana* in each generation following annual chlorsulfuron applications. Initial frequencies of chlorsulfuron resistant *A. thaliana* were 0.1%, 1%, 10%. Vertical bars represent standard errors.

all herbicide treatments where the gene frequency was initially 1% or 10% of the population. The rate of increase in resistance was significantly different ($P < 0.05$) between 1% and 10% although the changes occurring were larger for populations with an initial gene frequency of 10%. The rapid evolution of resistance observed in rigid ryegrass (*Lolium rigidum*) could be due to a high initial gene frequency of resistance. Estimates of initial gene frequency of resistance to diclofop-methyl for rigid ryegrass are 2×10^{-2} (Powles et al. 1997), considerably more than the underlying mutation rate of 1×10^{-9} (Haughn and Somerville 1990). Resistance has evolved in many rigid ryegrass populations in as few as three generations (Gill 1995). Simulations by Jasieniuk et al. (1996) indicate that even where herbicide efficacy is high (99% control) a difference in initial gene frequency of 1×10^{-1} may delay the appearance of resistance by one season. If selection by the herbicide is lower, in the order of 75% (more realistic when emergence after treatment is included), then the delay in herbicide resistance due to a lower initial gene frequency could be as much as four years.

The frequency of chlorsulfuron resistant *A. thaliana* increased most rapidly when chlorsulfuron was used every year of the rotation (Figures 2.2, 2.3 and 2.4). The rate of increase varied widely between generations and treatments. Chlorsulfuron resistance increased by up to 200% in a generation. Percentage increases were generally lower than the mean change for populations that started at an initial level of resistance of 10%. Rotation of chlorsulfuron with 2,4-D reduced the rate of resistance increase in years where 2,4-D was used. However, the rate of increase, in years when chlorsulfuron was used as part of the rotation, was similar to that of chlorsulfuron used alone. Mixtures of

