



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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A thesis submitted in partial fulfillment
of the requirements for the degree

of
Doctor of Philosophy
in
Crop and Soil Science

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APPROVAL

of a thesis submitted by

Robert Mayfield Davidson

This thesis has been read by each member of the committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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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 $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and $16^{\circ}\text{C} \pm 3^{\circ}\text{C}$ 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		
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

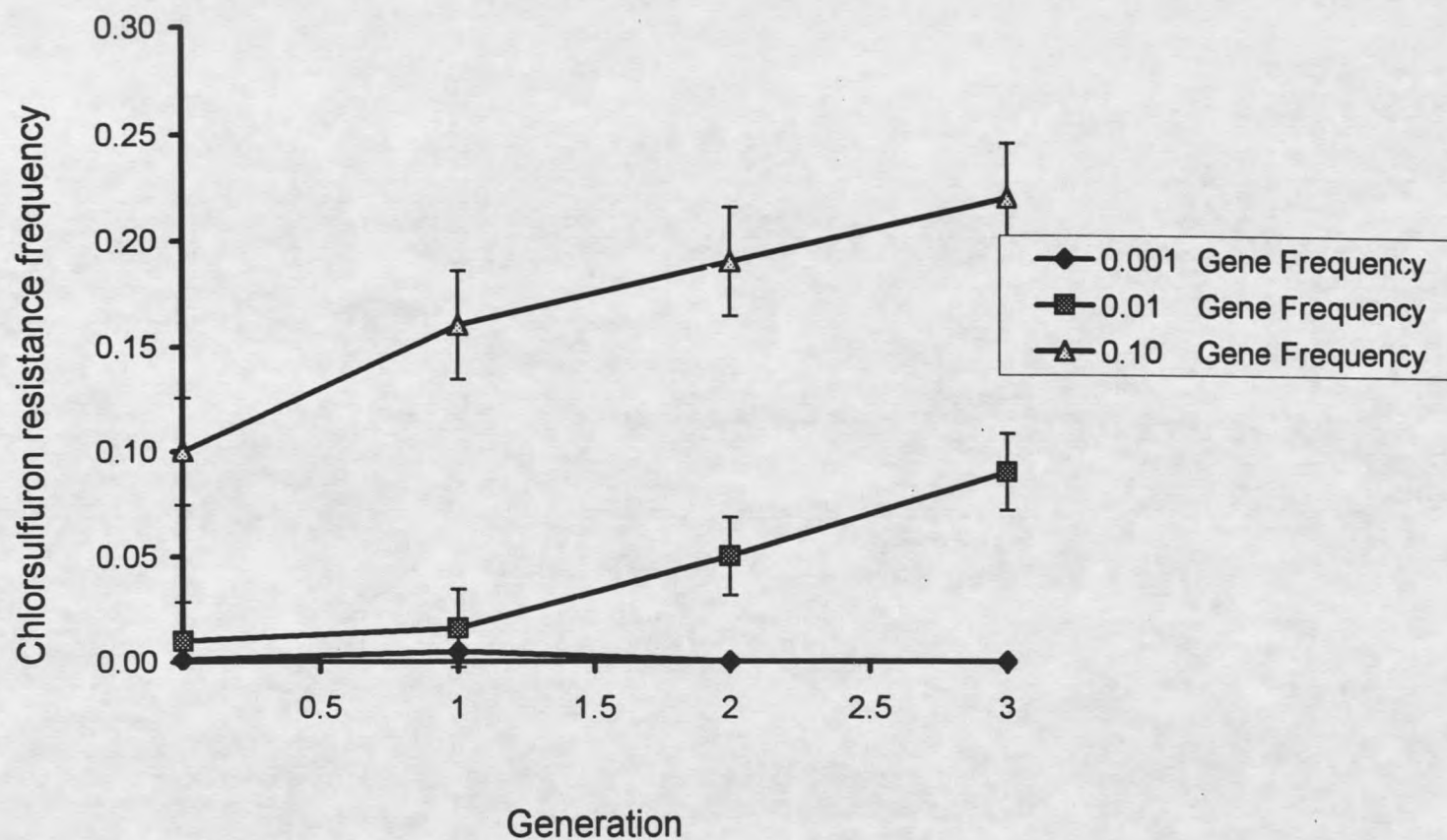


Figure 2.1. Frequency of chlorsulfuron resistant *A. thaliana* in each generation following annual chlorsulfuron applications. Initial frequencies of chlorsulfuron resistant *A. thaliana* were 0.1%, 1%, 10%. Vertical bars represent standard errors.

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

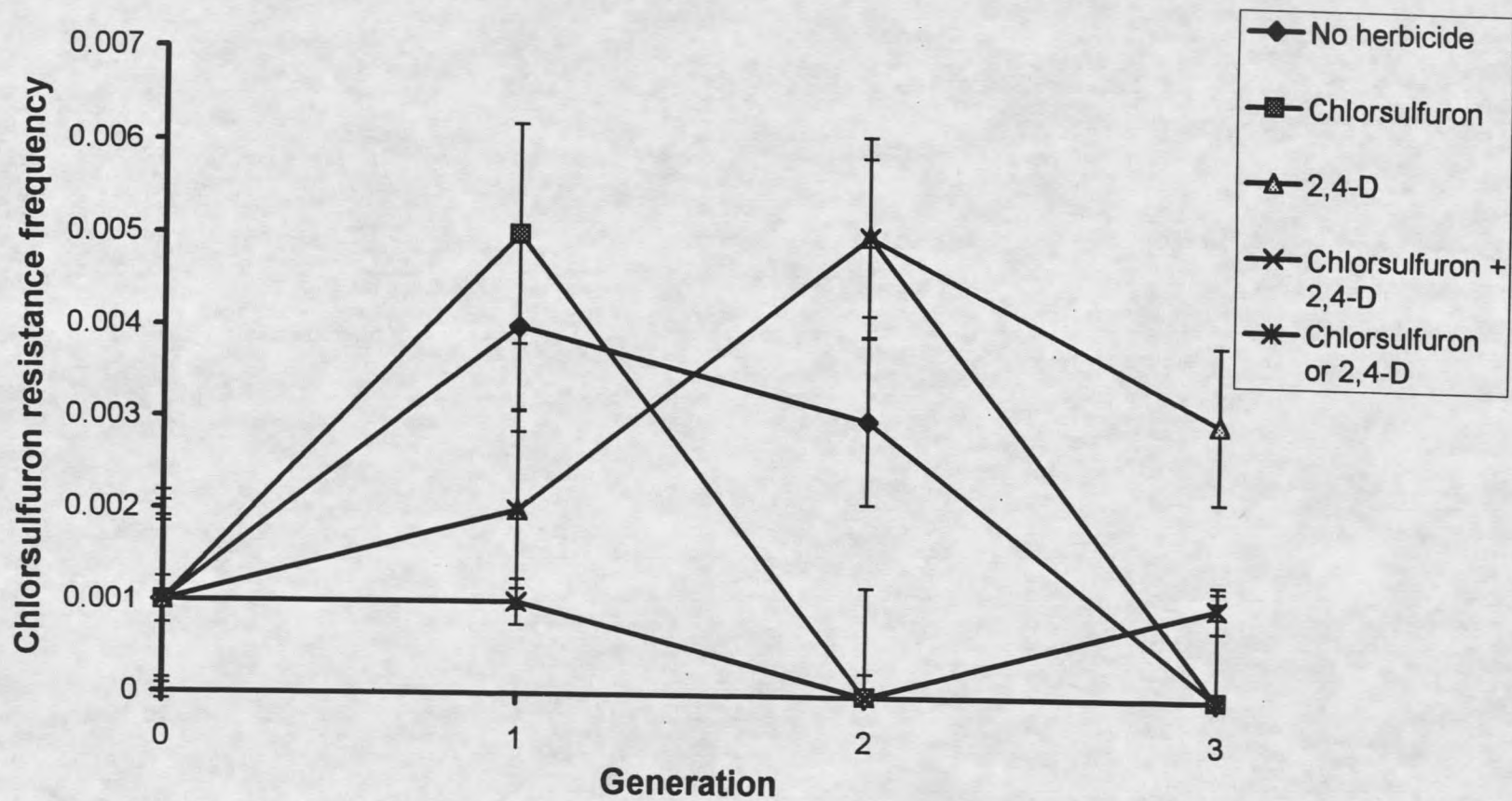


Figure 2.2. Frequency of chlorsulfuron resistant *A. thaliana* in each generation following different herbicide treatments. Initial frequency of chlorsulfuron resistant *A. thaliana* was 0.1%. Vertical bars represent standard errors.

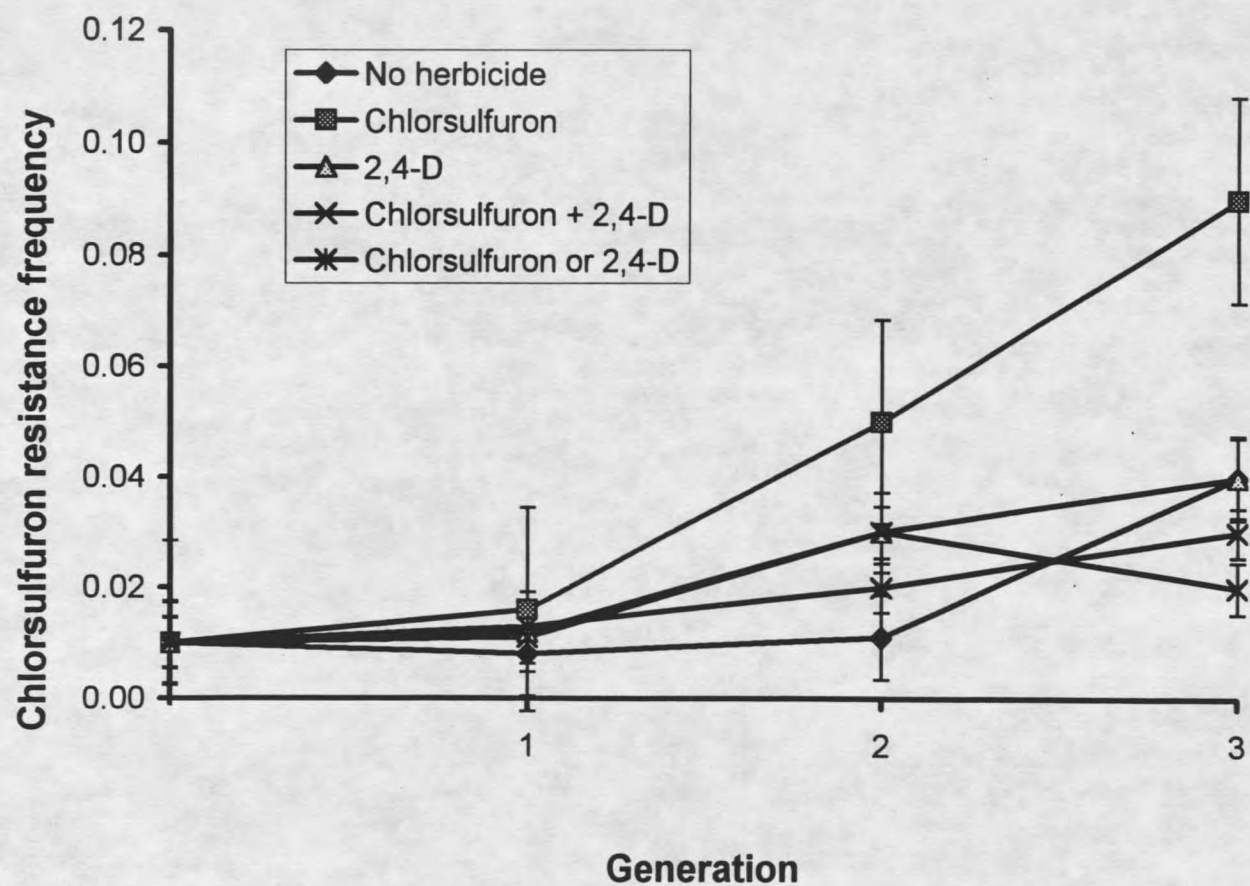


Figure 2.3. Frequency of chlorsulfuron resistant *A. thaliana* in each generation following different herbicide treatments. Initial frequency of chlorsulfuron resistant *A. thaliana* was 1%. Vertical bars represent standard errors.

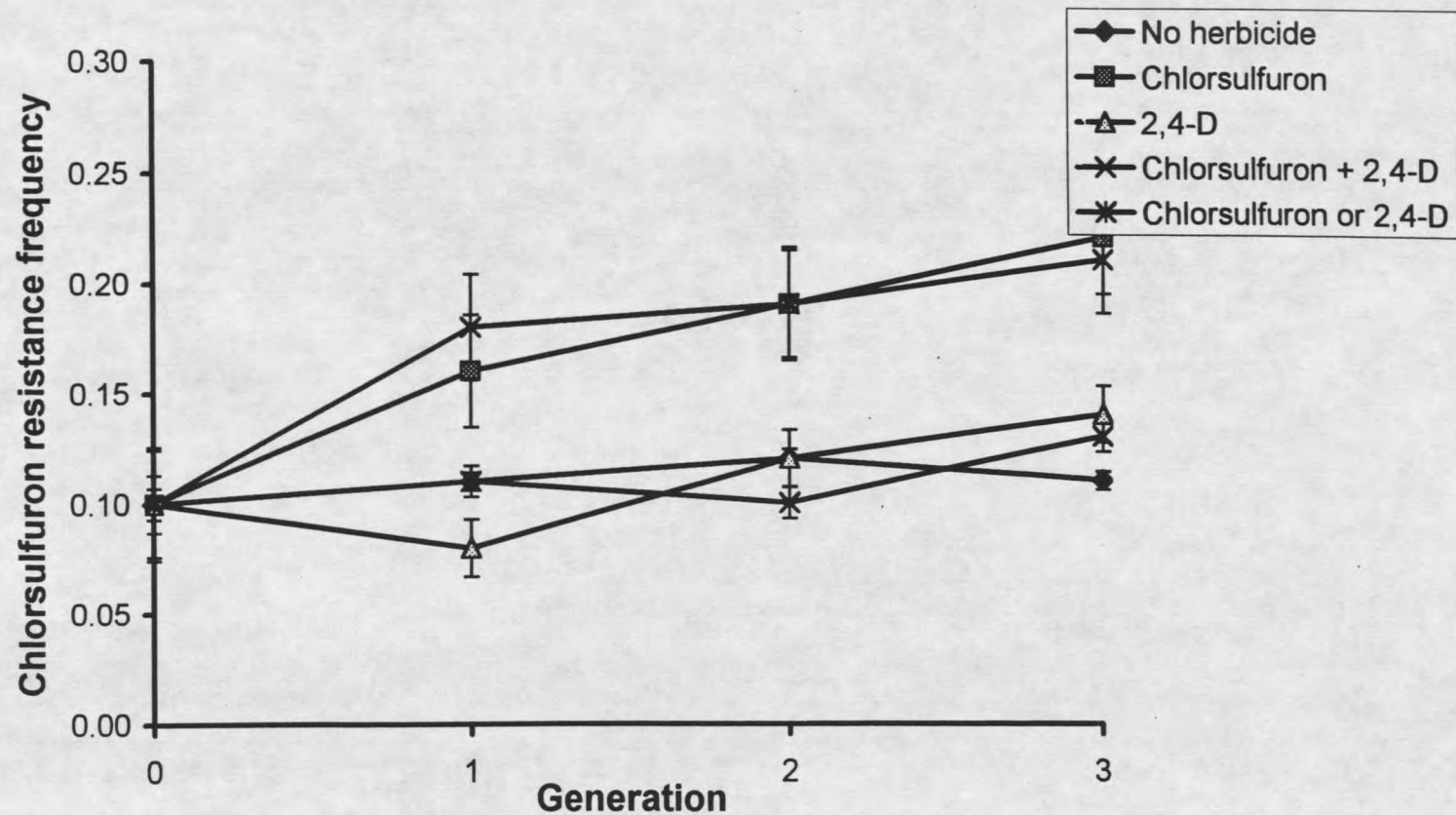


Figure 2.4. Frequency of chlorsulfuron resistant *A. thaliana* in each generation following different herbicide treatments. Initial frequency of chlorsulfuron resistant *A. thaliana* was 10%. Vertical bars represent standard errors.

2,4-D and chlorsulfuron maintained a low frequency of chlorsulfuron within the population with the frequency of resistance not significantly different from the unsprayed control ($P>0.05$). The variation observed among treatments, is in part due to environment by chlorsulfuron interactions that impacted the efficacy of chlorsulfuron observed at each time of spraying. Low seed numbers produced by generation two, due to heavy insect infestations, did not permit extensive testing of resistance. This also contributed to the variation observed.

Efficacy

Increased efficacy of chlorsulfuron was correlated with an increase in the rate of change of chlorsulfuron resistance (Figure 2.5). This is in agreement with predictions of herbicide resistance models (Gressel and Segal 1990; Maxwell et al. 1990). The increase in efficacy from 75% to 90% was associated with an increase in the average annual change in the resistance frequency from less than 10% to over 50%. The rate of change in the frequency of resistance was not significantly different between the higher levels of efficacy (85% to 95%). Higher efficacy was associated with higher rates of change in resistance frequency which is in general agreement with predictions from resistance models. Model predictions of resistance evolution suggest that an increase in efficacy from 85% to 95% would result in resistance development (resistance frequency $> 30\%$) in seven years instead of three years.

The addition of susceptible seed each generation, simulating refuges, had no significant ($P>0.05$) impact on the rate of increase in resistance frequency (Figure 2.5). The 100 seeds added to 100,000 seed, sown in each flat each generation, represents a

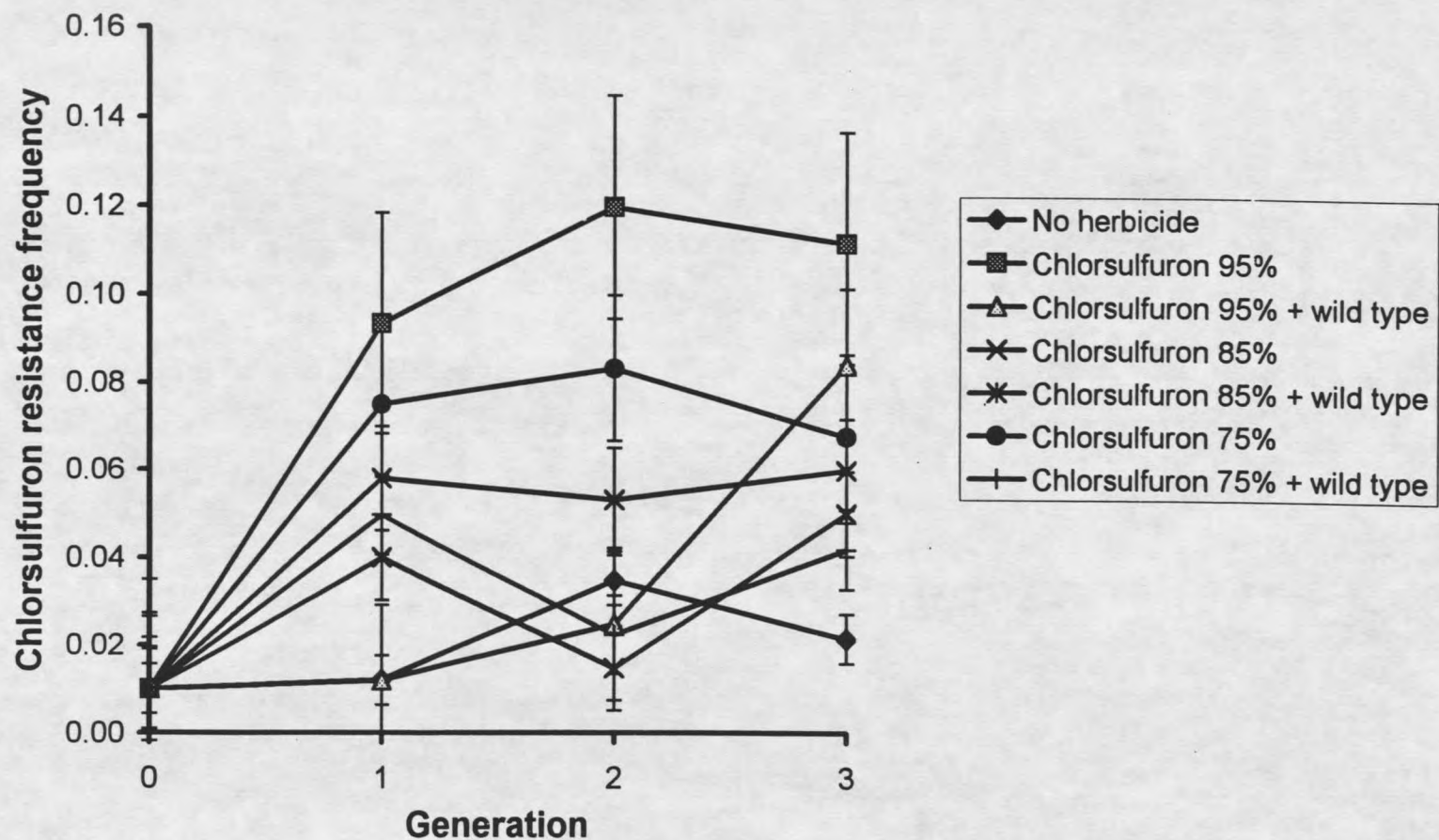


Figure 2.5. Frequency of chlorsulfuron resistant *A. thaliana* in each generation following chlorsulfuron application to 75%, 85% and 95% of the population. Susceptible seeds (100 seeds) were added for each generation. Vertical bars represent standard errors.

relatively small percent (0.1%) of the total population. If refuges within the field are widespread, the susceptible biotype could contribute a much higher percent of seed and pollen to subsequent generations, than those used in this experiment. Jasieniuk et al. (1996) indicated that refuges would be ineffective if selection intensity within the sprayed area is still high (99%) and resistance is conferred by a dominant gene. However, if resistance was conferred by a recessive gene in an outcrossing species, and herbicide efficacy only 75%, then the evolution of resistance could be delayed by several years (Jasieniuk et al. 1996). An alternative strategy that was evaluated by Ghersa et al. (1994) involved sowing susceptible seed into resistant populations. This would lead to relatively high levels of susceptible seed within the field, greater than 1: 1000 used in these experiments, leading to a shift in the gene frequency. The difficulty with this technique is that it can only be practically applied where the weed is also a desirable species in some part of the crop rotation. An example would be rigid ryegrass, which is a highly desirable pasture species in pasture ley farming systems of southern Australia (Smith and Levick 1974).

Mixtures and Rotations

The use of herbicide mixtures and herbicide rotations, although widely advocated as an effective strategy by many integrated weed management programs (Nalewaja 1981; Powles and Mathews 1991; Thill et al. 1991; Moss and Clarke 1994), has not been tested. This study found that rotation and/or mixtures of herbicides resulted in a decreased rate of resistance evolution in *A. thaliana* compared to control treatments (continuous chlorsulfuron) (Figure 2.6). There was no significant difference ($P > 0.05$) in the resistance

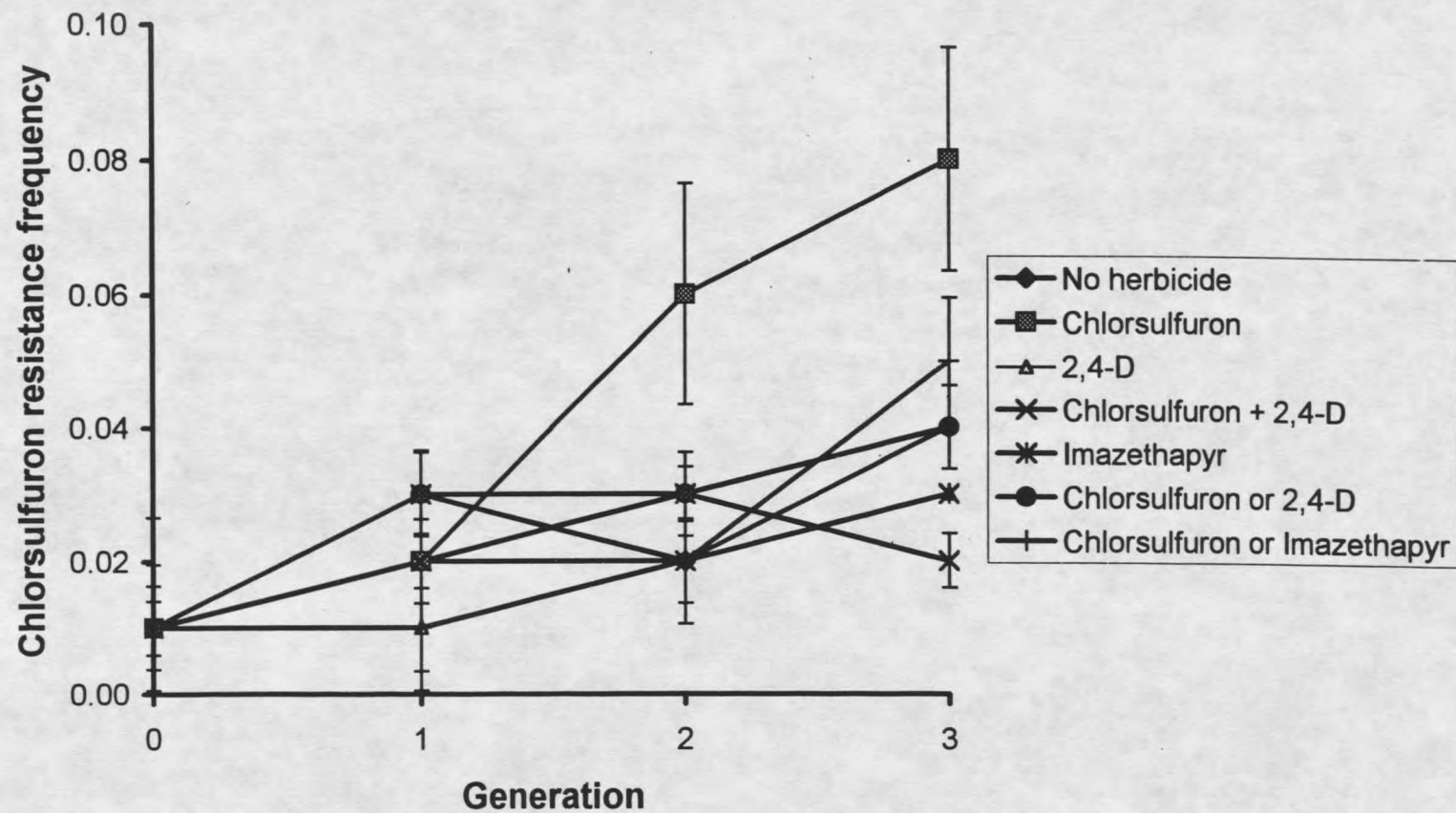


Figure 2.6. Frequency of chlorsulfuron resistant *A. thaliana* in each generation following application of chlorsulfuron alone and in mixture and rotation with 2,4-D plus imazethapyr. Vertical bars represent standard errors.

frequency between rotation and mixtures of herbicides. The increase in the resistance frequency was least where 2,4-D or imazethapyr was used in mixture with chlorsulfuron.

These results provide evidence that mixtures and rotations can be used to reduce the rate of evolution of resistance. The number of years that rotation or mixtures will extend the life of a herbicide is unknown. Only three generations of *A. thaliana* were examined and different species respond differently to selection by herbicides. The life of a herbicide would be extended further if fitness differences between susceptible and resistant biotypes existed. Model predictions by Gressel and Segal (1982) indicated that resistance evolution is a linear function of the number of herbicide applications. In the case of *A. thaliana*, therefore, if it took 10 generations to reach a resistance frequency of 0.5 with chlorsulfuron applied each year then it would take 20 generations to reach this level with either a rotation of chlorsulfuron with 2,4-D or imazethapyr.

The use of herbicide mixtures and rotations needs to be examined on a species by species basis. It is evident, with examples of cross resistance in species such as rigid ryegrass and black-grass (*Alopecurus myosuroides*), that herbicide management cannot be the only strategy for delaying resistance evolution (Kemp et al. 1990; Preston et al. 1996). Herbicide rotations and mixtures may be a risky strategies for resistance management because they may increase selection for cross or multiple resistance (Maxwell et al. 1990). The development of polygenic resistance in *Phalaris* also cautions against reducing efficacy to manage for resistance. The use of integrated techniques still provides the best options for management of resistance. McGauhey and Whalon (1992) state that resistance management associated with any species should integrate four strategies: (1) diversification

of mortality sources rather than relying on herbicides alone (2) reduce selection intensity (3) increase supply of susceptible individuals through the maintenance of refuges to enhance pollen or seed immigration.(4) prediction of resistance evolution through the use of diagnostic tools, monitoring and models. As not all cases of herbicide resistance evolution will follow the same path a combination of strategies is required to reduce the evolution of new herbicide-resistant populations and to provide effective management of existing resistant populations.

CHAPTER 3

PATTERN OF TRIALLATE-RESISTANT WILD OAT (*Avena fatua* L.) AT A REGIONAL, FIELD AND PATCH SCALE

Introduction

The number of weeds resistant to herbicides has exponentially increased since Ryan (1970) reported the first resistant weed, common groundsel (*Senecio vulgaris* L.). The number of weed species resistant to herbicides is currently estimated at more than 150 (Heap 1997). Changes in weed management are often required to cope with herbicide-resistant weeds which may result in higher costs of production and reduced economic return. Wild oat is an example of a serious weed of small grain cropping systems (Thurston 1954; Bowden and Friesen 1967; Thomas 1985) that has evolved resistance to herbicides (Powles and Howat 1990; Mansooji et al. 1992; Devine et al. 1993; O'Donovan et al. 1994; Kern et al. 1996). In Montana, wild oat has evolved resistance to triallate following more than 15 years of continuous use for weed control in malt barley production (Malchow 1995). The farming system has been changed to include the use of crop rotation, with alfalfa and canola, and the use of an alternative herbicide, imazamethabenz, in the cereal crops. Minor changes to the farming system represent a short-term solution as these strategies do little to manage for further evolution or the spread of herbicide resistance.

Development of integrated weed management (IWM) strategies has been suggested as the key to management of weeds (Thill et al. 1991; Dieleman et al. 1996) and herbicide resistance (Powles and Matthews 1991). Effective IWM strategies require a comprehensive understanding of both the spatial and temporal response of weed populations to environmental and human disturbance. Limited information is available on the structure and spatial dynamics of wild oat, one of the most studied weeds. A number of models exist that have incorporated a spatial component to weed dynamics based on assumptions of spatial arrangement (Kropff et al 1996). Maxwell and Ghera (1992) used a reaction diffusion type model to simulate the dispersal of weed seeds. Individual based models have also been developed (Pacala and Silander 1985) that allocate a location to each weed. No weed population model has yet incorporated the structure of real populations with the appropriate scale of processes acting on that population. Models that incorporate these factors should improve the predictive ability of weed dynamics and evolution of resistance.

The introduction of new genes into a metapopulation is due primarily to mutation or low frequency immigration events. Genetic change of patches within a metapopulation depend mainly on gene flow from other patches. Evolution of weeds in response to management practices, such as herbicide use, is highly dependent upon the genetic structure of the patch and the rate of gene flow between patches (Sokal et al. 1989; Maxwell et al. 1990; Jasieniuk et al. 1996). Spatial structure and effective population size can influence evolutionary processes such as the genetic drift and trait selection rate (Heywood 1991).

Small patches are associated with greater stochastic changes in gene frequency, such as changes due to genetic drift (Jain and Bradshaw 1966, Epperson 1990).

Triallate resistance in wild oat was found to be due to a reduced rate of metabolism of triallate to the active compound (triallate sulfoxide), in one population collected from the Fairfield Bench region of northern Montana, USA (Kern et al. 1996). No differences in uptake or translocation of triallate were detected between the triallate-resistant and susceptible biotypes of wild oat. All triallate-resistant populations tested to date are also resistant to difenzoquat (O'Donovan et al. 1994; Kern et al. 1996). Inheritance studies have identified that the mode of inheritance of resistance is due to two recessive genes (Kern et al. 1997). Both recessive genes are required for the triallate-resistant phenotype to be observed.

The objective of this study was to quantify and compare the pattern of phenotypic spatial variation of triallate-resistant wild oat at three spatial scales within the Fairfield region. An additional objective was to use the pattern in triallate resistance detected at each spatial scale to explain scale dependent processes. Patterns in plant location reflect the interaction of wild oat and the environment including the effect of agricultural management on resistance evolution and spread. A detailed knowledge of the spatial scale of processes is required to develop appropriate IWM strategies for delaying resistance evolution and minimizing resistance spread.

Materials and Methods

The distribution and structure of the triallate-resistant wild oat was determined at three scales in the Fairfield Bench region, Montana USA. The three scales were regional, field and patch. The regional scale was a geographically distinct region 20,000 ha in size, encompassing an irrigation district. The field scale was represented by three 0.36 ha sites and the patch scale by two 0.0025 ha sites. Sampling intensity and resolution differed between scales.

Sampling Scheme

In 1992, the Fairfield Bench region was divided into seventy 256 ha sampling areas, divided along section lines. Four fields were chosen at random from within each section, yielding a total of 280 fields of which 237 fields were sampled for resistance to triallate. The remaining fields were not sampled as they had been rotated to pasture. In 1993, 18 of the 237 fields were re-sampled for resistance. This was repeated in 1995 and 1996 when 30 fields were re-sampled in both year from the original 237 fields and the samples were tested for resistance to triallate. Wild oat seed was collected in each field by sampling using the "W" method (Thomas 1985) avoiding areas along fence lines and obvious herbicide skips. All seed collected from a field was bulked into a single sample.

Three fields were selected from known resistant areas on the Fairfield Bench for sampling at a field scale. A grid (60 m by 60 m) was located within each field and divided into 144 cells (5 m by 5 m). Two wild oat plants were identified within each cell, the center most plant and its nearest neighbor. The distance between these plants and the

center of the cell was recorded. All seed was collected from each plant and tested separately for triallate resistance.

In 1994, two patches less than 10 m in diameter were selected at random from fields determined to be resistant to triallate in 1992. A patch was randomly selected from within each field and each patch was sampled using a grid system (5 m by 5 m). Eight parallel transects were placed at random across the grid and 17 quadrats (0.0625 m^2) were located equidistant on each transect. All seed contained within a quadrat was collected and tested for resistance to triallate.

Resistance Tests

Wild oat sampled at the regional scale were tested for resistance using a greenhouse test of 105 seeds from each sample. The seeds were de-hulled (palea and lemma removed by hand) and the endosperm was punctured on the distal end to increase germination. The seeds were sown into rows within flats (45 cm by 61 cm by 9 cm) containing 6 cm of untreated 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 daylight with natural light supplemented with mercury vapor lamps ($165 \mu\text{E m}^{-2} \text{ sec}^{-1}$) at $25^\circ\text{C} \pm 3^\circ\text{C}$ and $16^\circ\text{C} \pm 3^\circ\text{C}$ for day and night temperatures, respectively. The experiment was watered every second day and fertilized weekly with Petersons solution (20:10:20; N, P and K by weight) at a rate of 75 ppm. The experiment was laid out as a completely randomized design with three replicates. Triallate was applied at $1.1 \text{ kg a.i. ha}^{-1}$ to flats containing 2.54 cm of greenhouse soil and this soil was thoroughly mixed in a rotatory mixer. Treated flats received 2.54 cm of triallate treated soil and untreated flats received 2.54 cm

of untreated greenhouse soil mix. The wild oat were harvested 28 days after planting. Triallate resistant and susceptible plants were identified by measuring individual plant fresh weights and comparing fresh weight with that of known susceptible and resistant plants. Large and small size classes were established based on the mean fresh weight per plant of resistant and susceptible plants within each flat. The treated class means were compared to each other and to the untreated mean fresh weight per plant of a field sample using a t-test ($P=0.05$) to determine if a sample was triallate resistant or susceptible (Malchow 1995).

Triallate resistance status of samples collected at field and patch scales was determined using a petri dish test (Kern et al. 1996) due to the large number of samples involved. Seed samples were prepared by de-hulling and piercing the seed on the dorsal side. Seeds were surface sterilized in a 1% bleach solution and imbibed in distilled water in darkness for four days. Twenty seedlings with coleoptiles 10 mm to 15 mm in length were selected and lengths recorded. Five seedlings were placed into each of four petri dishes. All petri dishes were 100 mm by 25 mm and contained one Whatman number 4 9 cm filter paper and 2 ml of distilled water. Seedlings contained in two petri dishes were treated with triallate by placing 1 μL of 100 ppm triallate on the tip of each coleoptile using a 10 μL syringe. The remaining two petri dishes were untreated. After 7 days the shoot length was recorded for the 10 replicated seeds for each treatment. Mean growth in shoots between the untreated and treated proportions of the sample were compared using a t-test.

Spatial Analysis

The location of wild oat samples and the percent resistance was determined for all samples collected at the regional and patch scale. Samples collected at the field scale did not have an exact location recorded for the plant sampled, as the plant chosen for testing for resistance was the plant closest to the center of a 5 m cell. Semivariogram analysis was used to determine the level of autocorrelation or aggregation of triallate-resistant wild oat at a regional and patch scale. Spatial autocorrelation was determined using the semivariogram statistic on arcsine square root transformed resistant proportions using the equation:

$$\gamma_h = \frac{1}{2N_h} \sum_{i=1}^N [Z(x_i) - Z(x_i + h)]^2$$

where γ_h is the semivariogram statistic for the sample locations separated by a distance h (meters). $Z(x_i)$ and $Z(x_i + h)$ are the arcsine transformed triallate resistant proportions at point x_i and $x_i + h$ and N is the number of pairs of sample sites separated by h (Cardina and Sparrow 1996).

Cokriging and cross-covariogram techniques were used to develop maps of resistance for sparse data collected from surveys in 1993, 1995 and 1996 using methods described by Cressie (1993). Cokriging incorporates information about the spatial distribution of resistance between fields sampled in 1992 (237 fields sampled), which was correlated to resistance values in later years (1993, 1995 and 1996) to estimate the resistance values for unsampled fields. The relationship between resistant fields in 1992 and other years was examined using the cross covariogram (Cressie 1993). If spatial

analysis indicated no spatial autocorrelation then maps were produced using inverse distance interpolation as the best alternative to kriging.

Semivariogram analysis of the resistance distribution was not appropriate for analysis of the field scale measurements of resistance. Individual plants were tested for resistance in this experiment resulting in a binomial data distribution (resistant or susceptible plants). Spatial point pattern analysis was used to determine if the resistant or susceptible plants within a cell were aggregated, regular, or completely spatially random. If the process responsible for the distribution of resistant or susceptible plants within the field is completely stochastic, then this can be characterized by a poisson process. It can then be concluded that the process is completely spatially random. The susceptible and resistant plants were analyzed using a program, developed by Hamilton et al. (1995), that tests for non random spatial patterns.

The L function, a measure of crowding, was calculated using the equation:

$$L(r) = [K(r)/\pi]^{1/2} - r$$

where r is distance and $K(r)$ is given by:

$$K(r) = \lambda - 1(E)$$

and λ as defined by Cressie (1993). The function $L(r)$ tends to be greater than zero when the true process leads to aggregation and less than zero where the process leads to a regular pattern (Cressie 1993). The L value was plotted for all point pair distances. Tolerance envelopes were also calculated to statistically test the hypothesis concerning

spatial randomness. Envelopes corresponding to the probability of 0.90, 0.95 and 0.99 were plotted (Hamilton et al. 1995).

Spatial analysis at the field scale also included analysis of the pattern of wild oat plants irrespective of resistance status using the distance measures of wild oat plants from the center of the cell. The analysis used the T-Square Index of spatial pattern (C) (Ludwig and Reynolds 1988) and is given by the equation:

$$C = \frac{\sum_{i=1}^N [x_i^2 / (x_i^2 + 1/2 y_i^2)]}{N}$$

where x_i is the distance from the center of the grid to the first plant and y_i is the distance from the first plant to its nearest neighbor. N is the total number of sample points. To test the significance of the departure from spatial randomness ($C=1/2$) the z value was computed ($P=0.05$) using the equation:

$$z = \frac{C - 0.5}{\sqrt{1/12N}}$$

The significance of z was obtained from a probability table for the standard normal distribution ($z = 1.96$ at $P=0.05$).

Results and Discussion

Regional Scale

Triallate-resistant wild oat were detected in 64% of the 237 fields surveyed in 1992 (Table 3.1). The frequency of fields that contained resistant wild oat increased to

94% in 1993. No survey for resistance was conducted in 1994 but between 1993 and 1995 the frequency of fields containing resistant wild oat had declined 17%. By 1996 the frequency of resistance had declined a further 12%. The changes observed between years in a single field fluctuated widely with both increases and decreases in resistance levels (Table 3.2). Malchow (1995) detected changes within individual fields of +100% in 1992 to -67% in 1993. The changes in triallate resistance from 1993 to 1995 were $\pm 100\%$. The changes from 1995 to 1996 were +63 % to - 47%. Sampling error may account for some of the fluctuations in resistance measurements, but it was also observed that large changes in resistance were often associated with large population fluctuations within the field (especially between 1995 and 1996). A reduction in triallate use occurred following 1992, removed the selection for triallate-resistant plants from many fields. No analysis was conducted to determine if the change in resistance frequency correlated with a change in herbicide use or other management practice.

Table 3.1. Frequency of fields located on the Fairfield Bench containing triallate-resistant wild oat.

Year sampled	Resistant fields (%)	Fields sampled
1992	64	237
1993	94	18
1994	unknown	none
1995	77	30
1996	65	30

Table 3.2. Annual rate of change in frequency of triallate-resistant wild oat within single fields. Samples were collected in 1992, 1993, 1995 and 1996.

Year sampled	Resistance change (%)			
	Average	Maximum increase	Maximum decrease	Standard deviation
1992-1993	+32	100	67	56
(1993-1995)/2	-10	21	47	27
1995-1996	0	62	46	34

The spatial distribution of the fields, sampled in 1992, 1993, 1995 and 1996, for triallate susceptible and resistant wild oat were analysed to determine if resistant fields were aggregated. Semivariogram analysis indicated the pattern of wild oat resistance was non-random at the regional scale (Figure 3.1) A spherical model best described the distribution of resistance at this scale ($r^2 = 0.64$). The range, which is the distance of autocorrelation of point pairs, was 1.47 km. Autocorrelation indicates that fields located less than this distance from each other were more likely to be of similar resistance frequency. The range corresponds well with the average size of farms (≈ 400 ha) within this region and the pattern of resistance was hypothesized to be the result of similar management practices between fields under the same manager. However, the average difference in resistance percentage between fields with the same manager was 55%, almost identical to the average difference between fields with different managers of 57%. This may reflect the similarities between managers in their herbicide use. Malchow (1995) determined that management of fields with respect to herbicide use was correlated to the evolution of resistance. Fields that had received more than 15 years of triallate usually contained triallate-resistant wild oat. The pattern of resistance observed in the surveys

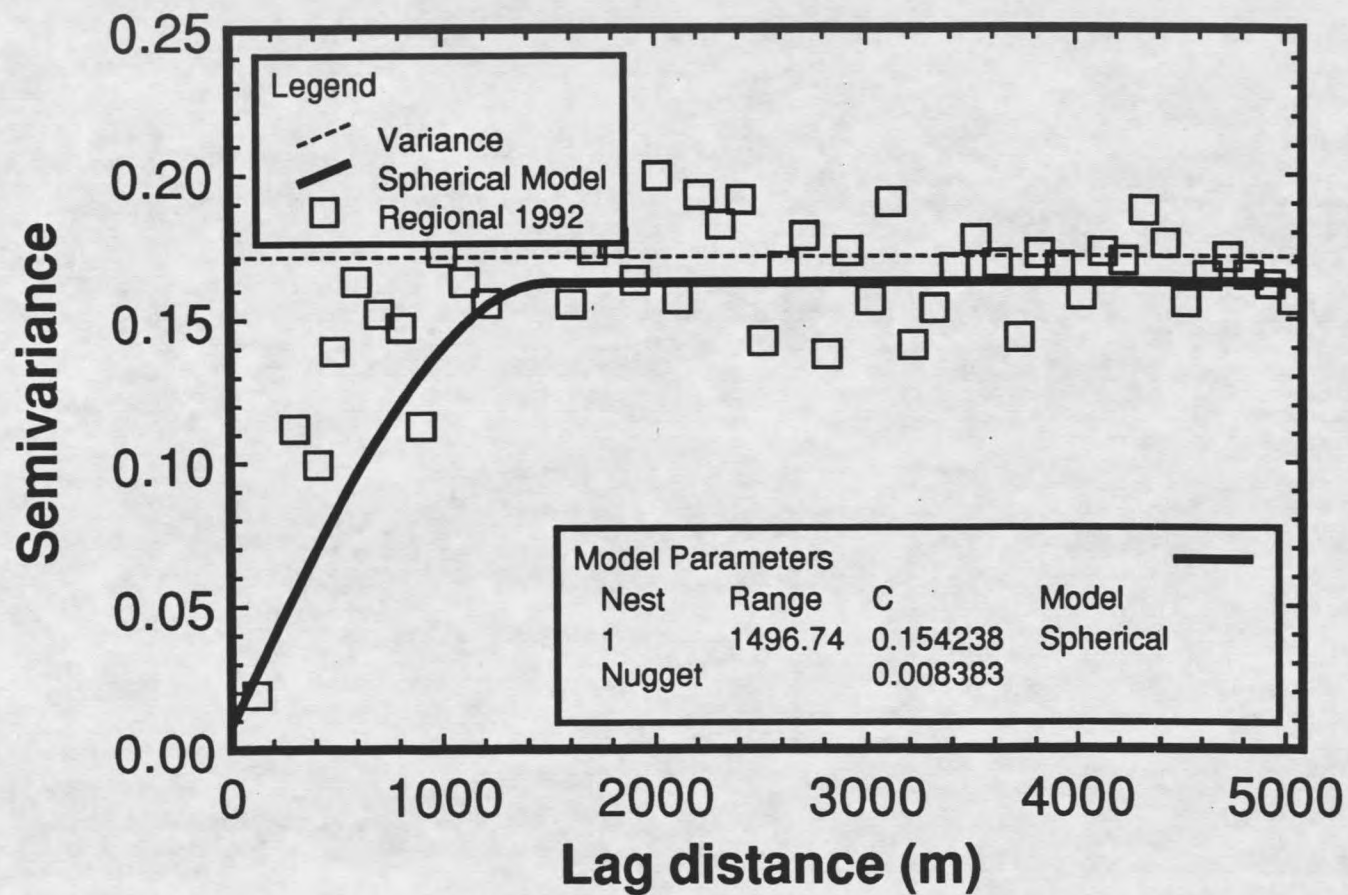


Figure 3.1. Semivariogram of triallate-resistant wild oat sampled at a regional scale in 1992. Resistant wild oat were detected in 64% of fields.

could also be explained as the spread of resistance from an initial point source on a farm. Machinery movement within farms acts as a vector for seed dispersal (Howard et al. 1991) and could increase the level of aggregation of resistance at a farm scale.

The pattern of aggregation, as seen in kriged and cokriged maps of the Fairfield region from 1992 to 1996, fluctuated (Figures 3.3, 3.4, 3.5, 3.6). The change in the pattern of aggregation between years mirrors the large temporal changes occurring with the resistance frequency within individual fields. Even with the fluctuations in pattern, areas containing a high frequency of triallate-resistant wild oat in 1992 were the same areas containing high frequencies of triallate-resistant wild oat in later years. The small number of sample locations contributed to variation observed in the pattern and further investigation is require to determine the optimum sample number for monitoring changes at this scale.

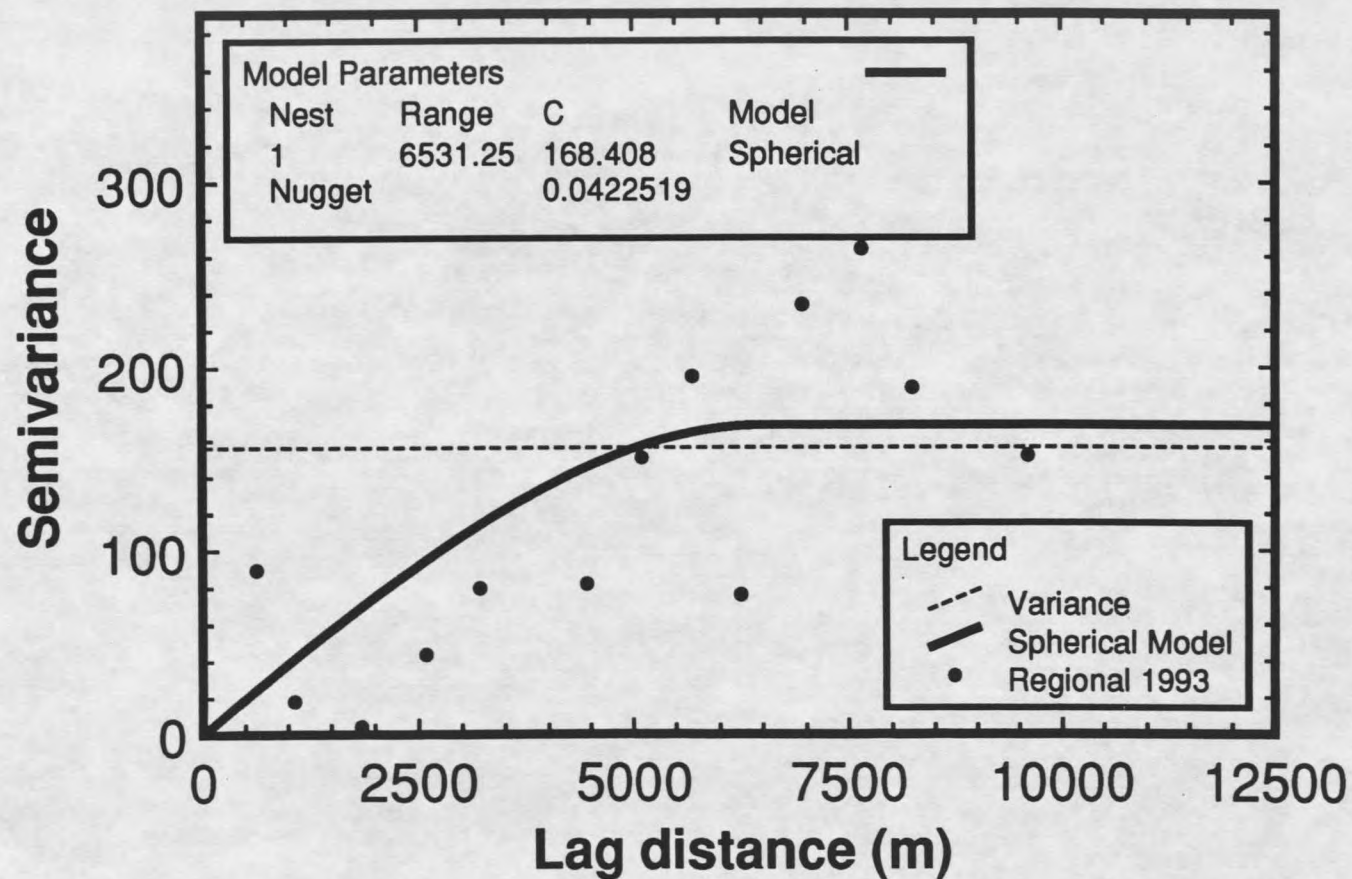


Figure 3.2. Semivariogram of triallate-resistant wild oat sampled at a regional scale in 1993. Resistant wild oat were detected in 94% of fields.

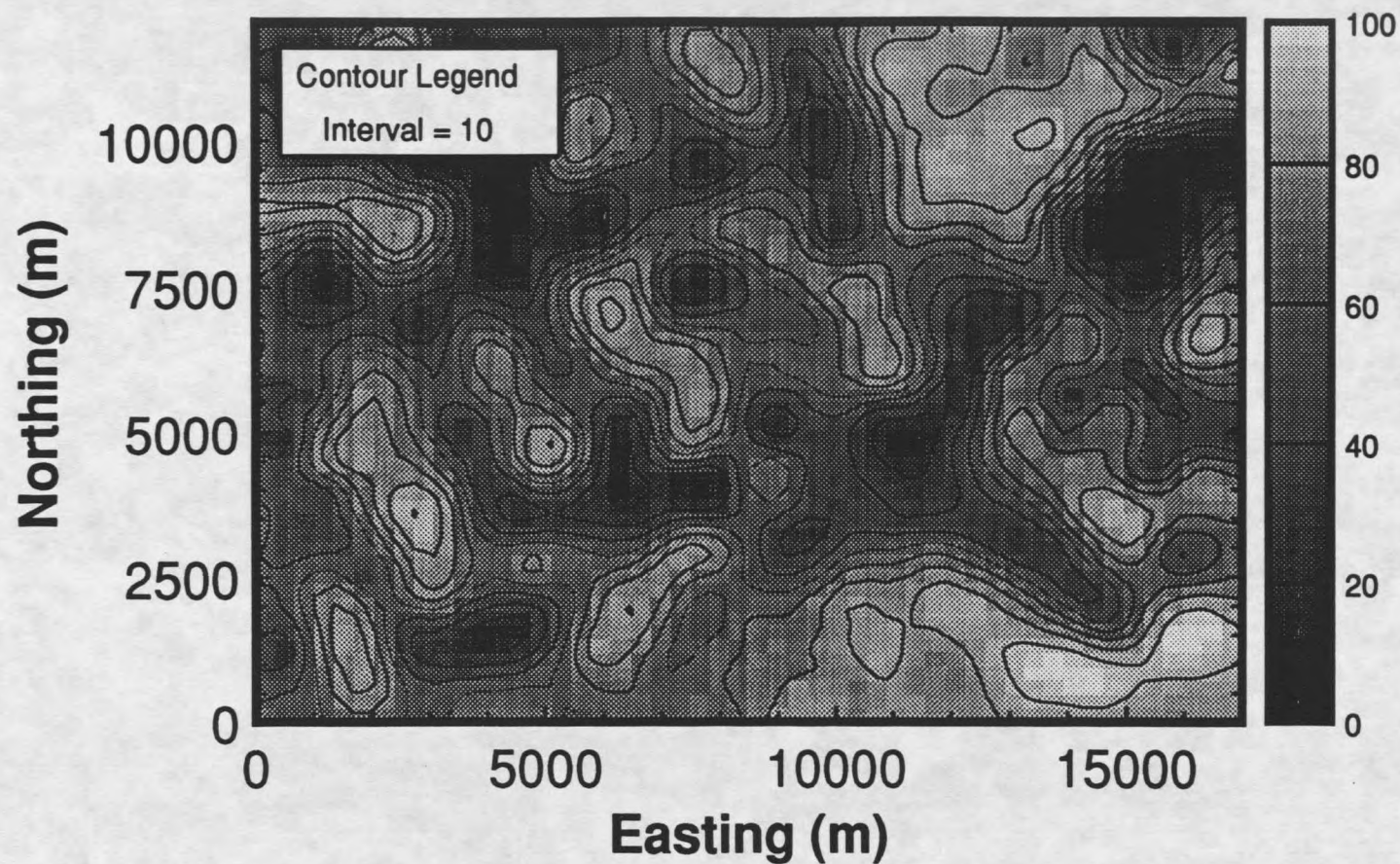


Figure 3.3. Kriged map of triallate-resistant wild oat on the Fairfield Bench in 1992. Resistance was detected in 64% of fields. Grey-scale legend indicates the percentage of resistant wild oat within the field.

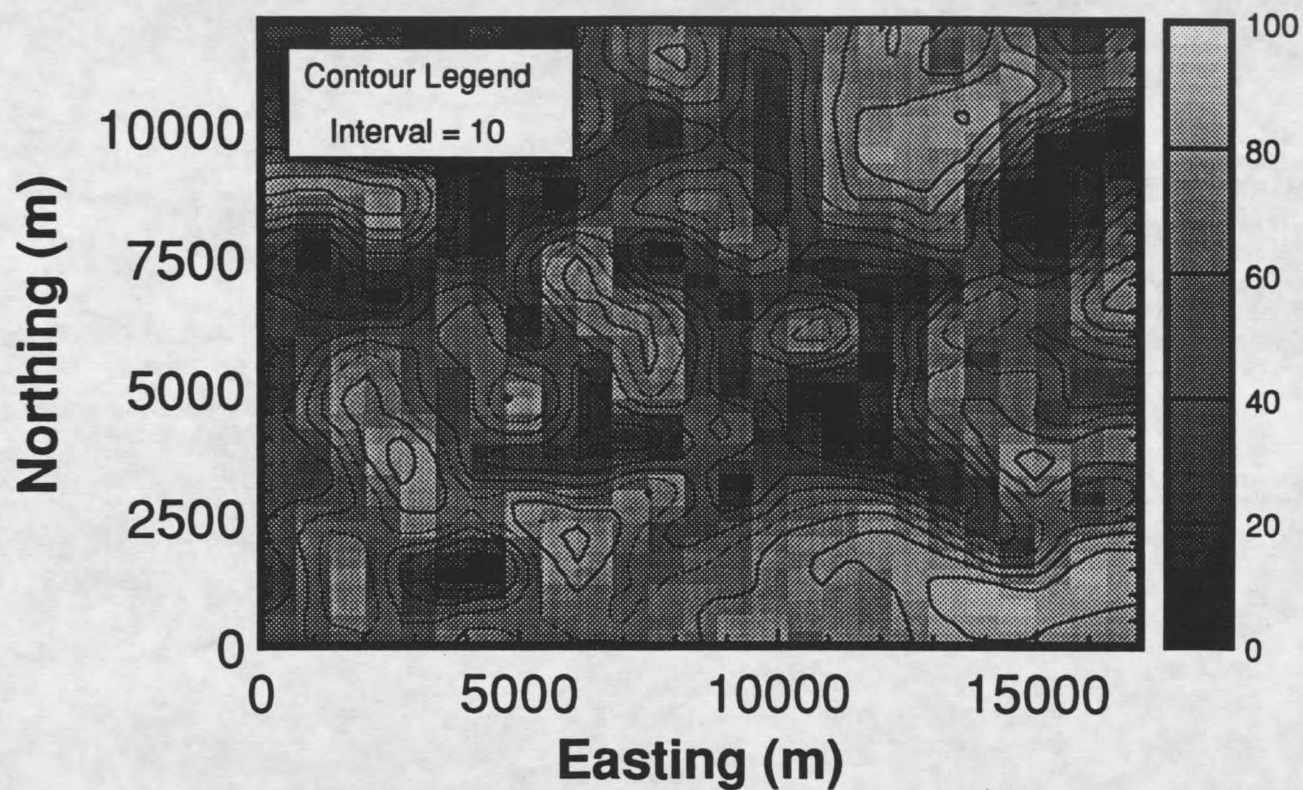


Figure 3.4. Cokriged map of triallate-resistant wild oat on the Fairfield Bench in 1993. Resistance was detected in 94% of fields in 1994. Grey-scale legend indicates the percentage of resistant wild oat within the field.

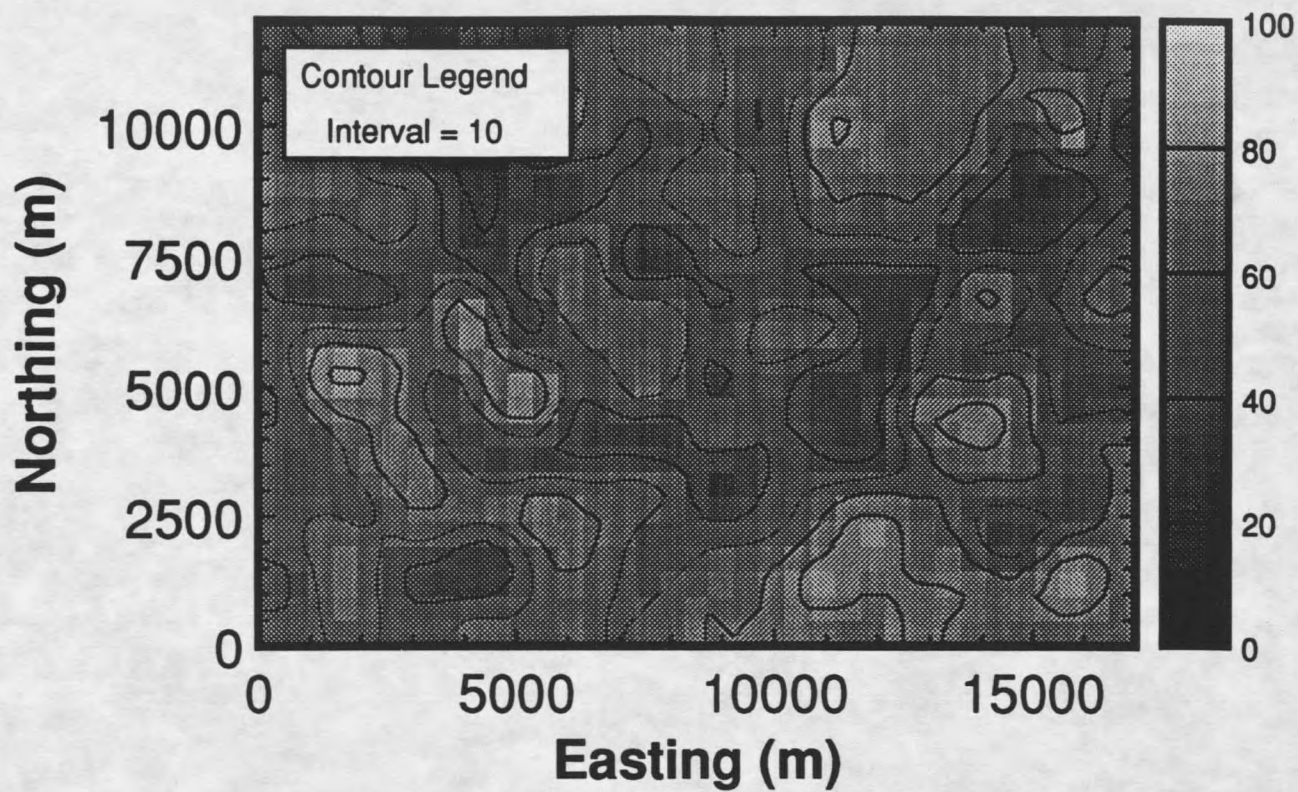


Figure 3.5. Cokriged map of triallate-resistant wild oat on the Fairfield Bench in 1995. Resistance was detected in 77% of fields in 1995. Grey-scale legend indicates the percentage of resistant wild oat within the field.

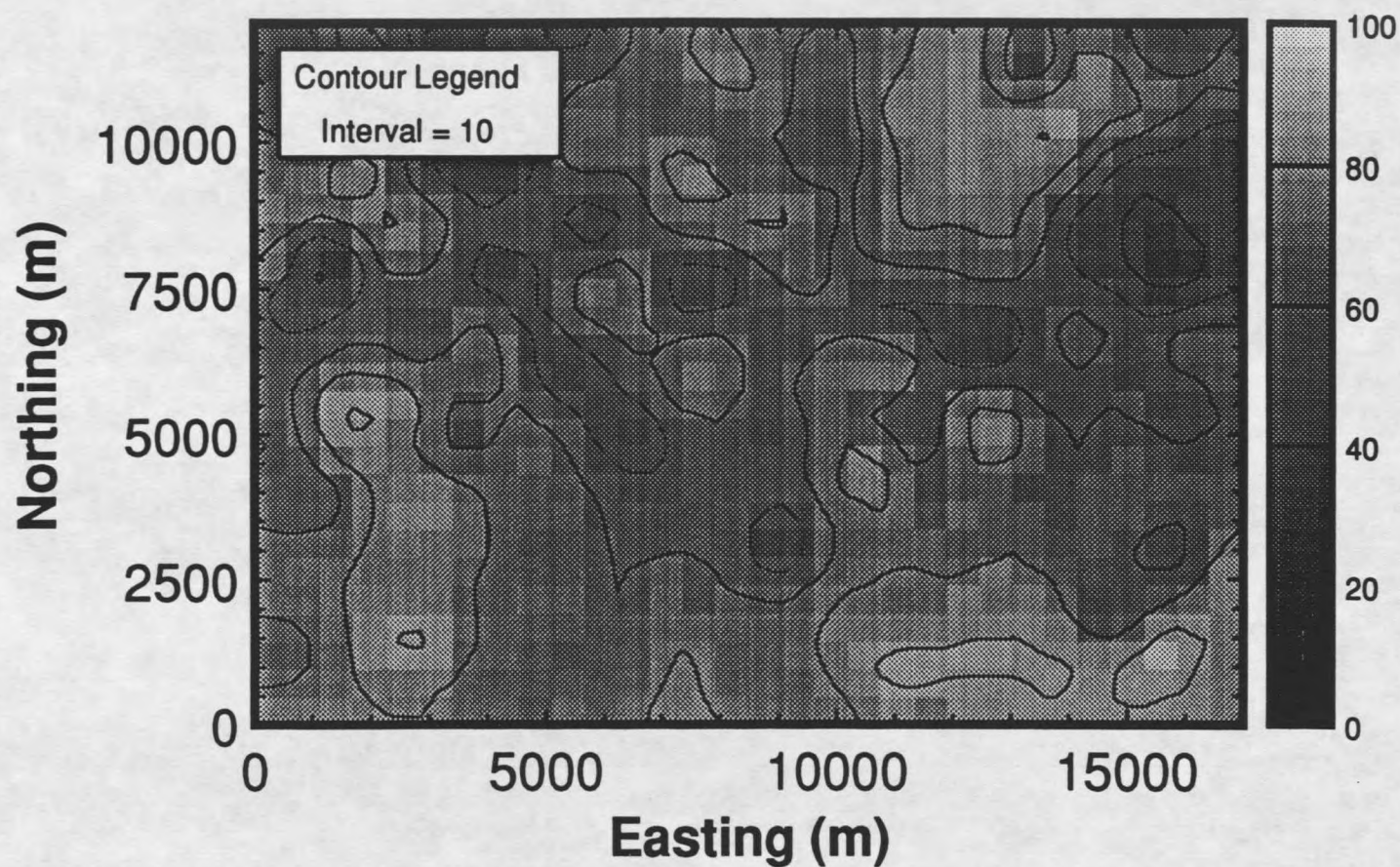


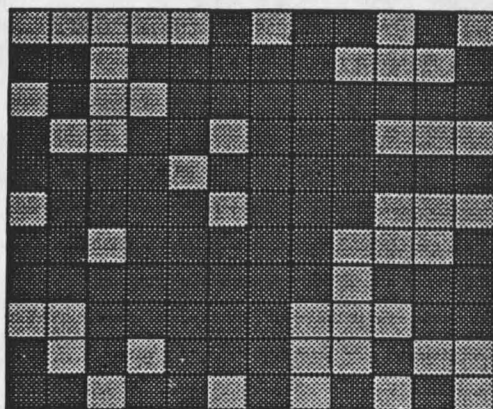
Figure 3.6. Cokriged map of triallate-resistant wild oat on the Fairfield Bench in 1996. Resistance was detected in 65% of fields in 1996. Grey-scale legend indicates the percentage of resistant wild oat within the field.

Field Scale

The pattern of triallate resistance within fields was determined in three fields in 1993 using spatial point pattern analysis. The null hypothesis used for this analysis was that resistance occurrence was completely spatially random. Susceptible and resistant plants were analysed separately for this procedure. Susceptible plants showed no significant aggregation at any of the sites and the null hypothesis was not rejected. Resistant plants at two of the sites (Site 2 and Site 3) were aggregated into patches of 7 m to 8 m in diameter with inter patch distances of approximately 22 m to 28 m determined by analysis using the $L(r)$ function (Figure 3.7). The existence of patches of this size may indicate that resistance has been present in the field for some time. Perhaps the continued use of triallate has enabled resistant plants to spread with minimal competition from susceptible plants creating a number of large aggregated patches. Conversely, the lack of aggregation of resistance at Site 1 may indicate that resistance has only recently developed. Resistant plants had not aggregated into patches greater than 5 m by 5 m as measured using the large grid size with the $L(r)$ function analysis.

The aggregation of wild oat based on density, without respect to resistance status, was also determined using the T-square index of spatial pattern. The level of aggregation at individual cells is shown as a contour map in Figure 3.8. If no pattern was present C would not be significantly different from 0.5. The value for Site 2 and Site 3 was 0.63 and 0.78 respectively, representing significant departure from spatial randomness. This is consistent with the findings of other spatial distribution studies (Marshall 1988; Hughes 1990; Wiles et al. 1992).

(a)



(b)

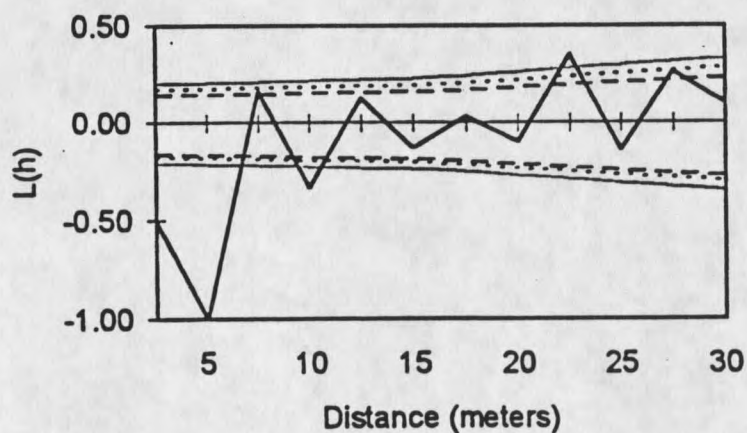


Figure 3.7. Map of triallate resistance within a sample grid (60 m by 60 m) at Site 2 (a). Resistant Susceptible. Plot of L values versus distance for the resistant cells at the same site (b). L function (—), $\pm 90\%$ envelope (---), $\pm 95\%$ envelope (...), and $\pm 99\%$ envelope(· · ·).

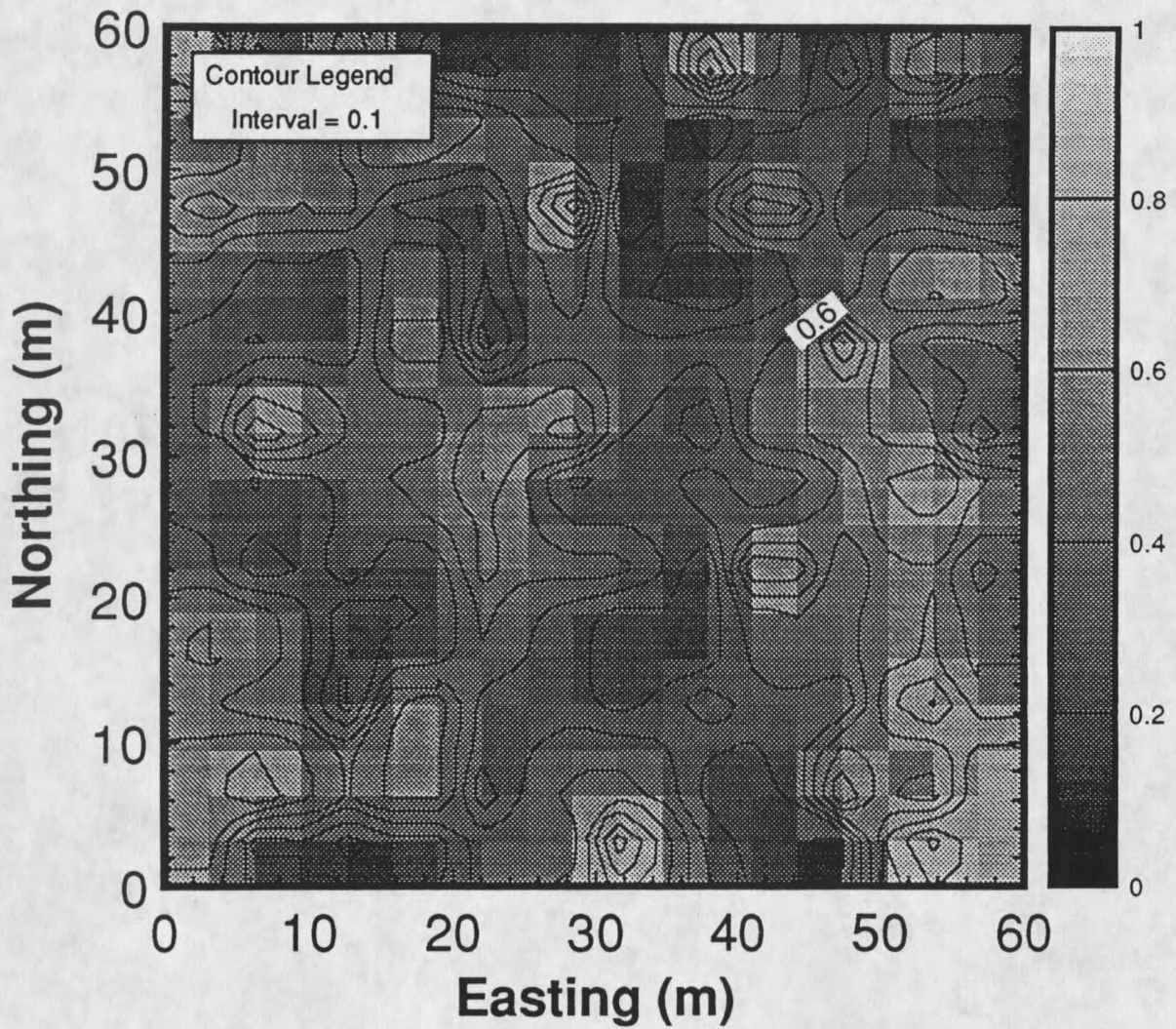


Figure 3.8. Interpolate map of aggregation (C value) at Site 2. C value was 0.63.

The level of aggregation at site 2 was higher where resistant plants were located. Conversely, at site 3, aggregation was higher where susceptible plants were detected. The dynamics involved at a field scale are obviously complex. The interaction of susceptible and resistant biotypes of wild oat could lead to the observed patterns if the resistant biotype was less fit than the susceptible biotype. For example, at Site 3 a high proportion of the cells were found to contain susceptible plants maximizing competition between the biotypes. Resistant plants would then be most successful (express higher fitness) in sites of low density (aggregation). At Site 2 resistant plants occupied a far greater number of the cells minimizing the opportunity for interaction with the susceptible biotype. This could represent a later stage in the spread of resistance in this area. It is unlikely that fitness was a major factor in controlling the dynamics of the population once a high frequency of resistance is present.

Patch Scale

Two patches of wild oat, each at different sites (Site 4 and Site 5), were sampled in 1994 to determine if aggregation of triallate-resistant plants occurred within patches. Both fields had received at least 10 years of triallate use prior to sampling, so it was assumed that resistant plants would have spread throughout the patch and be the dominant biotype. This would result in a patch that would be homogeneous for the resistant phenotype. However, a significant proportion of the plants tested were susceptible to triallate. Semivariogram analysis detected autocorrelation to a distance greater than 1 m in the patch at Site 4 (Figure 3.9) although none was detected in the patch at Site 5. This may indicate a greater level of disturbance and/or dispersal at Site 5 resulting in reduced

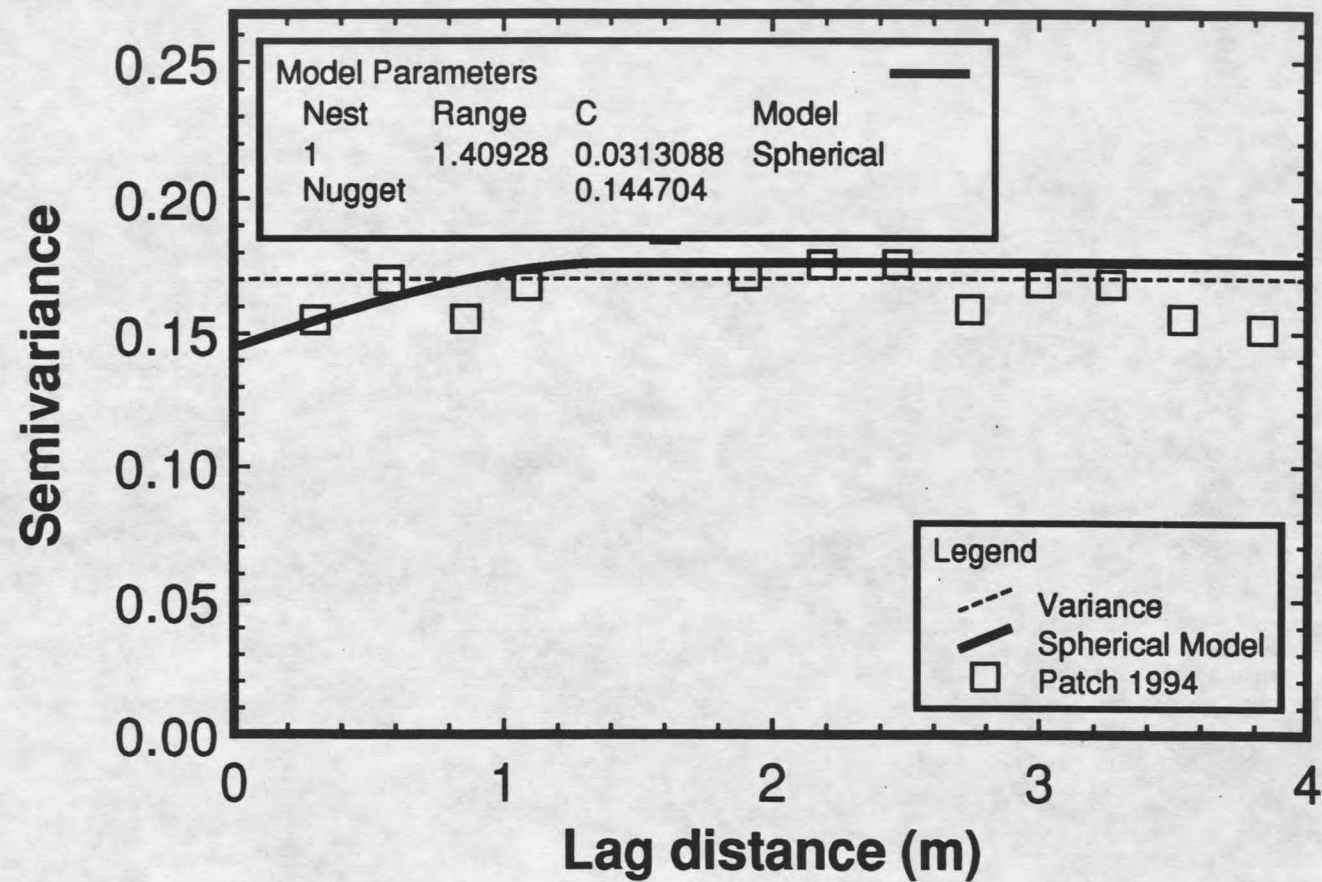


Figure 3.9. Semivariogram of triallate-resistant wild oat sampled at a patch scale in 1994 (Site 4).

genetic structure at this scale. The presence of autocorrelation at Site 4, within a patch, could be the result of later harvest times within this field. A late harvest would enable wild oat to drop more mature seed prior to disturbance by the harvester, leading to a more defined genetic structure. Metapopulation models need to accurately describe the genetic structure within patches. Few population models currently incorporate an internal patch structure (genetic or demographic). The historic assumption is that weed patches are a single gene frequency or density. The aggregation of resistance within a patch will influence, not only the rate of change of resistance within a patch, but also the gene flow occurring between adjacent patches within the population. The pattern of resistance is illustrated in Figure 3.10 and Figure 3.11.

Patterns of resistance were detected at the regional, field and patch scale. The distribution of triallate resistance at all scales was closely tied to the distribution of wild oat and the impact of agricultural practices. However, the pattern observed at each scale was different reflecting the different processes involved at each scale. Studies that integrate genetic and demographic components are required if we wish to fully understand the evolution and dynamics of weed populations under different agricultural management systems. Our understanding of weed dynamics within fields will be improved if we are able to create spatially realistic metapopulation models. Models of the dynamics of dispersal will become more complex due to the need to accurately depict dispersal at each scale; between fields, within fields and within patches. This study has identified appropriate scales at which metapopulations of wild oat should be studied and modeled.

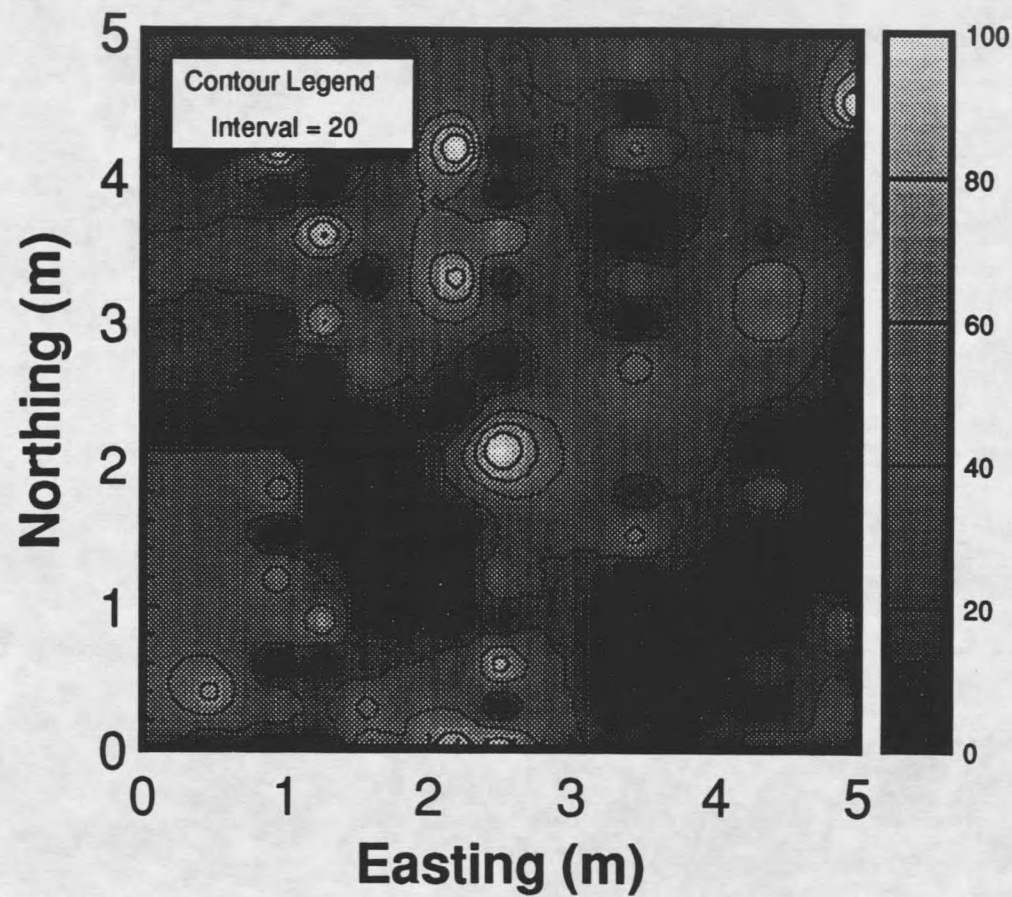


Figure 3.10. Kriged map of triallate-resistant wild oat within a 5 m by 5 m grid at Site 4. Grey-scale legend represents resistance percentage. Resistance levels ranged from 0 to 100%.

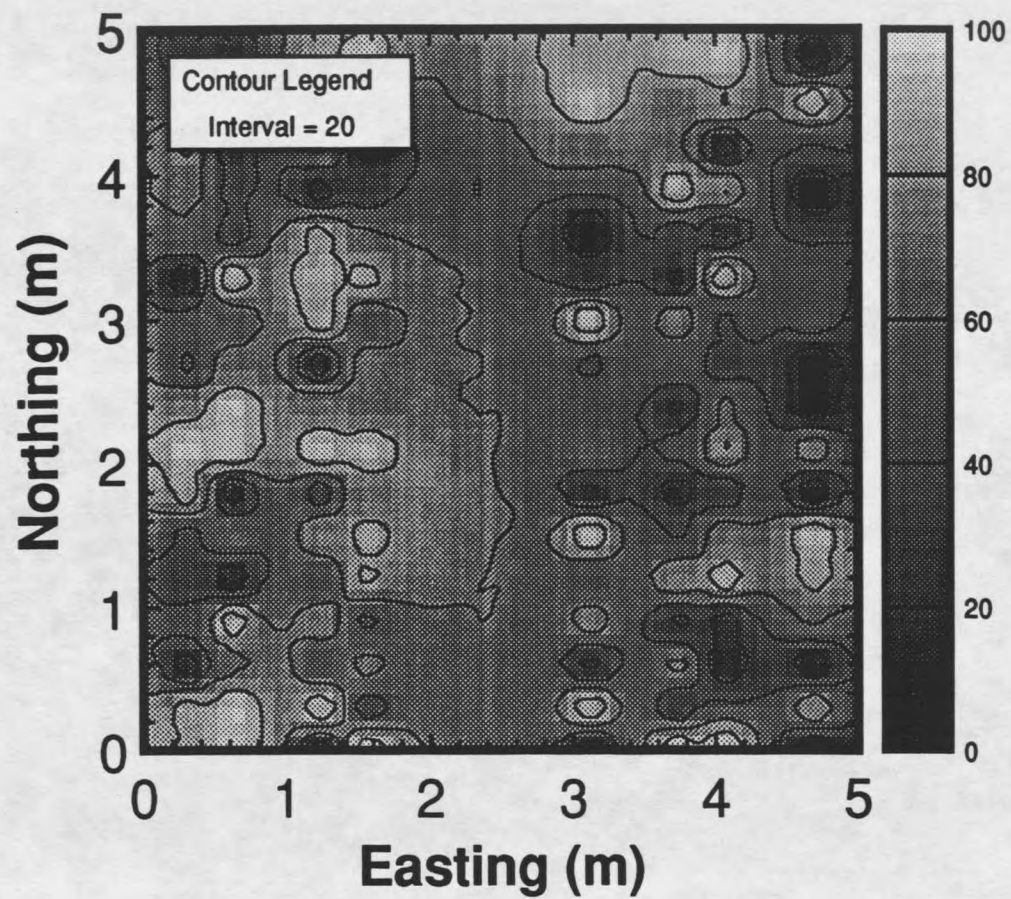


Figure 3.11. Inverse distance interpolation map of triallate resistance within a patch at Site 5. Grey-scale legend represents resistance percentage.

CHAPTER 4

STRUCTURE OF PATCHES OF TRIALLATE-RESISTANT WILD OAT (*Avena fatua* L.)

Introduction

The spatial pattern (demographic and genetic) of a weed population influences the dynamics of weeds and the appropriate strategies for management of the weed population. Simulation modelling, designed to determine management strategies for herbicide-resistant weeds, has identified two processes, gene flow and fitness, that significantly influence the rate of resistance development (Maxwell et al. 1990). The manipulation of these processes within the field has recently been suggested as a potential weed management tool (Roush et al. 1990; Jasieniuk et al. 1996). Ghera et al. (1994) found that management of diclofop-methyl resistant *Lolium multiflorum* could be achieved by altering the gene flow. They suggested that herbicide susceptible commercial ryegrass seeds could be sown into resistant populations of ryegrass as part of a crop rotation, resulting in a dilution of resistance. Gene flow can also be manipulated by a number of other agricultural practices including the use of pollen barriers within crop and the modification of tillage practices restricting the movement of the resistant gene(s). The pattern of gene flow is critical to weed population dynamics and to the effect of the weeds on the crop (Maxwell and Ghera 1992). However, even when gene flow remains

constant, variations in the spatial patterns of density can lead to differences in weed population dynamics (Czaran and Bartha 1992; Maxwell and Ghera 1992).

Weed patchiness has recently been documented to occur in many species (Van Groenendael 1988; Wiles et al. 1992). Patchiness of weeds exists in different patterns (Cardina et al. 1996) that have varying degrees of spatial stability from one season to the next (Wyse and Mortensen 1996). Many of the same weeds species have also demonstrated the ability to develop herbicide resistance under selection (Moss and Rubin 1993; Heap 1997). However, there is limited information available on the genetic structure within patches, despite the implications for manipulation of the gene pool to slow the development of herbicide resistance.

Weed patch existence in dynamic agro-ecosystems is the result of the interaction of underlying scale dependent processes including environmental heterogeneity and agricultural practices. Patchiness of weeds and other plants is often associated with the location of favorable conditions for survival and reproduction. The genetic structure of weed patches is affected by plant density, seed and pollen dispersal and by selection processes acting on the population. Selection, even at a small scale, can change the genetic structure of a patch. The level of change is dependent upon the spatial patterns of the heterogeneous environment, the intensity of selection, and the level of gene flow (Epperson 1990). The relative fitness of different genotypes is dependent on their location within the patch (Sokal et al. 1989). Where weed populations experience different selection intensities, as is the case with site specific weed management, local adaptations

and restricted gene flow may interact to promote greater genetic variation between metapopulations.

This study examined the within patch demographic and genetic spatial structure of triallate-resistant wild oat. Analysis using geostatistical techniques enables the detection of structure within patches (Epperson and Allard 1989; Cardina et al. 1995) and enables the formulation of hypotheses that identify the major factors contributing to within patch structure. It was hypothesized that farm machinery operations would enhance the initial spread of herbicide resistance throughout the field resulting in patches containing only triallate-resistant wild oat. In addition it was hypothesized that wild oat demographic and phenotypic structure would remain relatively stable in the later phases of resistance evolution.

Materials and Methods

Wild oat were sampled from fields located on the Fairfield Bench, a malt barley production region, in northern Montana. Four fields containing resistant wild oat were chosen from within the Fairfield region using information from previous surveys (Malchow 1995). All samples were collected from fields that had been sown to malting barley. Wild oat seeds were collected immediately prior to the harvest of the crop.

Sampling Scheme

Seven wild oat patches were sampled using a grid system at four different sites over three years. The grid size was 5 m by 5 m for all patches in 1994, but was increased to 10 m by 10 m in 1995 and 1996. The grid was positioned over each wild oat patch to

be sampled so that the center of the grid visually corresponded to the center of the patch. Eight parallel transects were randomly positioned across the grid and 17 quadrats (0.063 m²) in 1994, and 20 quadrats in 1995 and 1996, were sampled at regular intervals along each transect. All seeds on wild oat panicles within each quadrat were collected and maintained as a sample to be tested for triallate resistance. Numbers of panicles were also recorded from within each quadrat for estimates of wild oat density.

Resistance Test

Wild oat samples collected in 1994 were tested for resistance to triallate using a petri dish test (Kern et al. 1996). In 1995 and 1996 resistance was determined for each sample using a greenhouse pot test. Forty seeds from each field sample were removed and de-hulled (lemma and palea removed by hand). The endosperm was punctured by a needle to minimize dormancy and promote even germination. Ten seeds were sown in each of four pots (12 cm by 12 cm by 15 cm) previously filled with 12 cm of greenhouse soil mix (1:1:1 silt loam: washed sand: peat moss, by vol.). An additional 2.54 cm of triallate treated soil (1.1 kg ai ha⁻¹) was added to two of the pots for each sample. Untreated soil (2.54 cm) was added to the remaining two pots. Wild oat germinated and grew under a 14 hr day length regime of natural sunlight supplemented with mercury vapor lamps (165 $\mu\text{E m}^{-2} \text{sec}^{-1}$) with day/night temperatures of 24/16°C, respectively. Pots were watered daily and fertilized weekly with Petersons solution (20:10:20; N,P and K by weight) at a rate of 75 ppm.

Seedlings were counted 28 days after sowing and grouped into three categories based on size; small, intermediate and large. All seedlings in the intermediate and large

categories were determined to be resistant by statistical comparison of the distribution of seedling weights with the resistant and susceptible control groups. All small plants (less than 2 cm in height) were determined to be susceptible. Triallate susceptible seed was obtained originally from a non-dormant inbred line SH430 (Naylor and Jana 1976). These plants were grown at the Arthur Post Farm in Bozeman, MT, in 1992. An additional susceptible biotype was collected in 1994 from a natural population SRSF94, located along a roadside in the Fairfield region. RGH22 and RSFR94 both originated from triallate-resistant fields in the Fairfield region, were used as the triallate-resistant controls.

Data Analysis

Exploratory data analysis for detection of violations of the assumptions of stationarity in the wild oat density data, involved first testing for normality using the Shapiro-Wilks statistic (W). If stationarity is present, statistical properties of density are the same within the area measured, and spatial analysis can proceed. Density values for wild oat within the patch were highly skewed and were transformed using a $\log_e$ transformation for all except Site 2 in 1995. Box plots were used to assist in determining the presence of outliers. If a trend was present the data were median polished (Cardina et al. 1996) using the algorithm developed by Cressie (1993):

$$y'_{ij} = y_{ij}^t - y_{.j} - y_{i.} + y_{..}$$

where y'_{ij} is the detrended value, y_{ij}^t is the transformed value ($\log_e$ or arcsin square root) at row i and column j , $y_{.j}$ is the j th row median, $y_{i.}$ is the i th column median and $y_{..}$ is the median for the complete data set. A trend in the data, if not corrected, will result in

different estimates for the parameters of models fit to the semivariance values. The semivariogram will include the variation associated with the trend that may be unrelated to the processes that resulted in aggregation of wild oat. The distributions obtained following removal of trend (if required) were then examined again for normality and the presence of outliers. A semivariance analysis was also used for the resistance data, however, due to the nature of the percentage data, an arcsin square root transformation was used (Ahrens et al. 1990). The extreme values (0% and 100%) for the resistance data were so frequent that they could not be adequately adjusted by transformation and these sites were excluded from further spatial analysis. Statistical analyses of the panicle number (density) and resistance data were done by autocorrelation using the semivariogram statistic:

$$\gamma(h) = \frac{\sum_{i=1}^{n-h} [Z(x_i+h) - Z(x_i)]^2}{2[N(N-h)]}$$

where $\gamma(h)$ is the semivariance at a lag distance of h . x_i is the wild oat density or resistance for sample i and $x_i + h$ is the density and resistance value located at a distance (h) from x_i . N is the number of sample location pairs.

Isotropic semivariograms were constructed for all the data sets to identify the spatial structure and appropriate model to enable comparison of spatial parameters between sites and years. Using Uncert and GS+ a range of models were evaluated including linear, linear sill, spherical, exponential and gaussian. The spherical model provided the most consistent fit for all the data.

The spherical model:

$$\begin{aligned}\gamma(h) &= C_0 + C_1 [(3h/2a) - 1/2(h/a)^3] && \text{for } 0 < h < a \\ \gamma(h) &= C_0 + C_1 && \text{for } h \geq a\end{aligned}$$

where a is equal to the range and h is the distance between point pairs. C_0 is the nugget variance (y-intercept) and $C_0 + C_1$ equals the sill or asymptote.

Anisotropic analysis was also conducted on the density data. Semivariograms were calculated for four directions; 0° , 45° , 90° and 135° . This enabled the principle anisotropy (direction of strongest spatial autocorrelation) to be detected. Difference in the range of the spherical model was used as an indicator of the importance of directional processes such as harvesting or cultivation.

The semivariogram parameters of the spherical model were used to estimate density and resistance values for non sampled points. Block kriging was used to create a map based on the estimated weights to provide the best linear unbiased estimate of the the non sampled points. Trend that was previously removed was added back to kriged values and these were then back transformed. This produced a map reflecting best estimate values for triallate resistance and wild oat density. Correlation of patches between years was achieved by calculating the correlation coefficient between kriged maps. The kriged maps consisted of 400 estimated data points for density with each data point representing an area of 0.25 m^2 .

Results and Discussion

Spatial Distribution of Wild Oat

The maximum density of wild oat populations within a patch, as estimated by panicle number, ranged from 384 panicles m^{-2} to 1180 panicles m^{-2} . The maximum density of wild oat varied from year to year at the same site as well as between sites. The population at Site 2 had a maximum density of 1180 panicles m^{-2} , in 1995, and declined to 950 panicles m^{-2} in 1996. The mean density for Site 2 increased from 243 panicles m^{-2} in 1995 to 339 panicles m^{-2} in 1996 (Table 4.1). The variation in density between sites in the same year was similar to the variation observed over years. This reflects common management practices for control of wild oat between farms, particularly herbicide use patterns. Triallate was the only herbicide used for wild oat control within the Fairfield region on many farms up to 1993. The widespread development of resistance to this herbicide resulted in a change by many managers to an alternative herbicide, imazamethabenz. The cropping system has also undergone change from a monoculture of malting barley to include alfalfa and canola in a crop rotation, although the predominant crop remains malting barley. Tillage has often been used in the past for control of wild oat prior to sowing, and is still used widely even though it is no longer required for incorporation of herbicides.

Semivariogram analysis of patch density indicated a high degree of aggregation of wild oat plants. A representative semivariogram was plotted (Figure 4.1). The corresponding analysis for other sites and years are shown in Table 4.2. The level of aggregation that was found indicated that plants within a patch were influenced by other

plants, by competition and/or dispersal, up to a distance of 4.8 m (Table 4.2). This causes a decline in seed production per plant in areas where densities are high. If resistant plants are not randomly located within a patch then density of wild oat plants will affect the relative contribution of each biotype to the next generation, therefore altering the rate of change of triallate resistance frequency. The total size of patches was often greater than 10 m in diameter, indicating that the dynamics of the patch were influenced by a combination of processes occurring at a smaller scale. This study found aggregation up to 4.8 m, which is larger than the 2 m previously calculated for *Avena barbata*, by Rai and Jain (1982).

Table 4.1. Mean and standard deviation of wild oat density (estimated by panicle number) within patches, at four locations, from 1994 to 1996.

Site name	Year	Density	
		Panicle m ⁻²	
		Mean	SD ^a
Site 1	1994	345	241
Site 2	1994	120	83
	1995	243	149
	1996	339	190
Site 3	1995	210	160
	1996	110	105
Site 4	1995	120	80

^a Standard deviation.

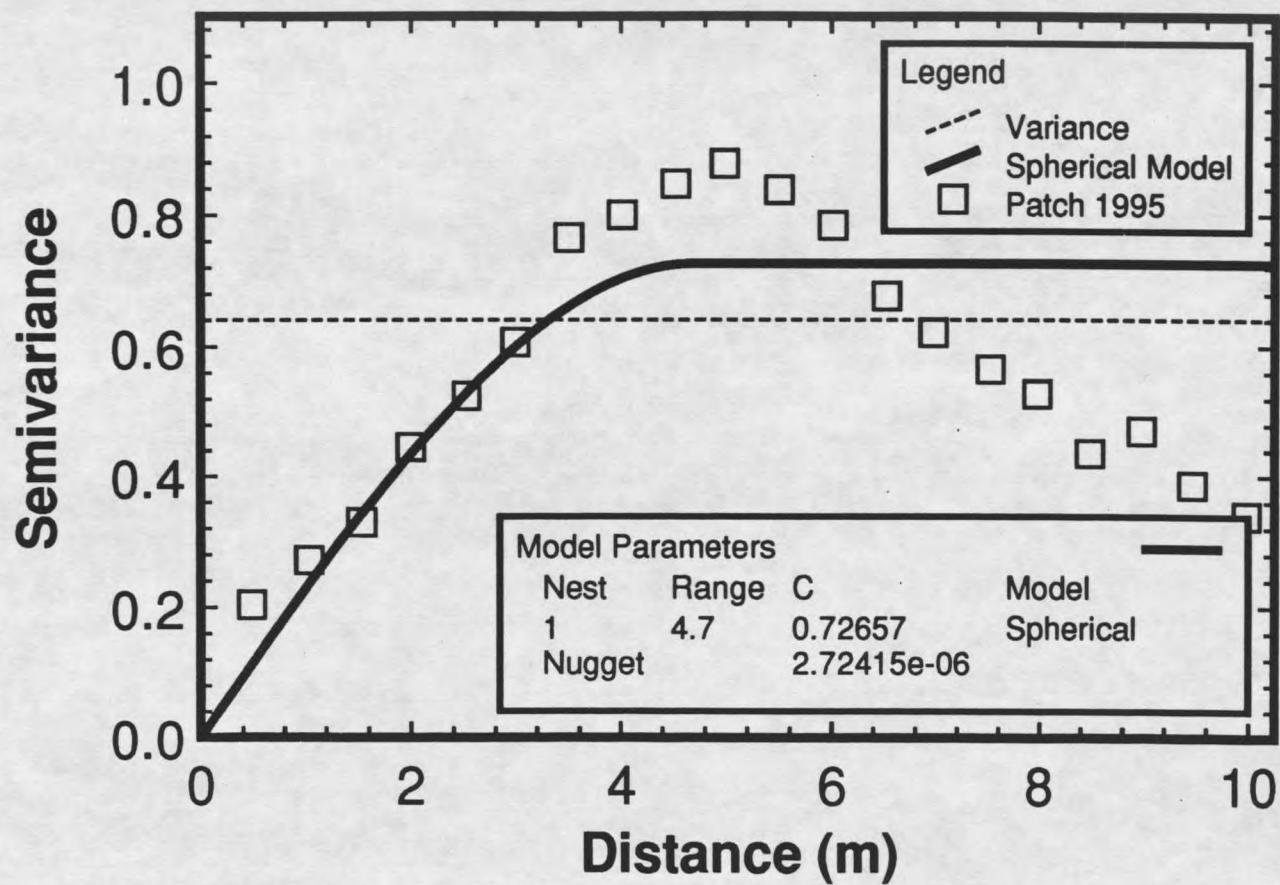


Figure 4.1. Isotropic semivariogram of density at Site 3 for 1995. Log_e transformed density (panicles m⁻²).

Table 4.2. Semivariogram parameters of the spherical models fit to density at four sites from 1994 to 1996.

Site name	Year	Range m	Nugget C_0	Sill (C_0+C_1)	r^2
Site 1	1994	3.1	0.1	0.6	0.87
Site 2	1994	7.3	0.5	1.8	0.95
	1995	2.4	^a 1.03×10^4	2.35×10^4	0.47
	1996	3.5	0.1	0.3	0.76
Site 3	1995	4.7	0.1	0.9	0.84
	1996	4.8	0.2	0.8	0.73
Site 4	1995	1.7	0.8	3	0.58

^aData were not log_e transformed

Density of wild oat was generally higher in the center of the patches (Figures 4.2, 4.3, 4.4, 4.5 and 4.6). The rate of change of density from the center to the edge varied between patches and years. In general, the larger the patch size the slower the rate of change in density observed from the center to the edge of the patch. The slow rate of change in density may be a reflection of density dependent processes acting in the center to limit the total number of panicles produced. The actual limit to panicle production per unit area would change with crop and seasonal conditions. The edges of patches in the field often appeared to be lobed, possibly indicating that the patch is expanding. In a highly disturbed landscape, as is typical of agroecosystems, the shape of the patch may reflect the continuous rebound of a population following herbicide use. Herbicides dramatically reduced the population numbers far below the carrying capacity, and the population compensated by maximizing the seed production of the remaining plants.

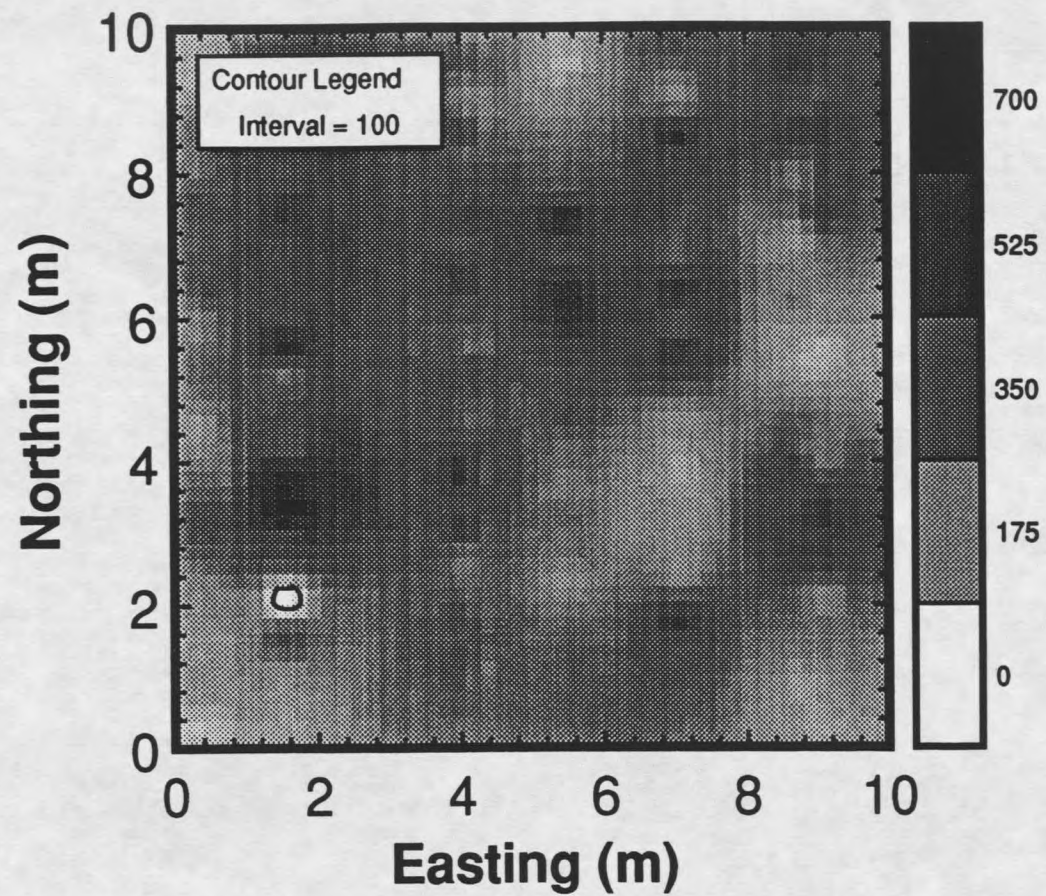


Figure 4.2. Map of kriged estimates of wild oat density at Site 2 in 1995. Grey-scale represents wild oat density (panicles m^{-2}).

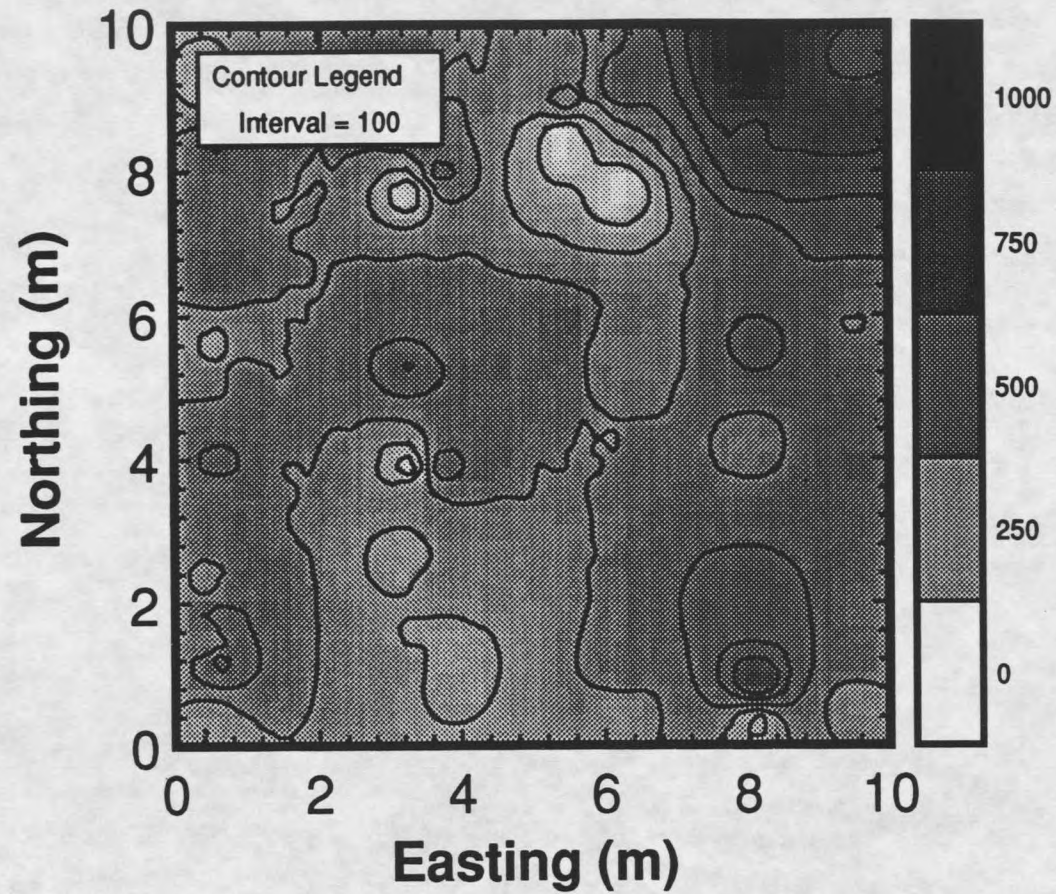


Figure 4.3. Map of kriged estimates of wild oat density at Site 2 in 1996. Grey-scale represents wild oat density (panicle m^{-2}).

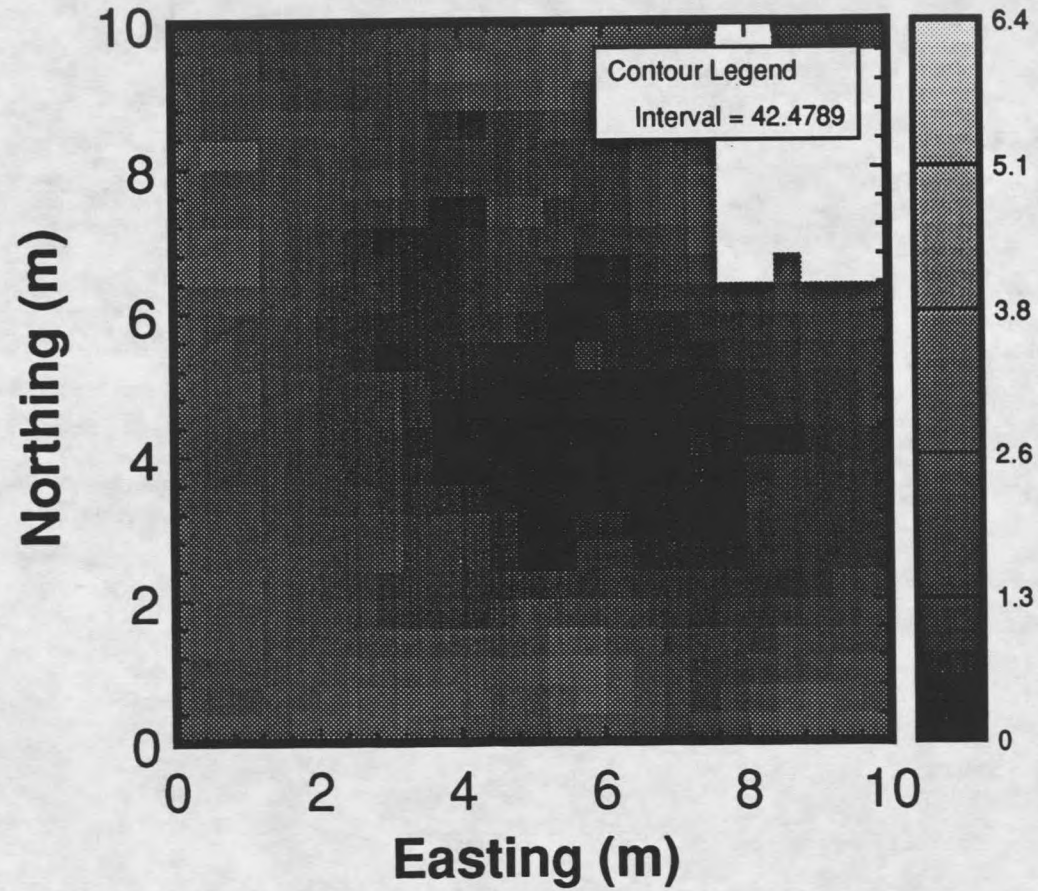


Figure 4.4. Map of kriged estimates of wild oat density at Site 3 in 1995. Grey-scale represents wild oat density (Log_e panicles m^{-2}).

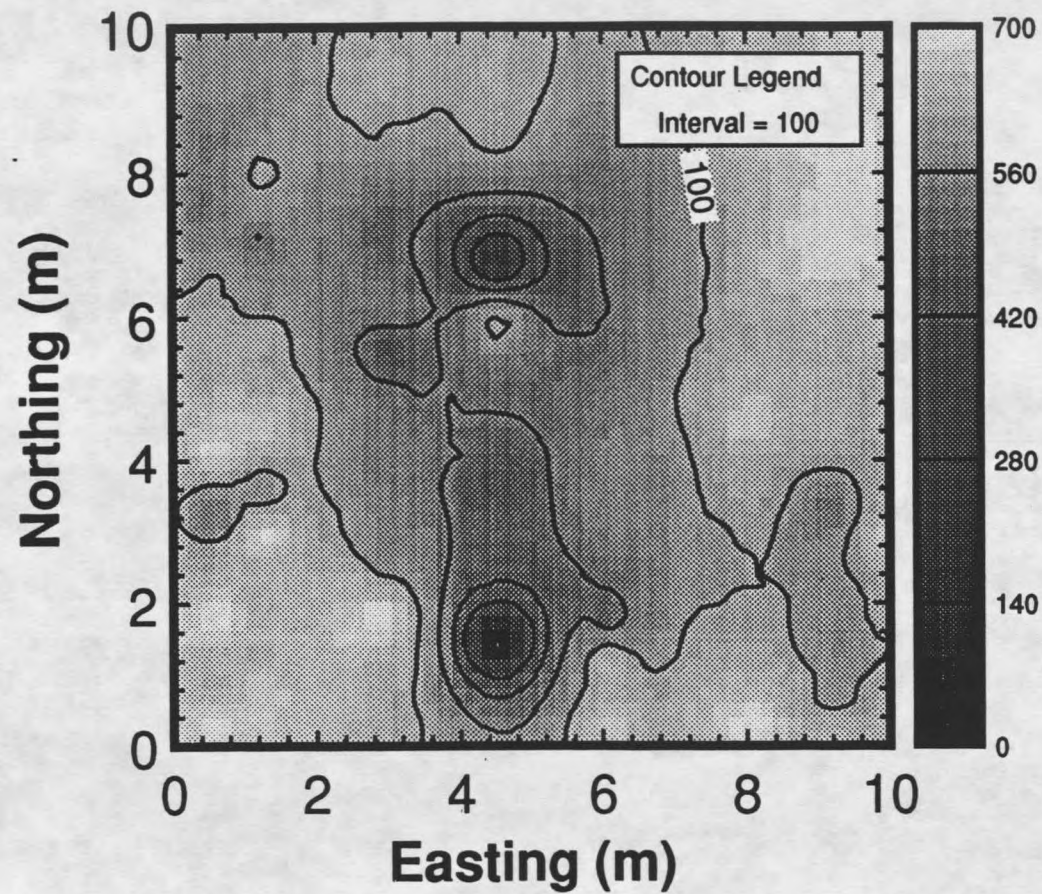


Figure 4.5. Map of kriged estimates of wild oat density at Site 3 in 1996. Grey-scale represents wild oat density (panicle m^{-2}).

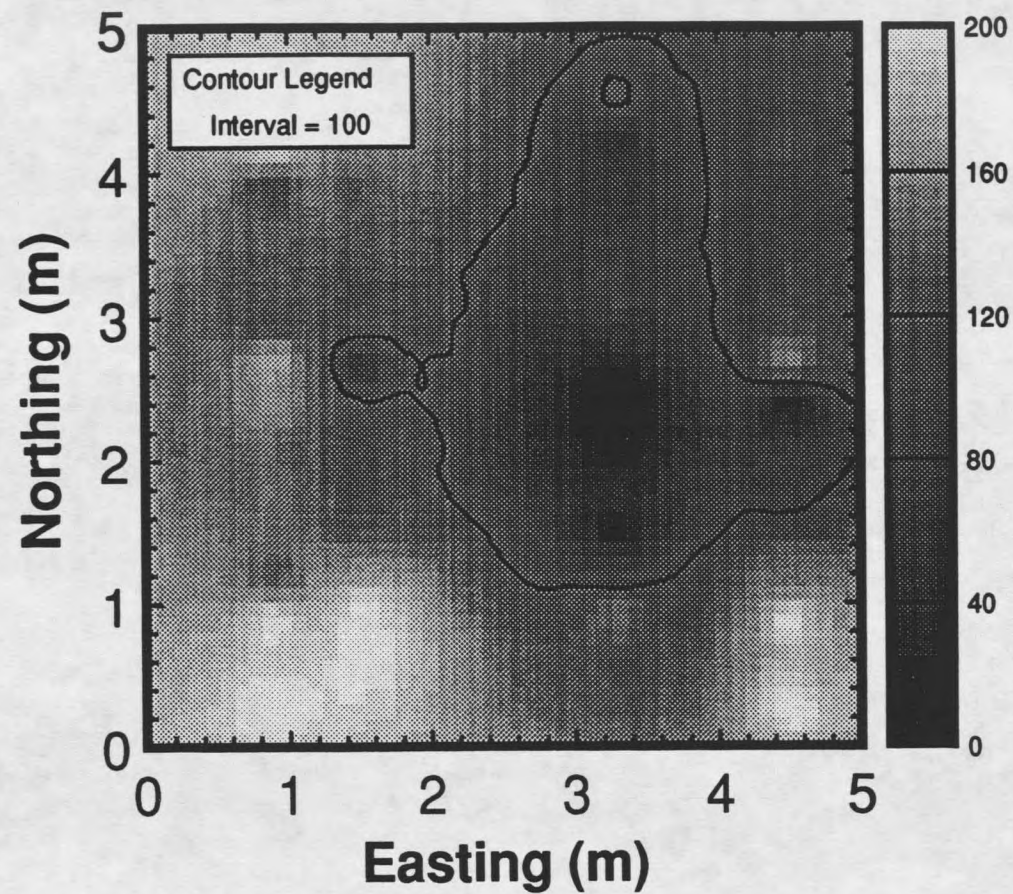


Figure 4.6. Map of kriged estimates of wild oat density at Site 4 in 1995. Grey-scale represents wild oat density (panicle m^{-2}).

Anisotropic analysis of patches indicated a high degree of directional autocorrelation. The range, as indicated by the semivariogram (spherical model), was consistently greater in the direction of harvester movement (Table 4.3). On average the range was 1 m greater in the direction of machinery movement. Dispersal direction is evident in the kriged maps of density at all sites and is seen as an oblong patch shape (Figures 4.2, 4.3, 4.4, 4.5, and 4.6). This supports the hypothesis that harvesting practice is a significant factor in the spread of wild oat within fields and contributes to the pattern observed. Cultivation has also been identified as a source of seed movement although at a smaller scale than harvest machinery (Howard et al. 1991). Estimates for the dispersal ability of different agricultural equipment indicate that the harvesting process has the greatest potential to disperse seed. Up to 25% of all wild oat seed produced in a year can be relocated in this process (Thill and Mallory-Smith 1997).

Genetic Structure of Patches

All patches sampled were composed of a mix of triallate susceptible and resistant wild oat even though the fields had been treated with triallate for over 10 years, with resistance detected at a high level two years before sampling (Table 4.4). The percentage of quadrats sampled that contained the resistant biotype, ranged from 4% to almost 100% with an average number of 35%. This is contrary to the hypothesis that the patches would be composed of either all susceptible or all resistant individuals depending upon the previous herbicide use and the origin of resistance within the field. It was hypothesized that resistance would expand from a central point (mother plant), and with continued selection, expand throughout the patch. Semivariogram analysis of the distribution of

Table 4.3. Semivariogram range of the spherical model fit to density values in one of two directions, 0 and 90°, where 90° corresponds to the direction of machinery movement. The angle of tolerance was set to $\pm 45^\circ$.

Site name	Year	Range	
		m	
		0°	90°
Site 1	1994	2.5	3.2
Site 2	1994	^a >4	>4
	1995	no difference	
	1996	1.93	3.24
Site 3	1995	3.6	6.98
	1996	no difference	
Site 4	1995	0.12	1.04

^a The range estimated was greater than the area of the patch measured.

Table 4.4. Mean and standard deviation of percent resistance of wild oat within patches, at four locations, from 1994 to 1996.

Site name	Year	Resistance	
		Percent	
		Mean	SD
Site 1	1994	79	27
Site 2	1994	55	23
	1995	82	19
	1996	1.4	0.5
Site 3	1995	1.7	0.3
	1996	3.9	1.2
Site 4	1995	36	5.4

^a Standard deviation.

resistance indicated that where autocorrelation was present it was rarely further than 1 m (Figure 4.7). Only at Site 2, in 1994, was the r^2 value for the semivariogram fitted model greater than 0.2. The lack of autocorrelation greater than 1 m may be the result of a high level of dispersal by previous harvest and tillage operations which resulted in mixing of susceptible and resistant seed (Figures 4.8 and 4.9). An alternative explanation of the patchy pattern of resistance within wild oat patches is that triallate resistant seed was planted with the crop. This alternative was not studied.

Epperson (1990) found that both selection of genotype and dispersal of seed contribute to genetic structure. The level of gene flow necessary to override the underlying genetic structure is difficult to predict. If gene flow was limited one would expect a prominent genetic pattern (Barbujani 1987). In outcrossing species homozygous genotypes would become concentrated within the patches and would be surrounded by heterozygotes (Sokal et al. 1989). In predominantly self-pollinating species, such as wild oat, the level of heterozygotes within the patch would remain at a high level. There have been few studies of the impact of different types of selection on genetic pattern. Epperson (1990) simulated populations with limited dispersal and direction selection and found that these factors resulted in a decline in patch size and spatial and temporal correlation of genetic variation. The wild oat patches sampled at Fairfield have undergone a high intensity of selection with a high level of dispersal due, primarily, to harvesting equipment. This has resulted in large patch sizes but little autocorrelation of resistance within patches.

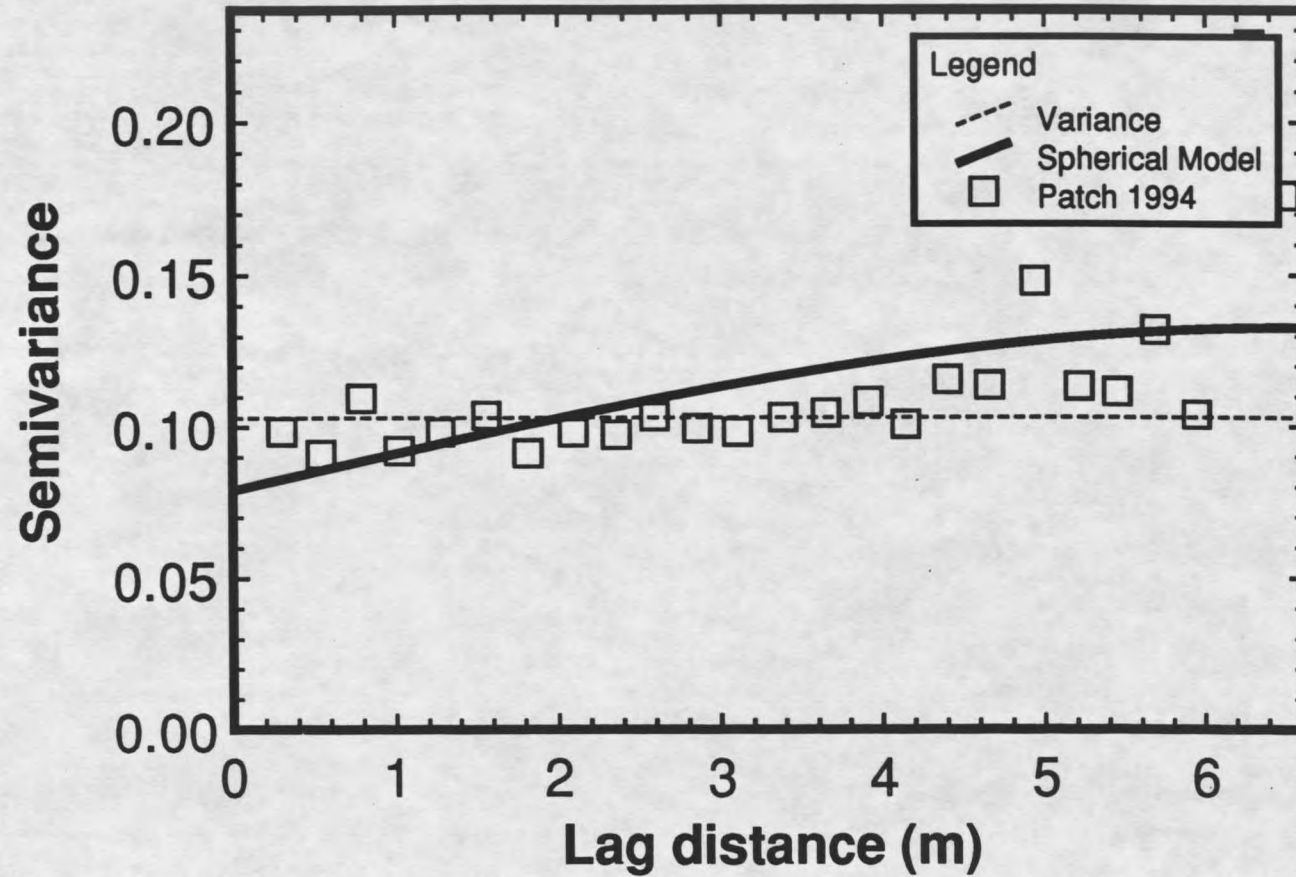


Figure 4.7. Isotrophic semivariogram of resistance at Site 2 for 1994. Arcsine square root transformed resistance values.

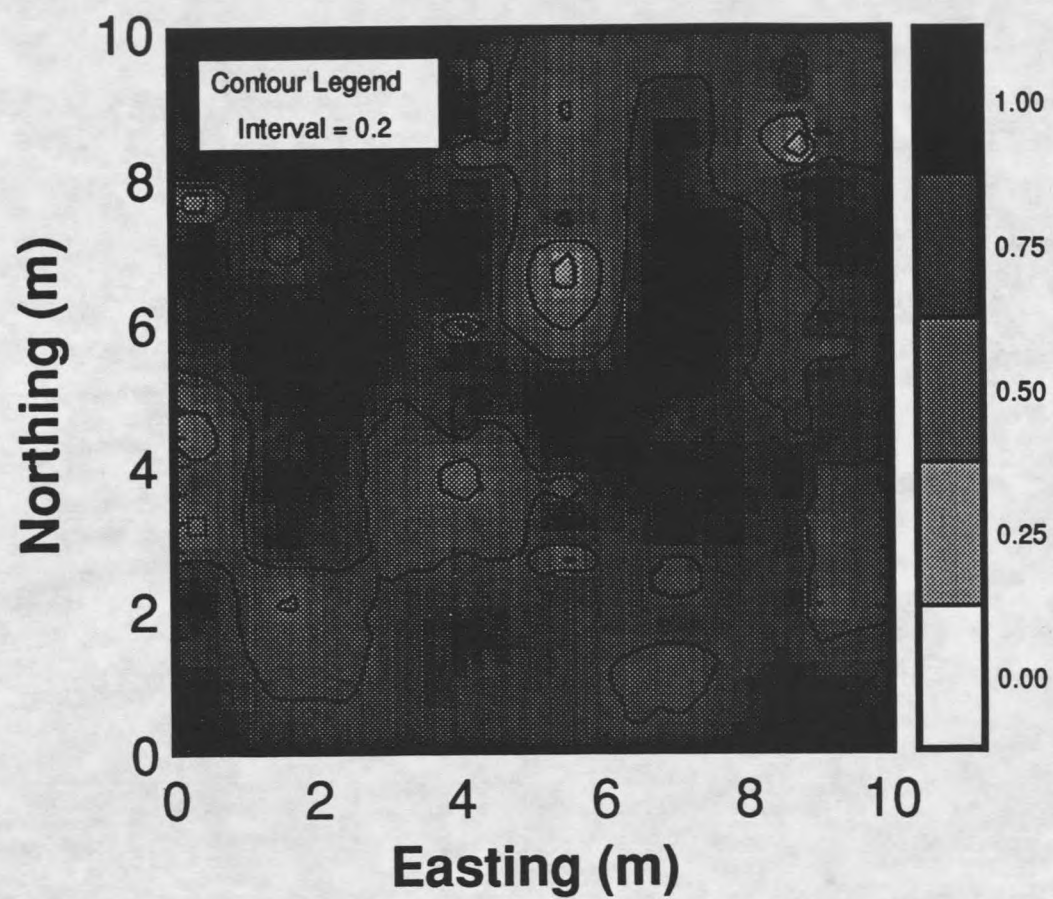


Figure 4.8. Interpolated map of triallate resistance at Site 2 1995. Grey-scale represents proportion of resistant wild oat.

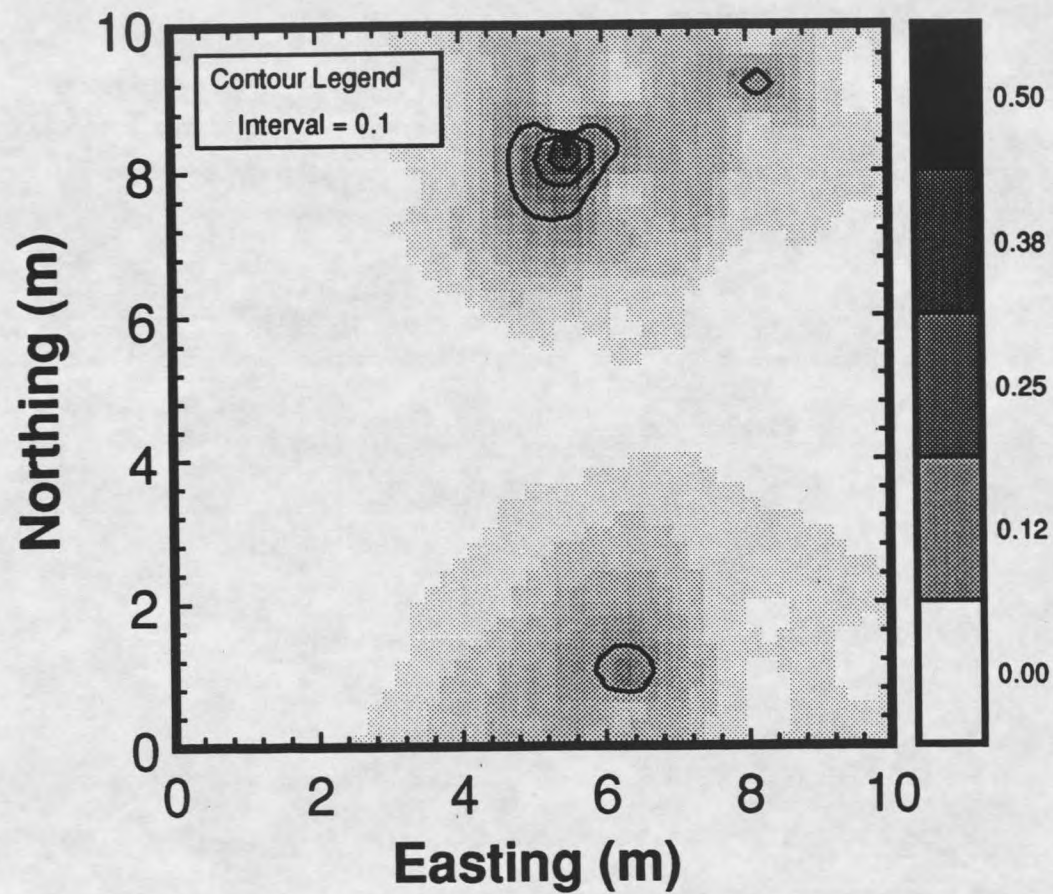


Figure 4.9. Interpolated map of triallate resistance at Site 2 in 1996. Grey-scale represents proportion of resistant wild oat.

Correlation of Patches Between Years

The density of a patch was more stable than its resistance status. Areas of high density, in 1995 at Site 2 and Site 3, correlated with areas of high density in 1996. However, the magnitude of density varied between years. The correlation coefficient between standardized densities at Site 2, 1995 and 1996, was 0.67 and at Site 3, 1995 and 1996, was 0.54. A high correlation coefficient indicates stability in the structure of the patch. The stability is a function of the capacity of the seed bank to buffer changes due to environmental fluctuations between years, the dispersal that is occurring within and outside the patch boundaries, crop and weed management practices, and environmental spatial heterogeneity. If initial location of the patch is strongly influenced by small scale variation in habitat (safe sites for wild oat), it may act as a source of seed to the surrounding areas. If the habitat surrounding the patch is not as suited to colonization by wild oat, then seed dispersed outside the patch will be less likely to increase patch size. Patch stability will, therefore, be increased.

The resistance level was low in 1995 at Site 3 and remained so in 1996. The level of resistance changed dramatically between years at the Site 2 location between 1995 and 1996 (Table 4.4). It is not evident how such a large change in resistance can occur (82% to 1.4%) in one year. A possible explanation is that the delay in sowing in 1996, due to wet weather, enabled the control of many wild oat prior to sowing. If germination times differ between the susceptible and the resistant biotypes, in this case favoring the early germination of the resistant biotype, as observed in other cases of herbicide resistance (Dyer et al. 1993), then the frequency of resistance could decrease rapidly. Another

explanation, again linked to the delay in sowing, is that fitness differences between the biotypes were enhanced as a result of increased intraspecific and interspecific competition, often observed within late sown crops resulting in a shift in frequency towards the susceptible biotype.

Correlation of Resistance and Density

Triallate-resistant plants were often at higher frequencies at patch boundaries and areas of low density at Site 1. This relationship was not as clear at other locations or years. However, at Site 3 1995, all of the 16 cells containing triallate-resistant plants were at densities lower than 300 panicles m^{-2} . In areas where few susceptible plants were present resistant plants were able to obtain a high density. At Site 2 1995, the median level of resistance for cells above 300 panicles m^{-2} was 90% compared to 85% below this density. One explanation for this pattern of resistance is that resistant biotypes are less fit and are unable to compete with the susceptible biotypes at high densities. The role of dispersal in shaping a patch becomes even more important when fitness differences exist, as dispersal of a resistant biotype with reduced fitness into an area of low density increases the rate at which resistance will develop.

This study identified the density and genetic structure that exists within patches. Aggregation of wild oat could influence the rate of change of resistance in a patch. If resistant wild oat are located at patch boundaries where densities are relatively low, as observed in this study, they will contribute a disproportionate amount of seed to subsequent generations. Management of resistant wild oat should aim to enhance fitness differences present between susceptible and resistant biotypes and limit gene flow within

and between patches. This can be achieved by reduced tillage which will reduce the dispersal of seed. Reduced seed dispersal decreases the probability that it will find an unoccupied site, therefore, decreasing the likelihood of survival. Increasing sowing density of the crop on and around wild oat patches will also limit gene flow and enhance fitness differences by acting as a barrier to seed and pollen flow and increasing the level of interspecific competition. If increasing the crop seeding density rate increases lodging or has other negative impacts on yield, seeding rate could be limited to the wild oat patch edges where resistance is most likely to increase in frequency and become a source of spread. This study illustrates the importance of examining weed populations in a spatially explicit manner to identify the appropriate scale of processes affecting the dynamics of the population and determine the impact that management decisions will have at a field scale.

CHAPTER 5

FITNESS OF TRIALLATE-RESISTANT AND SUSCEPTIBLE BIOTYPES OF WILD OAT (*Avena fatua* L.)

Introduction

Weed populations are dynamic. Changes in genetic structure can occur in just a few generations as illustrated by the rapid evolution of herbicide resistance in weeds. Rates of increase of resistance have been studied by observing the growth and interactions between herbicide-resistant and herbicide-susceptible biotypes and the use of theoretical models (Gressel and Segel 1978; Gressel and Segel 1982; Maxwell et al. 1990; Thompson et al. 1994, Jasieniuk et al. 1996). Models have indicated that the change in the population is dependent upon several factors including the initial frequency of the resistant biotype, nature of inheritance of resistance, efficacy of the herbicide, application frequency of the herbicide, the relative fitness of resistant and susceptible genotypes, and the environment (Maxwell et al. 1990; Holt and Thill 1996). A major objective of many studies of competition, between susceptible and resistant biotypes has been to improve our understanding of the rate of evolution of herbicide resistance occurring in weed populations in the field (Thompson et al. 1994; Gill et al. 1996). Theoretical models have indicated that differences in fitness between susceptible and resistant biotypes in the absence of herbicide are a major factor determining the rate of resistance evolution (Gressel and Segal 1978; Maxwell et al 1990; Jasieniuk et al. 1996). If resistant biotypes

are significantly less fit than susceptible biotypes, it is theoretically possible to reduce the rate of evolution of resistance and manage existing resistant populations simply by reducing herbicide use.

The fitness of an individual is a measure of the relative reproductive success of one genotype over another within a specific environment (Silverton and Lovett-Doust 1993). To determine fitness differences between susceptible and resistant biotypes, isogenic lines (plants that differ only with respect to the resistance gene) should be compared under the same environmental conditions that resistance developed (Gill et al. 1996; Jasieniuk et al. 1996). Environmental conditions include crop competition. In practice this is difficult to achieve as the creation of near isogenic lines requires a number of years of breeding (Gressel and Ben-Sinai 1985) which delays any evaluation of fitness differences. A second complicating factor relates to environment-by-genotype interactions. The response of many plants is dependent upon the environment under which they are grown (Zuberi and Gale 1976; Somody et al. 1984; Maddox and Cappuccino 1986). An example of genotype-by environment interactions was published by Zuberi and Gale (1976). They examined 20 inbred lines of poppy (*Papaver dubium*) in different soil types that had different nutrient status and found that the biotype that produced the greatest leaf number at 10 weeks, in a high nutrient environment, was the same biotype that produced the smallest plant under a poor nutrient environment. Even with this knowledge of environment interactions, fitness differences are not normally determined under the same field conditions that the resistant biotype evolved. This is primarily because methods used for competition and fitness evaluations require intensive sampling

and are more amenable to greenhouse studies or locations on nearby farms. Despite these difficulties, many resistant biotypes have been identified that are less fit than susceptible biotypes in the absence of the herbicide (LeBaron and Gressel 1982; Jasieniuk et al. 1996).

Fitness differences have mostly been detected in weeds that have evolved resistance to triazine herbicides (Holt 1996). The reduced growth and competitive ability of triazine resistant biotypes is due to a mutation that decreases the rate of electron transfer to QB in photosystem II (Bowes et al. 1980). Decreased electron transfer resulted in decreased photosynthetic efficiency. Not all triazine resistant biotypes are less fit (Warwick and Black 1981; Jansen et al. 1986). Jansen et al. (1986) found that the triazine resistant lambsquarters (*Chenopodium album*) biotype was more vigorous in both non-competitive and competitive environments. They attributed the increased fitness to normal genetic variation observed within many weed species from different geographic regions.

Fitness differences have been more difficult to detect in weeds that have evolved resistance to herbicides other than the triazines. Weeds resistant to acetolactate synthase and acetyl coenzyme-A carboxylase inhibiting herbicides have shown equal competitive abilities of resistant and susceptible biotypes (Alcocer-Ruthling et al. 1992; Thompson et al. 1994). However, the evaluations of kochia (*Kochia scoparia*) biotypes by Thompson et al. (1994), and prickly lettuce (*Lactuca serriola*) by Alcocer-Ruthling et al. (1992) were not conducted in competition with a crop. In weeds resistant to dinitroaniline herbicides, fitness differences were difficult to detect. The fitness of dinitroaniline resistant goosegrass (*Eleusine indica*) biotypes have been evaluated under non competitive

and competitive conditions in the field with no significant differences (Murphy et al. 1986). However, this is not in agreement with work conducted by Valverde et al. (1988) who found that the resistant biotype was less competitive and had a reduced reproductive weight. The difference between study results could be attributed to genotype-by-environment interactions as all studies were not conducted under the same environmental conditions.

Resistance of wild oat to triallate has become widespread in a malt barley production region of northern Montana, USA (Malchow 1995). Management strategies for triallate-resistant wild oat need to be developed to minimize the impact of resistance. Fitness differences will affect the choice of management strategies following the widespread occurrence of resistance in the region. The objectives of this research were three fold. The first objective was to evaluate the fitness of triallate-resistant and susceptible biotypes of wild oat using traditional greenhouse and field experiments. The second objective was to compare these results with changes in triallate resistance frequency that occurred at a regional level within farmers fields. The change in frequency of resistance was then evaluated using the resistance simulation model (RSIM), developed by Maxwell et al. (1990), to determine the likelihood that changes were due to fitness differences. The third objective was to evaluate fitness using an on farm evaluation of field populations of triallate resistant and susceptible wild oat contained within patches. A comparison of the findings utilizing the different methodologies will provide valuable information to assist in the future planning of research studies with the objective of

identifying fitness differences between herbicide-resistant and susceptible weed populations.

Materials and Methods

Plant Material

Seed of the resistant biotype (F284-R) used in growth analysis and replacement series experiments was obtained from plants located in a triallate-resistant population in a field on the Fairfield Bench of northern Montana, USA. The seed was germinated and grown under greenhouse conditions and tested for resistance to triallate using methods similar to those described by Davidson et al. (1996). The susceptible biotypes used in these experiments originated from two sources. PF93-S was a susceptible biotype from the Fairfield region in northern MT and SH430-S was a susceptible non dormant inbred biotype (Naylor and Jana 1976) grown at the Arthur Post farm in Bozeman MT, USA in 1993. An additional 250 biotypes of wild oat were collected during surveys of the Fairfield Bench, from 1991 to 1996, and were tested for resistance to triallate.

Non-competitive Environment

Growth analysis studies were conducted in both greenhouse and field environments in 1993 and 1994. In the greenhouse, plants were grown in pots 42 cm in diameter by 46 cm in depth, with a 12 hr day length. Pots were watered as needed to maintain field capacity and fertilized weekly using Petersons solution (20:10:20, N P and K by weight) at a rate of 75 ppm per liter. Five plants from each biotype were selected at measurement time coinciding with different plant development stages; first leaf, second leaf, third leaf,

tillering, and seed head emergence. Measurements including leaf area and dry shoot biomass were carried out at each harvest. The experiment was a completely randomized design with five replicates. Transformation of the leaf area measurements, using the natural logarithm ($\log_e$), was required. Analyses of the relative growth of resistant and susceptible biotypes was estimated using the Richards function (Venus and Causton 1979). The Richards function is a generalization of the logistic function and is given by the equation:

$$\ln Y = \ln A - \ln(1 + \exp(-K(X - X_0))) / N$$

where Y is the predicted growth measure, A is the asymptotic maximum growth measure, N controls the point of inflection, X and X_0 represent time (days after planting) and K is a parameter without biological significance.

In field experiments, located at the Arthur Post farm near Bozeman MT, plants were grown individually in 1 m² plots. The experiment was laid out as a randomized complete block design with four replicates. Nitrogen fertilizer, in the form of urea (34 kg ha⁻¹), was applied to the experimental area just prior to seeding. Plots were irrigated at three separate times during the growing season to promote germination and development. Plots were hand weeded at regular intervals to ensure a competition free environment. Four plants of each biotype were measured at each harvest time. Plants were cut at the soil surface and measurements of growth, including leaf area and dry shoot biomass, were carried out. Leaf area was calculated using a leaf area meter. Dry shoot biomass was measured following drying at 80°C for a minimum of 24 hrs. Five harvests were conducted over the growing season at 27, 38, 45, 55 and 76 days after planting. An

additional harvest at maturity was used to measure seed production. This involved daily collection of seed over a two week period. Seed number was determined by counting each sample by hand. Data were $\log_e$ transformed prior to fitting the Richards function with non-linear regression (Proc NLIN, SAS 1992).

Competitive Environment

Responses of triallate susceptible and resistant biotypes were examined in a competitive environment with malting barley using three methods: a replacement series design, a survey method combined with model simulation, and within-field examination of seed production by the resistant and susceptible biotypes under different environmental conditions. All experiments examining the fitness of resistant and susceptible biotypes in competition were located on the Fairfield Bench in northern MT.

Replacement Series A replacement series design (deWit 1960; cited by Silvertown and Lovett-Doust) with single planting densities was used to evaluate fitness differences in a triallate susceptible and resistant biotype. Wild oat seed was sown at a density of 121 seeds m^2 in monocultures of susceptible and resistant biotypes, and a single mixture of 50% resistant and 50% susceptible seed. Seed was sown within 1 m^2 plots in a grid layout of equal row and column spacing. There were four replicates of three 1 m^2 plots (resistant monoculture, susceptible monoculture or mixture) in a randomized complete block design. The experiment was repeated each year for three years. The populations were sown in a field of irrigated malt barley. Prior to the harvesting of the malt barley, wild oat plants were removed and tiller number, dry

biomass, seed number and weight were measured. An analysis of variance statistical test was used to determine if significant differences existed between the resistant and susceptible biotypes for all the fitness measurements.

Survey Seeds of wild oat were collected from fields located on the Fairfield bench in northern Montana using the W method (Thomas 1985). Plant numbers sampled varied depending on density but a minimum of 200 plants per field were collected. Fields sampled varied between years with 44 fields sampled in 1991, 236 in 1992, 18 in 1993, 30 in 1995 and 30 in 1996. Samples were tested for triallate resistance using a greenhouse test (Malchow 1995). The percent of wild oat seed resistant to triallate was determined for each sample. Fields sampled with no resistant wild oat seed were classed as susceptible and fields where any resistant seeds were detected were classed as resistant. The percentage of fields containing triallate-resistant wild oat was then calculated.

The RSIM model of Maxwell et al. (1990) was used to examine the rate of change in the frequency of fields containing triallate-resistant wild oat in the Fairfield region. Population parameters required by the model for wild oat were derived from life history measurements in threshold experiments conducted in Montana (Maxwell et al. 1994). The pattern of use of triallate in the Fairfield region was based on survey data (Malchow 1995) and discussions with chemical distributors. Triallate was assumed to be applied from 1978 for a period of 17 years. No triallate was applied in 1994 or in the years following due to widespread triallate resistance (resistance levels of 94% were detected in the 1993 survey). The efficacy of triallate on the susceptible biotype was set at 85%, which was the estimated field performance for this herbicide in this region. Gene flow

from outside populations was set to zero as gene flow would be predominately occurring within the system at this scale. Resistance was initially assumed to be a dominant trait based on prevalence of this mode of inheritance in other resistant weeds (Jasieniuk et al. 1996). However, one triallate-resistant population has been identified where resistance was reported to be controlled by two recessive genes (Kern et al. 1997). It is unknown at this stage if this is representative of the resistant populations on the Fairfield Bench, therefore the assumption of mode of inheritance was not changed. The RSIM model was used to quantify the fitness difference necessary to account for the observed changes in frequency of resistant fields. Two cases were evaluated to determine the role of fitness. Case 1: No difference between the susceptible and resistant biotypes. Case 2: Resistant biotype was assumed to be 50% less fit than the susceptible biotype. The actual value for fitness was based on the seed production estimated from the 1993 replacement series experiments. The maximum seed production of the resistant biotype was set at 1300 seeds m^{-2} representing a 50% reduction compared to the susceptible biotype.

Within Field Seed Production Wild oat seed production was calculated within six patches located in four different fields on the Fairfield Bench. Grids of 5 m by 5 m in 1994 and 10 m by 10 m in 1995 and 1996, were centered over a single wild oat patch within each field. Approximately 140 seed samples, each from an area of 0.0625 m^2 were collected within each grid. Panicle number was counted, as a measure of density for each seed sample that was collected. The samples of wild oat were tested for resistance to triallate using a petri dish test in 1994 (Kern et al. 1996) and a greenhouse pot test in 1995 and 1996 (Chapter 3). Seed from each quadrat was counted and the seed production

per meter square was calculated. A surface was fit, using least squares estimates, to visually examine the relationship between the dependent variable seed production and the independent variables density and the frequency of triallate resistance. Three of the six patches sampled contained insufficient resistant samples (less than 20) to permit surface fitting. Samples from the remaining patches were categorized based on the frequency of triallate-resistant wild oat. Samples collected from quadrats in each patch were grouped into three categories: susceptible, mixed and resistant. Susceptible samples contained less than 25% resistant seed, mixed samples contained between 35% and 65% resistant seed, and resistant samples contained greater than 75% resistant seed. Due to the limited number of samples in each category, data from the three patches were combined. The seed production data were standardized by dividing seed production per quadrat by the maximum seed production for the respective sites. This process, although necessary, prevents further analysis of seasonal variation in biotype response. Regression analysis was used to determine the relationship between density and seed production for the resistant, susceptible and mixed populations. Transformation, using the natural log ($\log_e$), of seed production and density was required to satisfy the assumptions of independent and constant variance and normality of residuals.

Results and Discussion

Non-Competitive Environment

The growth of resistant and susceptible biotypes, as measured by leaf area, was similar in the greenhouse growth analysis experiments. By day 56 in the greenhouse

experiment, leaf area was 400 cm², 540 cm², and 470 cm² for the biotypes F27-R, F37-S and SH430-S, respectively (Figure 5.1). Growth analysis in the field showed a similar trend with the susceptible biotypes growing more than the resistant biotypes. In the field experiments in 1994 leaf area at 55 days after planting was 257 cm², 380 cm² and 890 cm² for the biotypes F284-R, PF93-S and SH430-S respectively (Figure 5.2). The susceptible biotype SH430 at 55 days in the field experiment obtained a significantly larger leaf area compared to the other susceptible biotype PF93-S and the resistant biotype F284-R. At 76 DAP both susceptible biotypes had significantly larger leaf area than the resistant biotype F284-R (Figure 5.2). The results for biomass accumulation also indicated no differences in the greenhouse growth analysis experiment (Figure 5.3). However, as with leaf area, the biomass of the susceptible biotypes in the field experiment were significantly ($P < 0.05$) larger than the biomass of the resistant biotype by day 76 (Figure 5.4). Seed production for the susceptible and resistant biotypes did not show the same pattern as that of biomass accumulation. There were no significant differences ($P > 0.05$) between any of the biotypes despite the large differences between mean values (Figure 5.5).

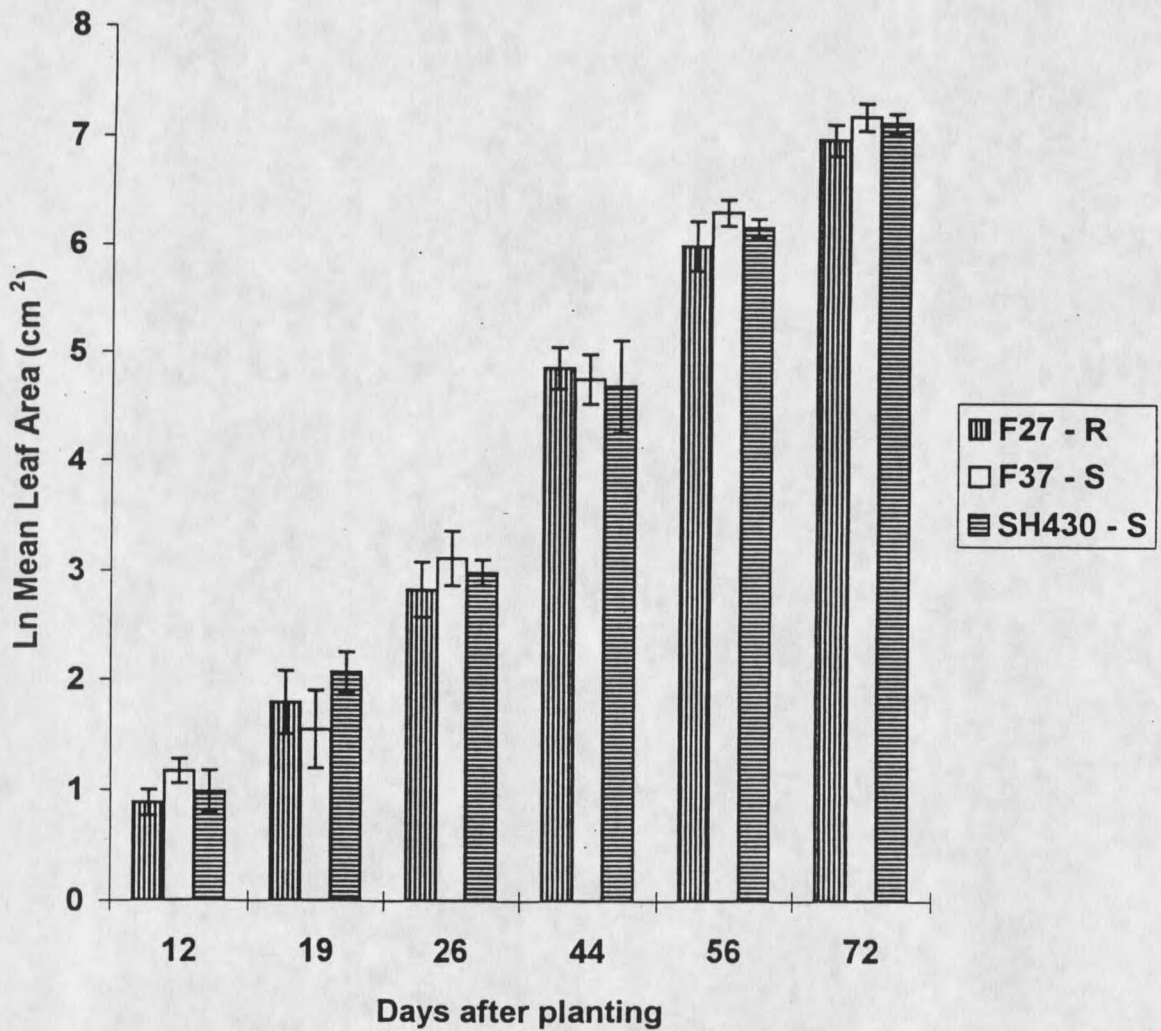


Figure 5.1. Mean leaf area of triallate-resistant and susceptible biotypes of wild oat grown in greenhouse conditions in 1993. Six harvest times ranged from 12 to 72 days after planting. Vertical bars represent standard errors.

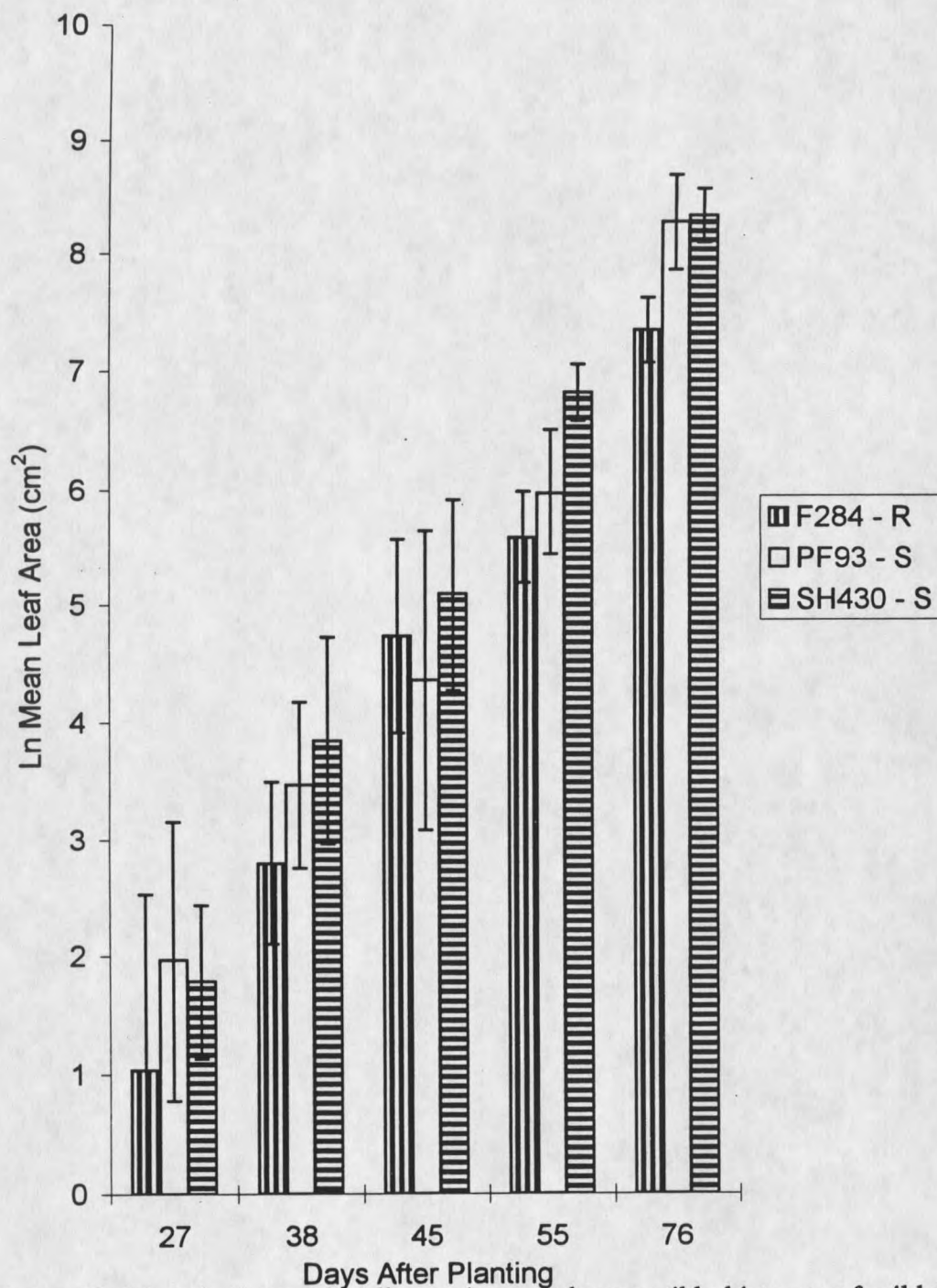


Figure 5.2. Mean leaf area of triallate-resistant and susceptible biotypes of wild oat grown in field conditions in 1994. Five harvest times ranged from 27 to 76 days after planting. Vertical bars represent standard errors.

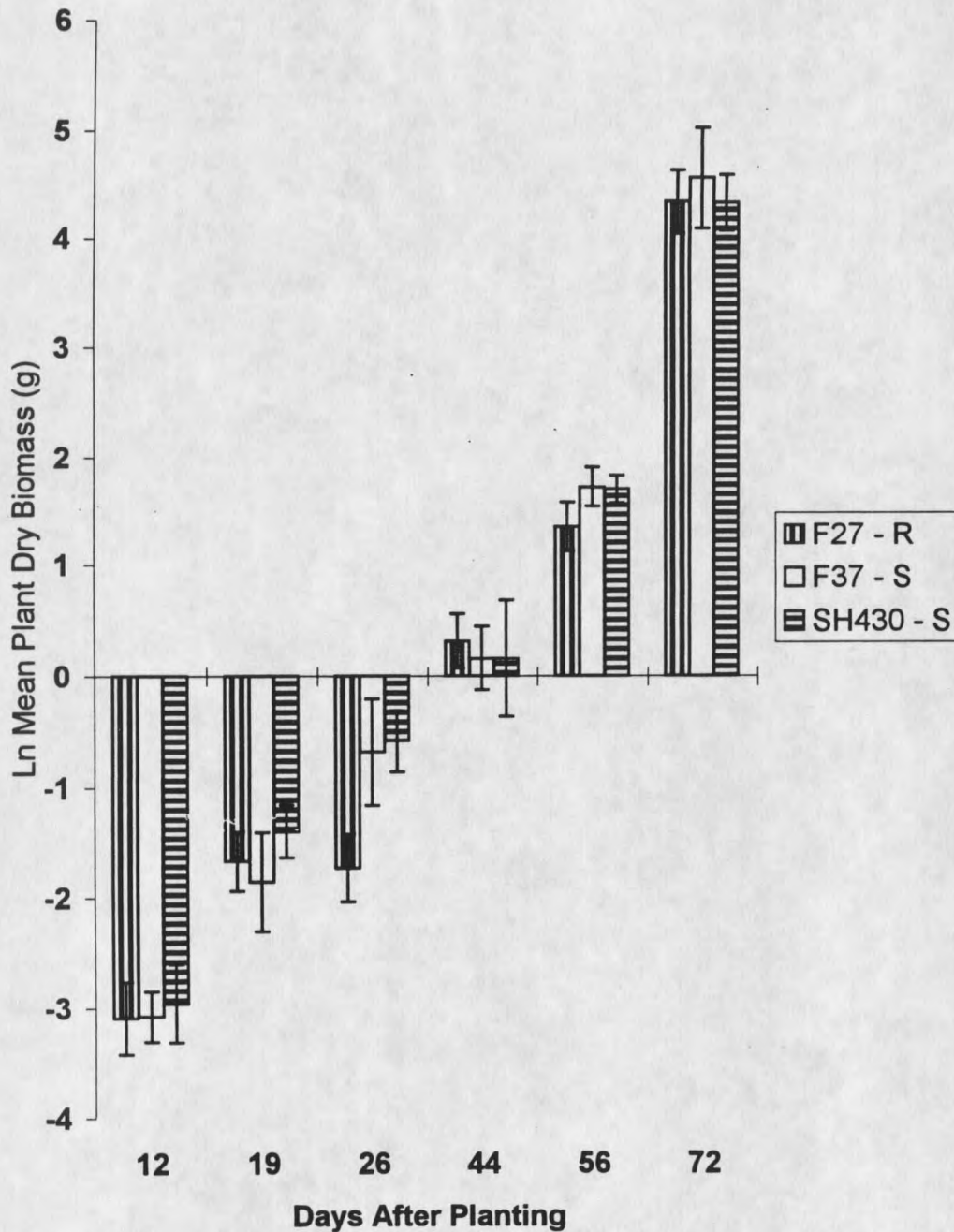


Figure 5.3. Mean plant dry weight of triallate-resistant and susceptible biotypes of wild oat grown in greenhouse conditions in 1993. Six harvest times ranged from 12 to 72 days after planting. Vertical bars represent standard errors.

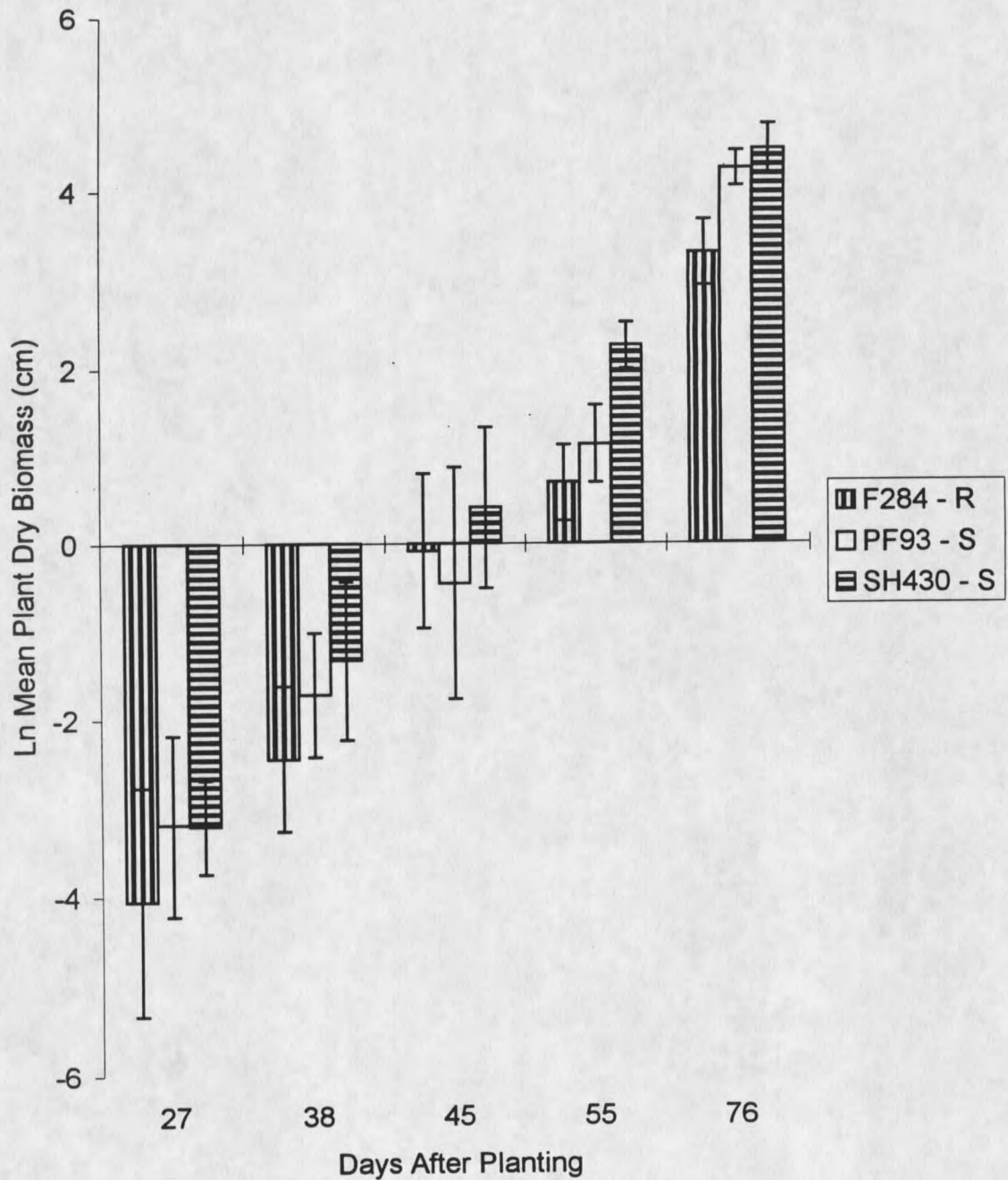


Figure 5.4. Mean plant dry weight of triallate-resistant and susceptible biotypes of wild oat grown in field conditions in 1994. Five harvest times ranged from 27 to 76 days after planting. Vertical bars represent standard errors.

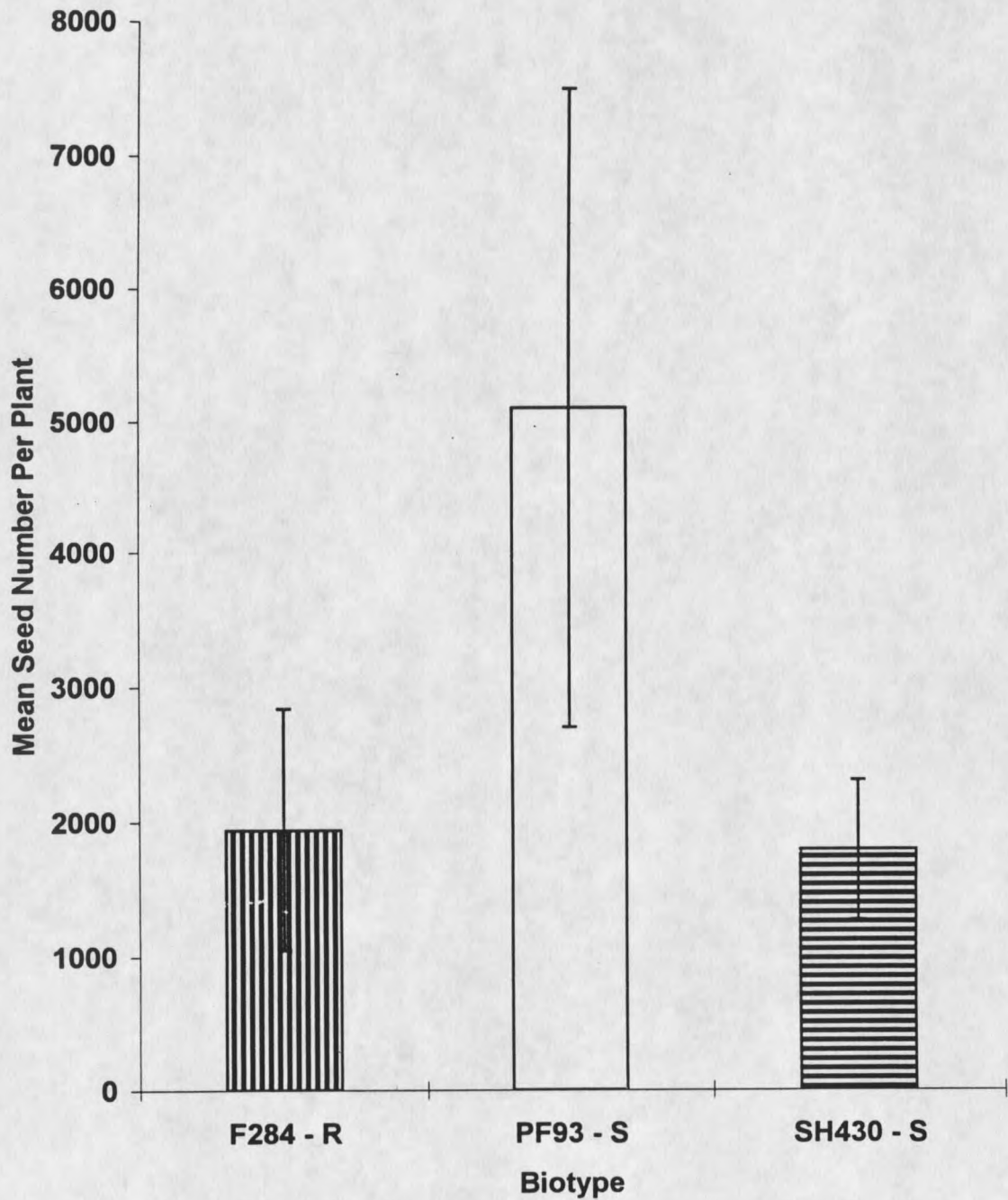


Figure 5.5. Mean seed production per plant for triallate-resistant and susceptible biotypes of wild oat grown in field conditions in 1994. Vertical bars represent standard errors.

Competitive Environment

Replacement Series The number of tillers produced per plant by the resistant biotype ranged from 10.3 tillers per plant in 1993 to 2.4 tillers per plant in 1994 (Figure 5.6). Tiller production per plant of the resistant biotype was not significantly different from tiller production per plant of the susceptible biotype. Biomass per wild oat plant at harvest in monoculture and mixture was also not significantly different ($P>0.05$) (Figure 5.7). Mean biomass production per plant ranged from over 15 g in mixture in 1994 to only 0.3 g per plant in the resistant monoculture in 1993. Seed production also fluctuated widely between years. In 1993, which corresponded to a year with low biomass production, mean seed production for the resistant biotype was less than 20 seeds per plant (Figure 5.8). In 1994, seed production for the resistant biotype was over 225 seeds per plant (Figure 5.9). In 1995, seed production was again low with approximately 70 seeds produced per resistant plant (Figure 5.10). Differences in seed production between resistant and susceptible biotypes also varied in magnitude and direction between years. In 1993, resistant plants produced an average of 20 seeds per plant less than the susceptible plants. In 1994, the trend was reversed with the resistant biotype producing approximately 70 mean seeds per plant more than the susceptible biotype. In 1995, seed production of the two biotypes was almost equal with the resistant biotype producing 70 seeds per plant and the susceptible biotype producing over 80 seeds per plant. However, in no year were the differences in seed production significant. The lack of significant differences between biotypes at this density does not preclude the existence of differences at other densities.

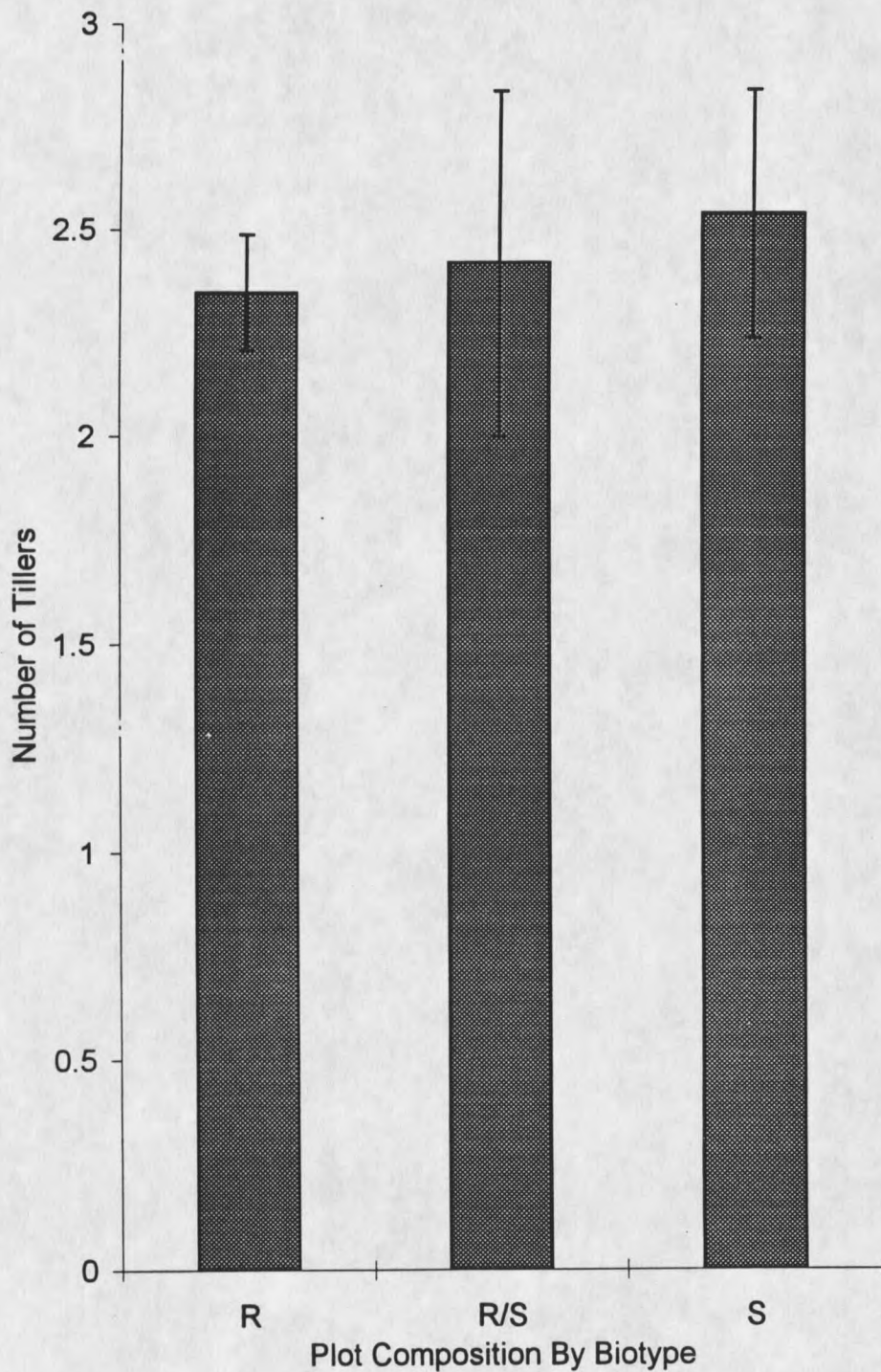


Figure 5.6. Mean number of tillers per plant grown in monocultures and mixtures of triallate-resistant and susceptible wild oat. Plants were grown in field conditions on the Fairfield Bench in 1994. Vertical bars represent standard errors.

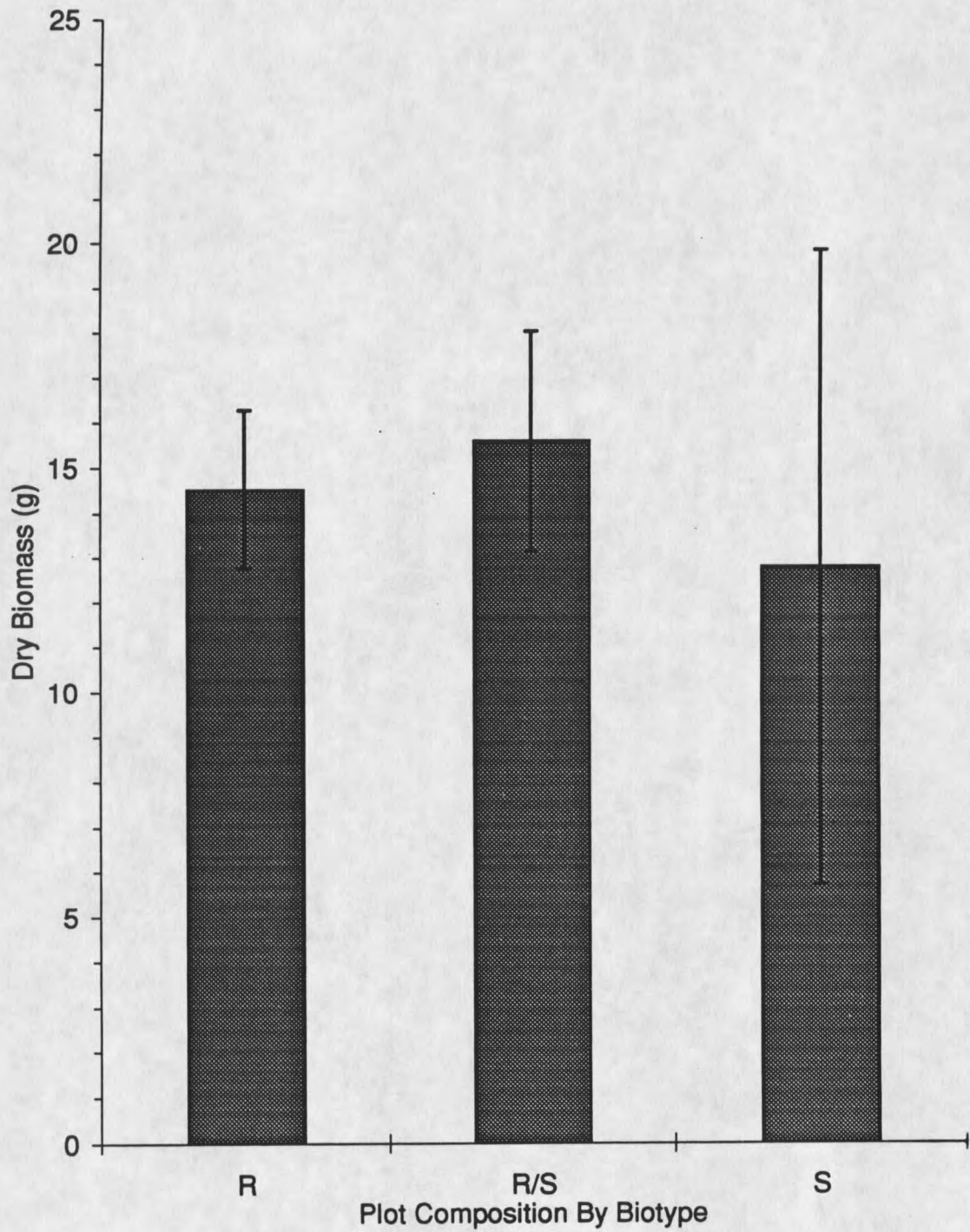


Figure 5.7. Mean plant dry weight per plant grown in monocultures and mixtures of triallate-resistant and susceptible wild oat. Plants were grown in field conditions on the Fairfield Bench in 1994. Vertical bars represent standard errors.

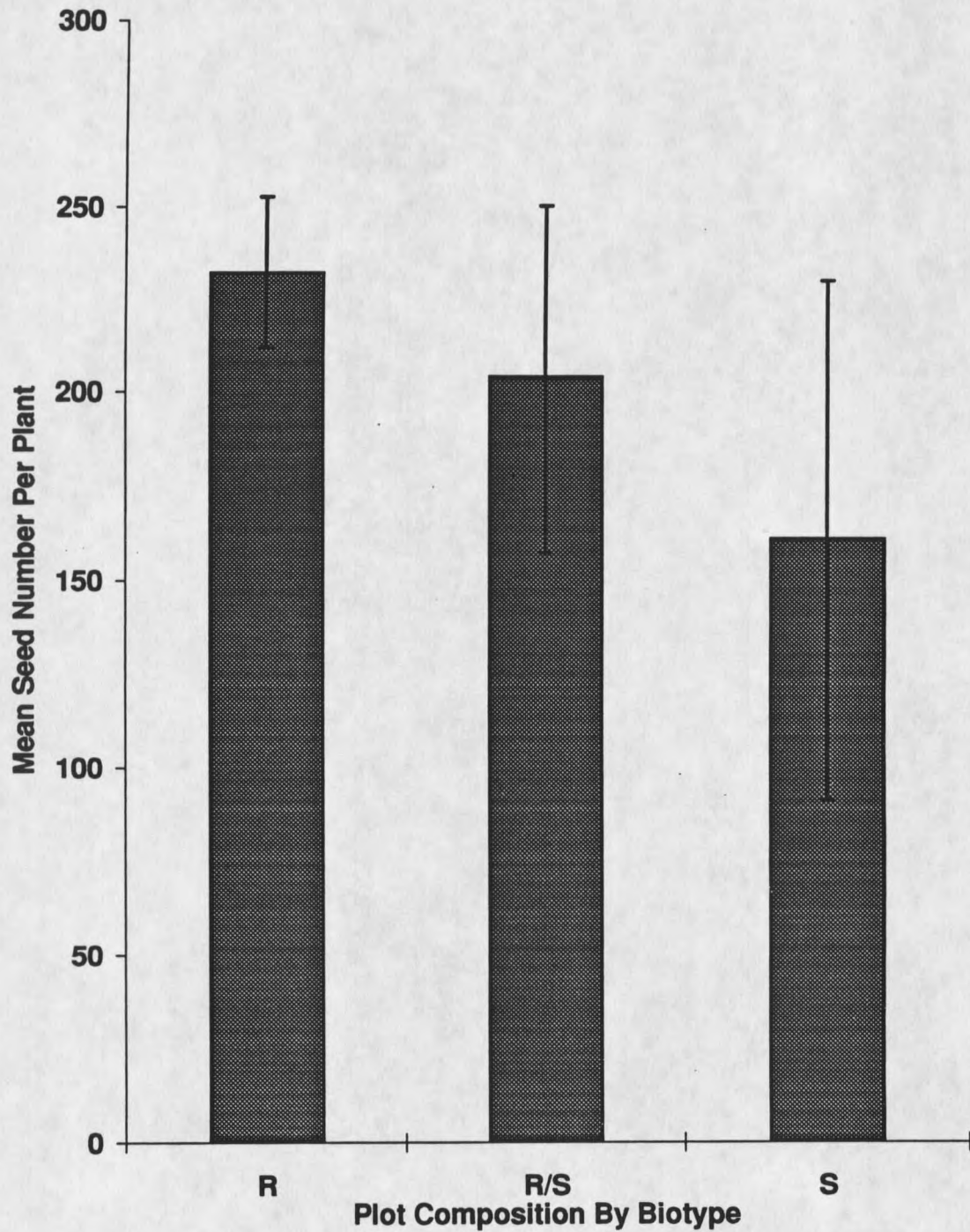
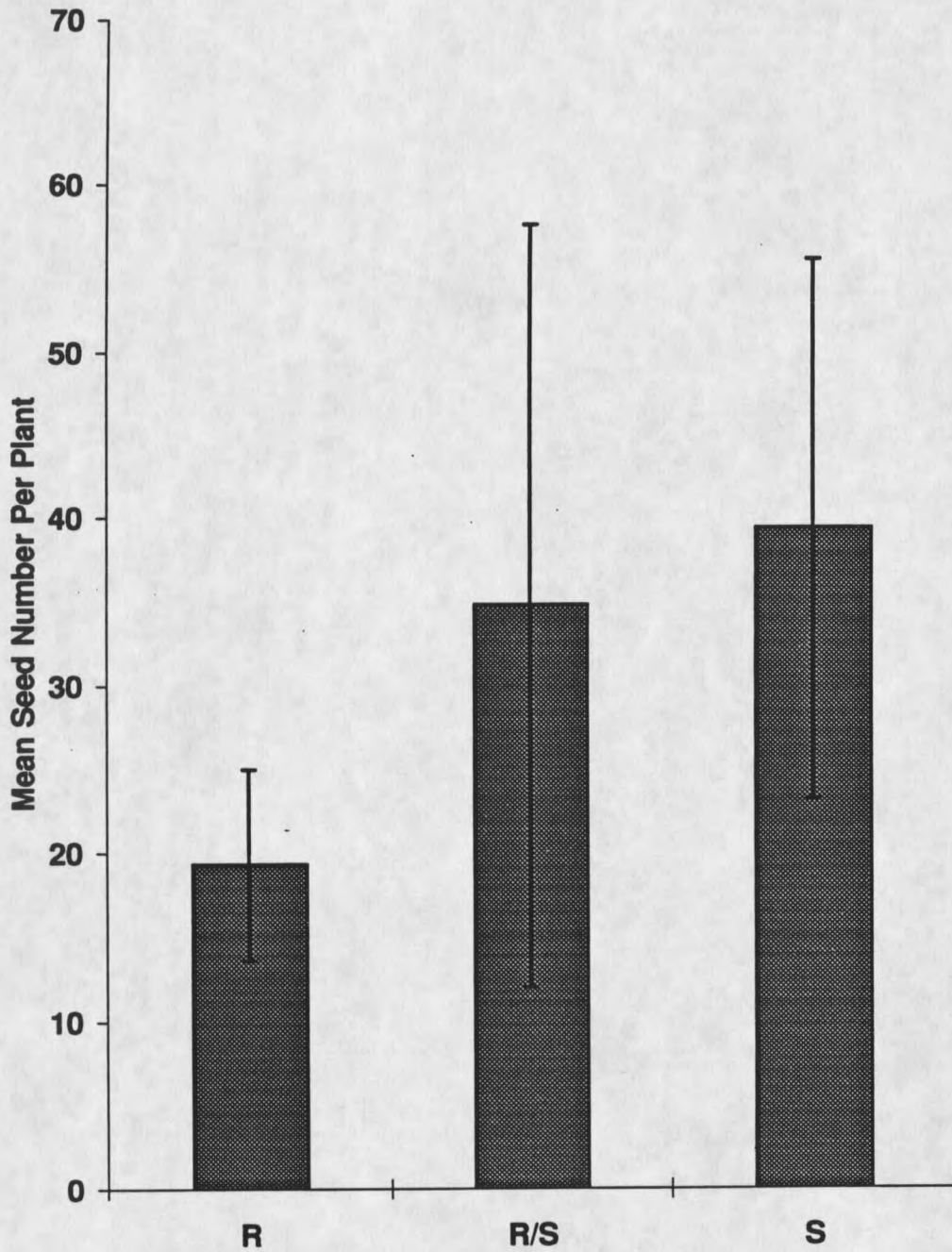


Figure 5.8. Mean seed number per plant grown in monocultures and mixtures of triallate-resistant and susceptible wild oat. Plants were grown in field conditions on the Fairfield Bench in 1993. Vertical bars represent standard errors.



Plot Composition By Biotype

Figure 5.9. Mean seed number per plant grown in monocultures and mixtures of triallate-resistant and susceptible wild oat. The plants were grown in field conditions on the Fairfield Bench in 1994. Vertical bars represent standard errors.

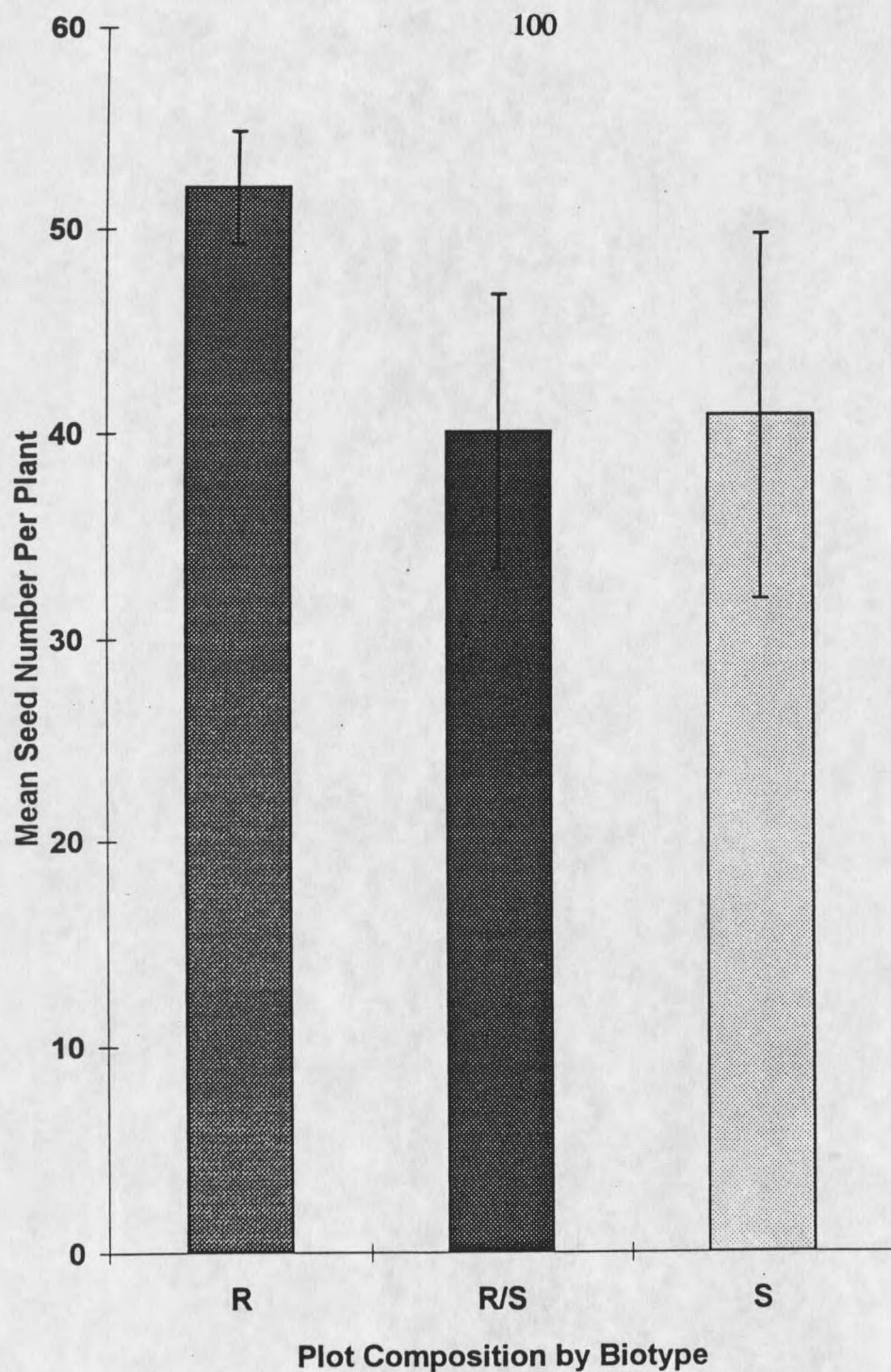


Figure 5.10. Mean seed number per plant grown in monocultures and mixtures of triallate-resistant and susceptible wild oat. The plants were grown in field conditions on the Fairfield Bench in 1995. Vertical bars represent standard errors.

Environmental conditions influence plant response significantly so it is not surprising to see dramatic differences in plant performance between years in field experiments. This information contributes little to our understanding of resistance or management of wild oat as only one resistant and one susceptible biotype from the Fairfield region were examined in either the greenhouse or field experiments. Gill et al. (1996) demonstrated the importance of examining a number of biotypes of both susceptible and resistant plants. They measured a number of components of fitness in 28 biotypes of rigid ryegrass (*Lolium rigidum*) including dormancy, rate of emergence, growth and development, and found that because of the variation in biotypes, unrelated to resistance status, different conclusions could be drawn on the relative fitness of resistant plants. The outcome depended on the combination of resistant and susceptible biotypes chosen for comparison.

Survey The number of fields containing triallate-resistant wild oat on the Fairfield Bench increased with continued use of triallate until 1993 when 94% of fields contained triallate-resistant wild oat (Figure 5.11). The resistance level declined after 1993 to 65% in 1996. Triallate was used on fewer than 5% of fields from 1993 onwards. The increase in the frequency of resistant fields with triallate use followed an exponential curve that is typical of model simulations of rate of resistance evolution (Maxwell et al. 1990). The rapid decline in the resistance frequency from 1993 onwards, although consistent with decreased fitness in the resistant biotype, could be due to negative cross resistance or may be related to the mode of inheritance of resistance. Malchow (1995) examined the effect of imazamethabenz on the change in the frequency of triallate-resistant wild oat following

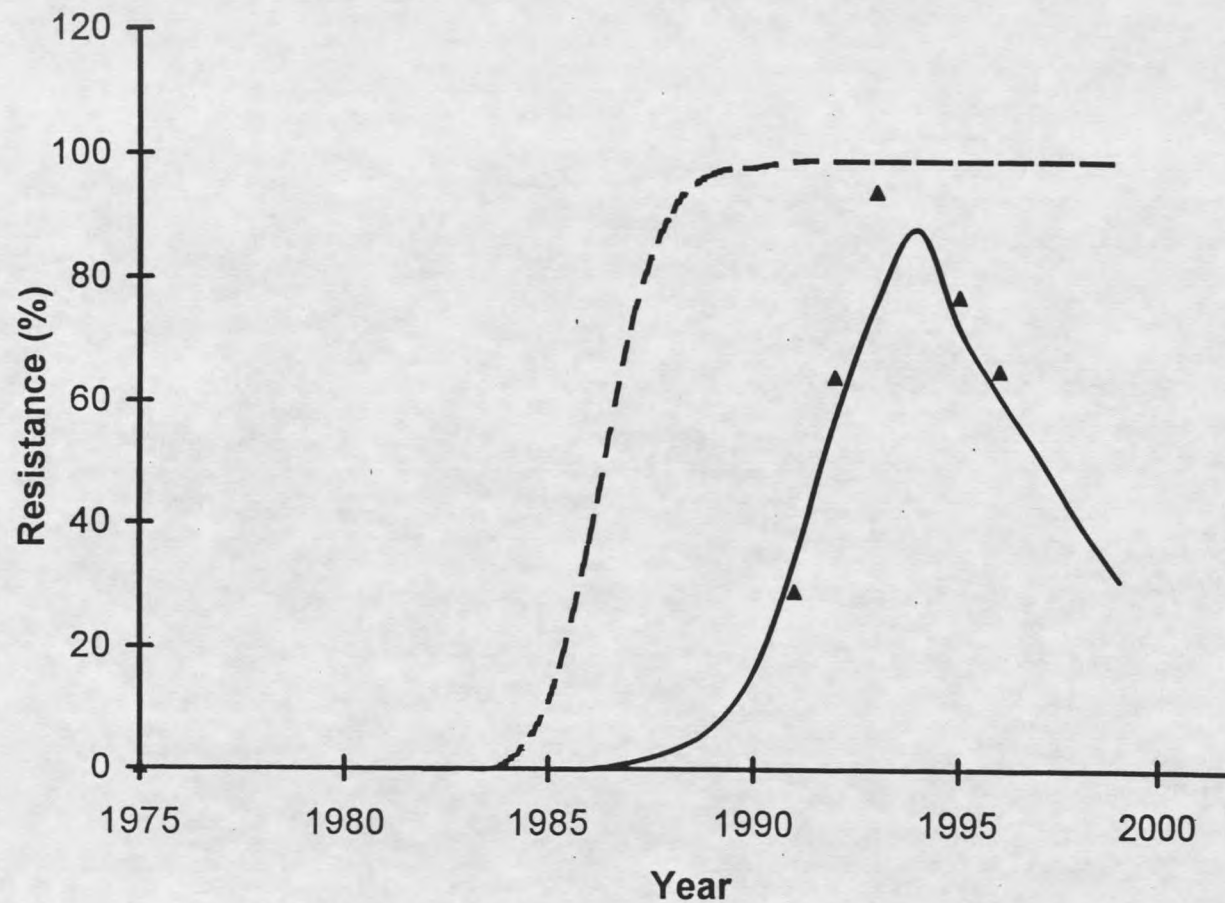


Figure 5.11. Observed (▲) change in frequency of fields containing triallate-resistant wild oat, on the Fairfield Bench. Model predictions for change in the frequency of triallate resistance when no fitness difference was assumed (---) and when a 50% reduction in fitness of the resistant biotype was assumed (—).

one year of use. No significant decline in the frequency of triallate resistance was observed. It is therefore unlikely that negative cross resistance is involved in the decline in frequency of triallate resistance observed in the survey.

The RSIM model was used to evaluate changes in triallate resistance frequency occurring under two different scenarios: (1) no fitness difference between resistant and susceptible biotypes in the absence of triallate, and (2) resistant biotypes are less fit (produce 50% fewer seeds than susceptible biotypes. In the first simulation, the mode of inheritance was assumed to be a single dominant gene with the fitness of the resistant biotypes equal to that of the susceptible biotypes. The simulation predicted that resistance would evolve in 90% of the fields in under 10 years of triallate use (Figure 5.11). Resistance would remain at a high level even when triallate use was discontinued (1994 onwards). The rate of increase in frequency of resistance was faster than that measured on the Fairfield Bench. The level of resistance also remained high even after triallate use was discontinued. This did not correspond to the change in frequency that was observed in the field. In the second simulation, fitness differences between the resistant and susceptible biotypes were included in the model. With a fitness difference of 50% the model predicted a rapid decline in the frequency of triallate-resistant wild oat when triallate was no longer used. The rate of decline was similar to that observed in the field. The slow evolution of resistance (15 years for resistance in 90% of fields) was more closely correlated with observed changes in the field.

The impact of resistance resulting from two recessive genes based on predictions using the RSIM model was not investigated as the current capabilities of this model do

not allow the investigation of two gene systems. However, by examining simulations on the two gene systems by Roughgarden (1996) it appears that two recessive genes would result in a slower rate of resistance evolution compared to a single dominant gene. The slow rate of evolution could only be compensated for by assuming a higher level of efficacy of triallate and reducing the difference in fitness between the two biotypes. The segregation of the two recessive resistant genes, after triallate use discontinued, could lead to a rapid decrease in the level of resistance within the field which would be consistent with observed changes in the field. Further investigation of mode of inheritance is required to determine if a two gene recessive system is typical of triallate-resistant wild oat populations. The likelihood that recessive genes are responsible for resistance is greatly enhanced in a self-pollinating species and cannot be dismissed (Charlesworth 1992).

Within Field Seed Production Many characteristics have been measured to determine fitness differences between resistant and susceptible biotypes. However, fitness differences are ultimately measured as a proportional difference in offspring produced by each biotype. Survey data, combined with simulation modeling, suggested that the triallate-resistant biotype may be less fit than the susceptible biotype. If this is the case, fitness differences should be detectable within fields. Seed production of both resistant and susceptible plants was examined at six sites (patches) at a range of plant densities. Of the sites examined, only three patches contained a satisfactory mix of resistant and susceptible frequencies and tiller densities. The relationship between resistance, tiller density and seed production was investigated (Figures 5.12, 5.13 and 5.14). The graphs

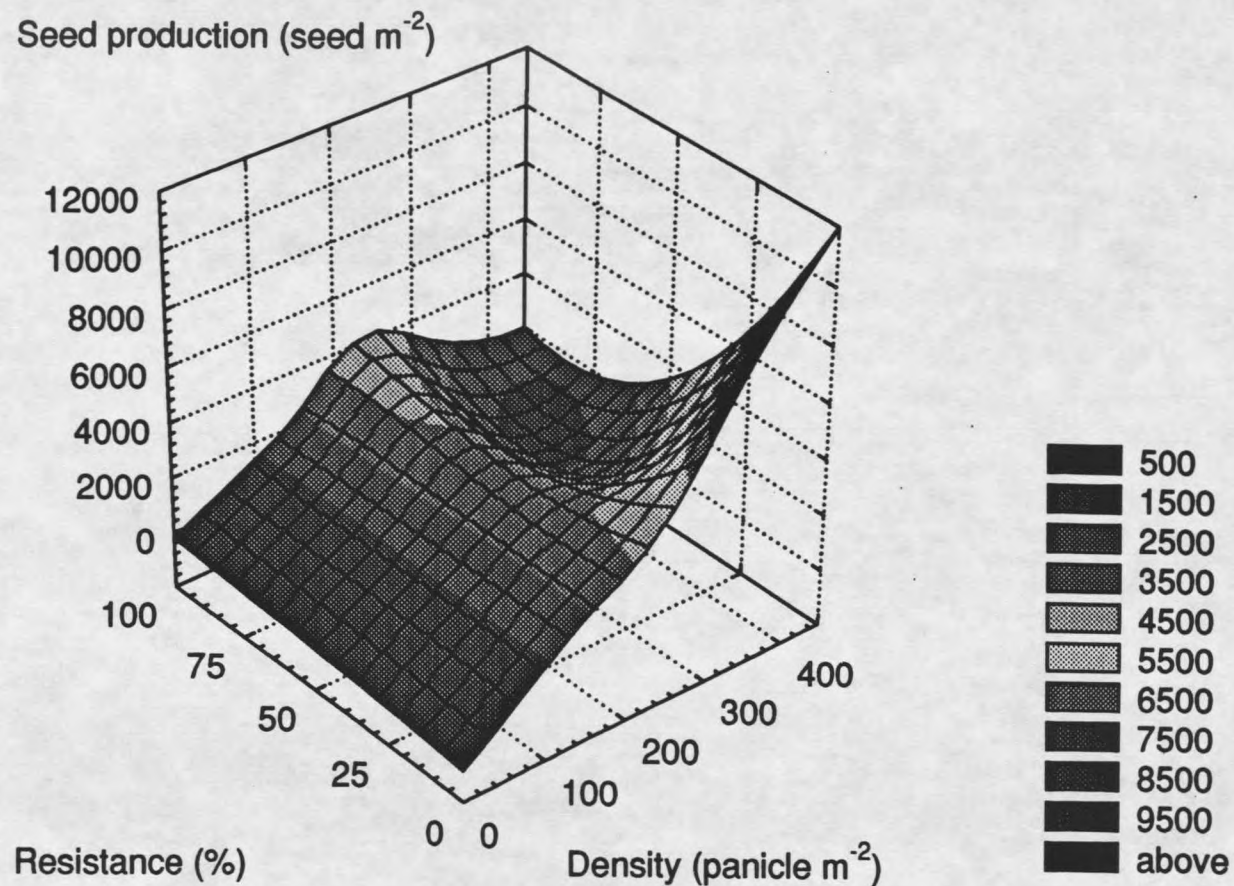


Figure 5.12. Least squares surface plot of the relationship between seed production, density (panicle number) and triallate resistance of wild oat from a patch located on the Fairfield Bench (Site 1. 1994). Grey scale legend represents seed production (seeds per m^2).

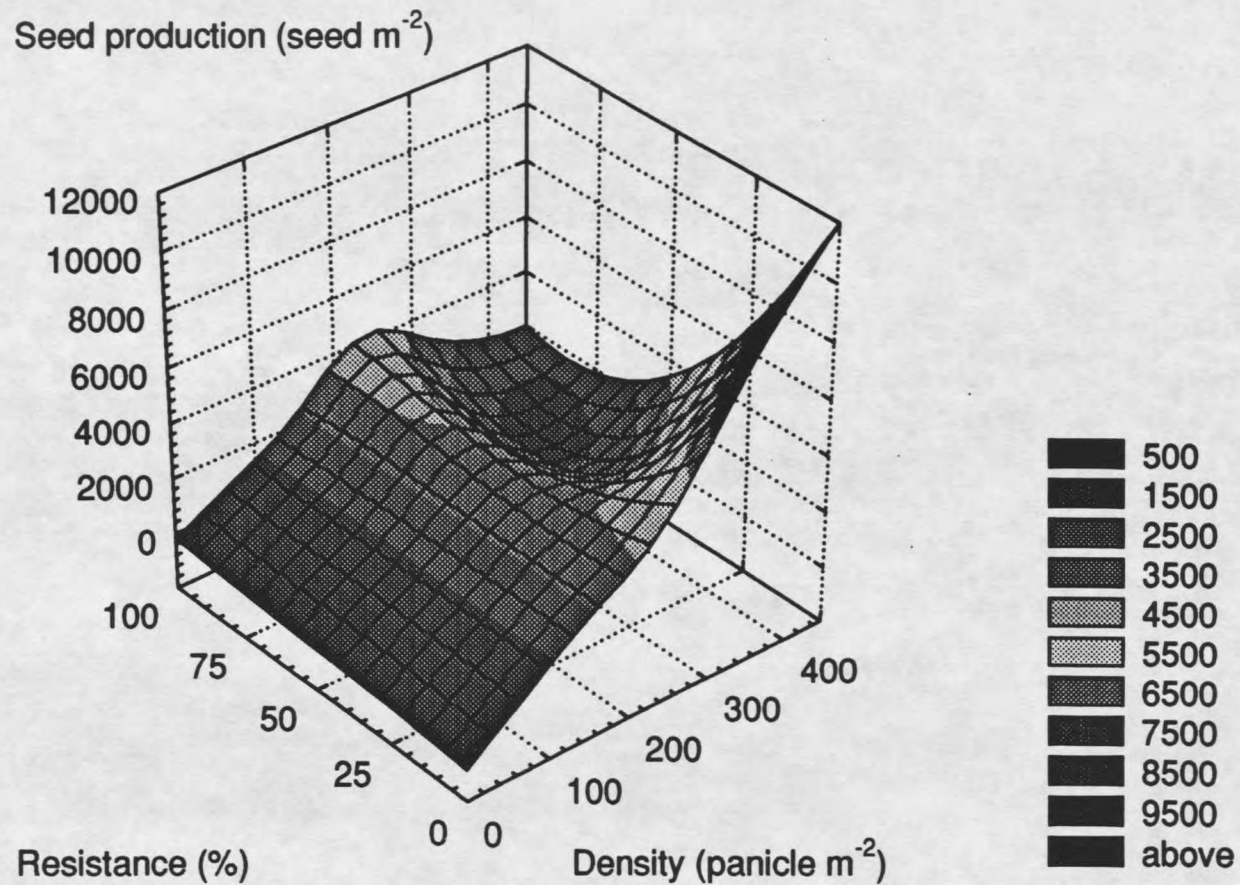


Figure 5.13. Least squares surface plot of the relationship between seed production, density (panicle number) and triallate resistance of wild oat from a patch located on the Fairfield Bench (Site 2. 1994). Grey scale legend represents seed production (seeds per m^2).

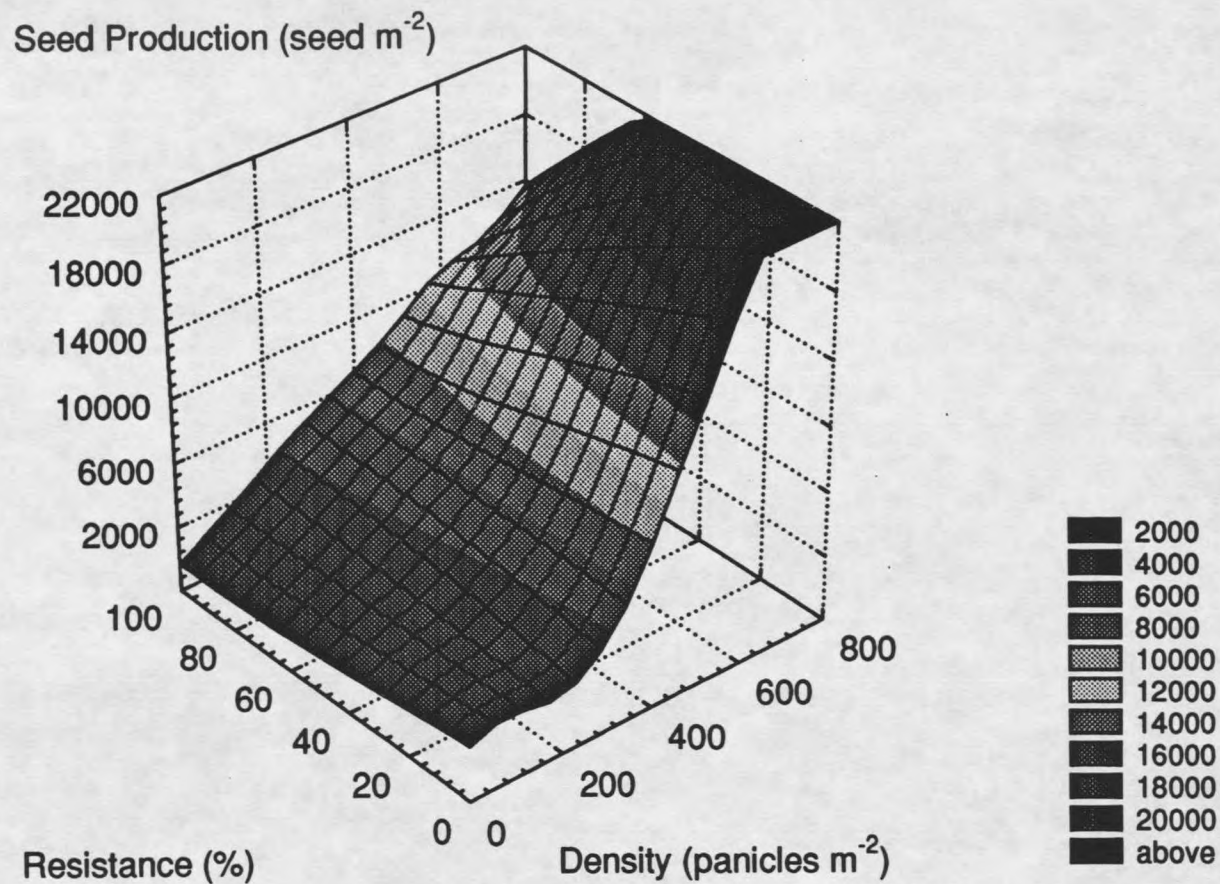
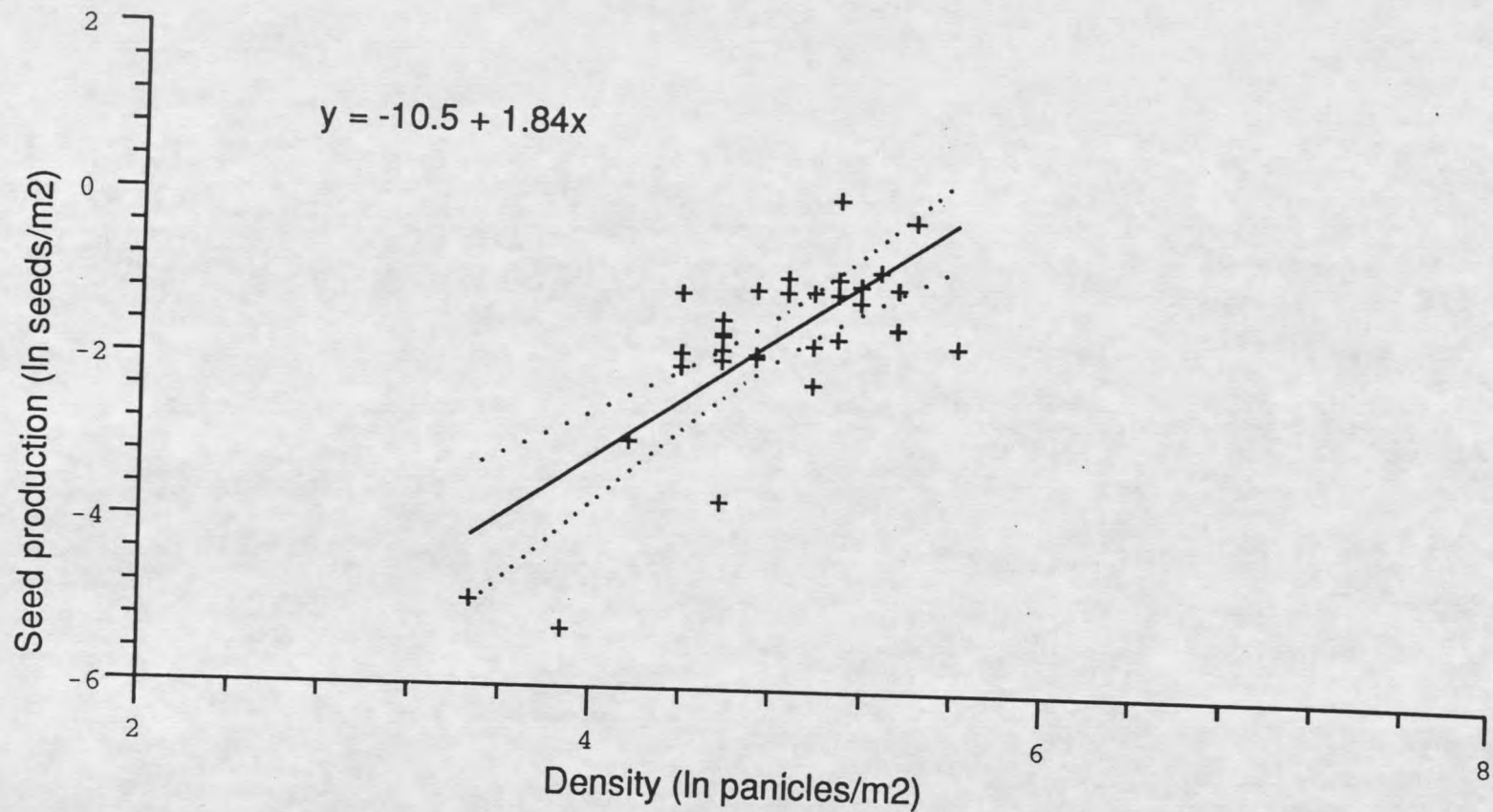


Figure 5.14. Least squares surface plot of the relationship between seed production, density (panicle number) and triallate resistance of wild oat from a patch located on the Fairfield Bench (Site 2, 1995). Grey scale legend represents seed production (seeds per m^2).



provide visual evidence of a difference in seed production of the resistant and susceptible biotypes at different densities. At low densities resistant and susceptible biotypes performed equally in terms of seed production in monoculture and in mixtures. However, in mixtures at high densities, seed production was less than either the resistant or susceptible plants in monoculture for Site 1. Seed production of the susceptible and resistant biotypes at high densities was not significantly different ($P>0.05$). At Site 2 and 3 seed production levels were much higher reflecting better conditions for growth. At Site 2 the susceptible biotype produced approximately 50% more seed per unit area at high densities. At Site 3 the difference in seed production between biotypes was greater with the resistant biotype producing approximately 12,000 seeds per meter squared compared to over 18,000 by the susceptible biotype at the same density.

Regression analysis was used to characterize the change in response of resistant and susceptible biotypes in monoculture and mixture with increasing density. The slope of the line of seed production versus density was significantly different between the susceptible and the resistant biotype (Figure 5.15 and 5.16). The slope was less for the resistant biotype compared to the susceptible biotype. This indicates that seed production per unit area did not increase as rapidly for the resistant biotype as it did for the susceptible biotype which may lead to the conclusion that the susceptible biotype was more fit than the resistant biotype. The slope of the line showing the relationship between seed production and density for the mixtures was not significantly different to that of the resistant biotype in monoculture (Figure 5.17). If competition and seed production are high, then the resistant biotypes will exhibit a fitness difference, producing significantly

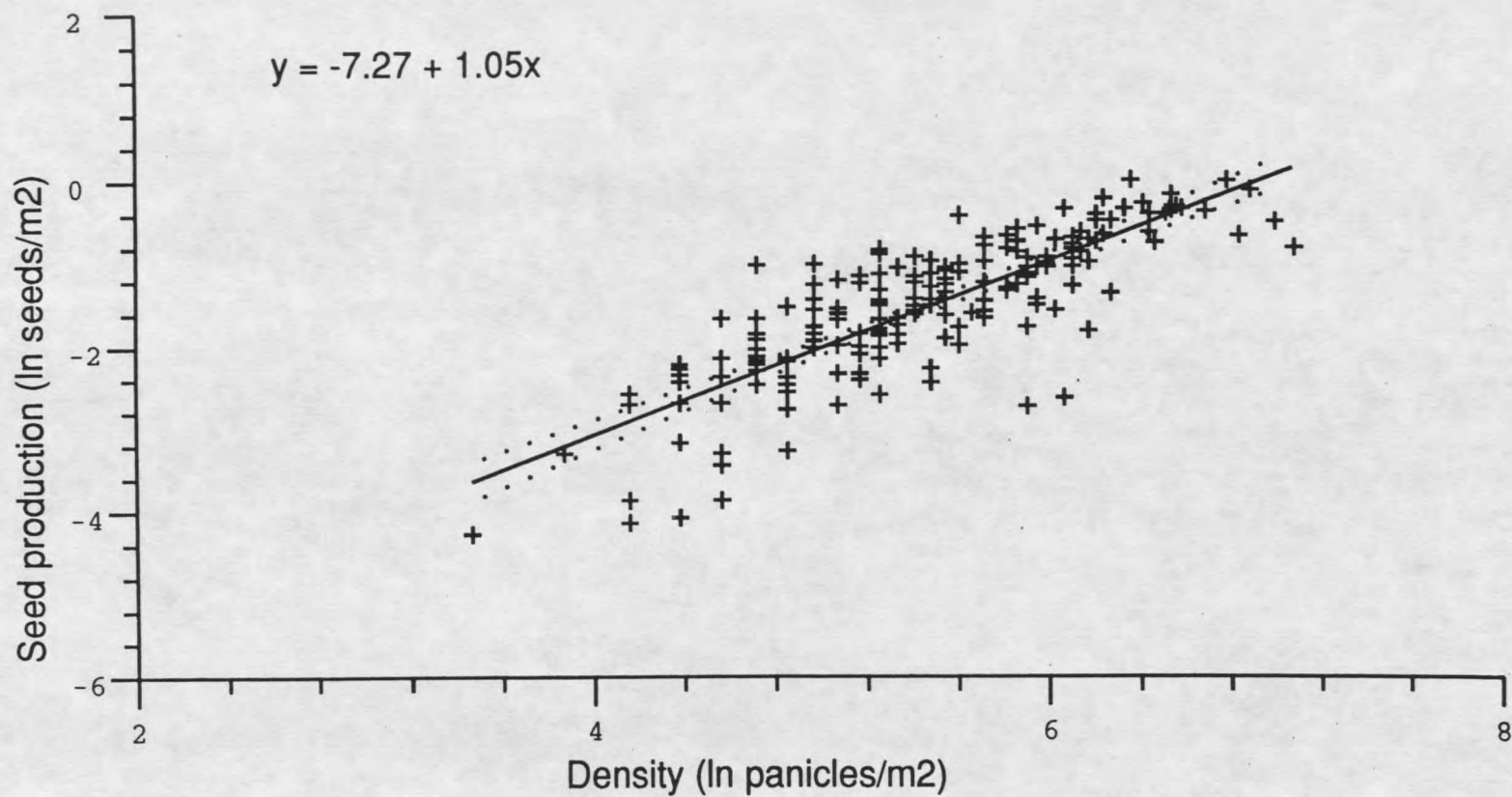


Figure 5.16. Seed production of triallate-resistant biotypes of wild oat in response to increasing wild oat density.
 $R^2 = 0.66$.

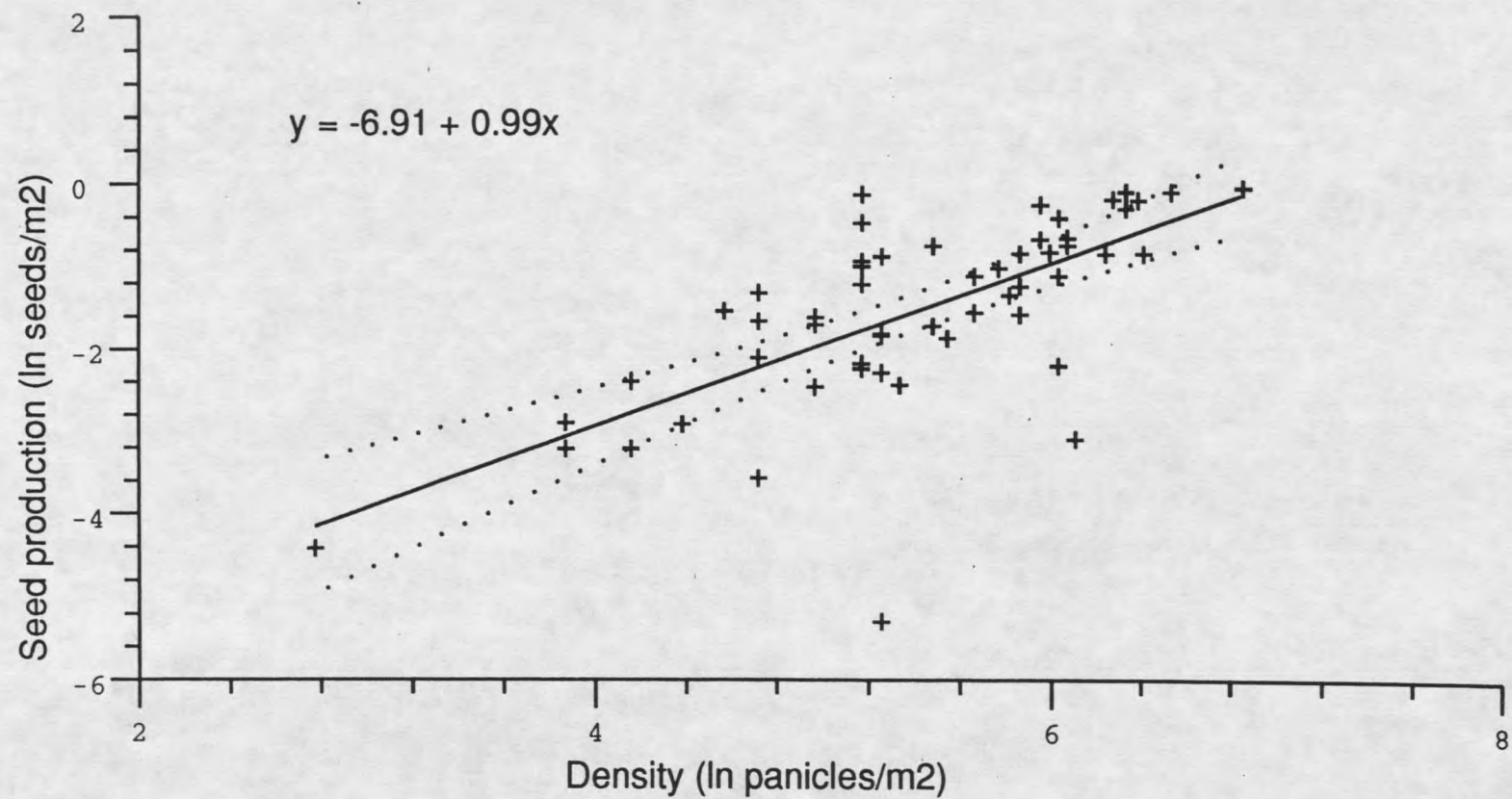


Figure 5.17. Seed production of mixtures of triallate susceptible and resistant wild oat biotypes in response to increasing wild oat density. $R^2 = 0.47$.

less seed than the susceptible biotype, when grown in a mixture. It is likely that the threshold value for density, where differences between biotypes can be observed, will vary with management practices (eg. crop sowing density) and season.

The results of the survey and the within field evaluation of native populations of resistant and susceptible wild oat (isogenic lines) do not agree with those obtained using either growth analysis or replacement series experiments. This highlights the importance of using isogenic lines and understanding environment interactions. Genotype by environment interactions are extremely important in determining the direction of resistance change, yet cannot be easily detected using current experimental techniques. Evaluating fitness at one location, under conditions that resemble neither the farming operations nor the environmental conditions, is unlikely to indicate the true relative importance of differences in fitness. Evaluation on farm permits evaluation of multiple populations under the conditions that affect their continued evolution.

A multitude of management practices have been recommended to delay and/or manage herbicide resistance in integrated weed management programs (Thill et al. 1991; Young et al 1994; Powles et al. 1997). The early detection of fitness differences, afforded by field studies and surveys, will provide better parameters for predictive models leading to more timely recommendations of management strategies for delaying and managing herbicide resistance.

CHAPTER 6

SAMPLING STRATEGIES FOR DETECTION OF HERBICIDE-RESISTANT WEEDS

Introduction

Integrated weed management programs and decision analysis systems require a greater level of monitoring of weed populations in the field. The efficient management of weed populations requires the ability to accurately predict the effect of the weed on the crop. Determining potential yield losses and long term economic thresholds requires estimates of weed density (Pannell 1990; Maxwell 1992). Weed control is then only carried out if weed density exceeds an economic threshold (Cousens et al. 1986). Knowledge of the frequency of resistance within the field is also required before a decision on weed control is made. However, unless cost effective monitoring strategies for density and resistance identification are developed, weed control decisions will continue to be based on estimates with low precision.

A number of sampling strategies have been used to sample weed populations in the field. Thomas (1985) used a W pattern in a weed survey in Saskatchewan. The sampling pattern commenced 100 paces from the edge of the field. A W pattern was followed within the field with each leg 100 paces long. Five locations were sampled along each leg of the W. Quadrats of 0.25 m² were used at each sample point. In an evaluation of the effect of sampling intensity on density estimates for grasses, Marshall (1988)

determined weed density using a systematic grid counting weeds in four 0.25m² quadrats. Sampling intensity varied from 2.5 points per hectare (25 sample locations) to 35 points per hectare (407 sample locations). Marshall (1988) used interpolation to create a map of the weed population to obtain an estimate of the density within the study area. Recent studies by Johnson et al. (1996) and Gould (1996) utilized the knowledge that many weed distributions can be described by the negative binomial distribution in development of appropriate sampling techniques. Both studies concluded that improvements in the sampling techniques were possible over the traditional methodology by using a sequential sampling methodology. The number of samples to be collected was based on the previous samples. This technique could not, therefore, be applied to sampling for resistance, as the resistance status of each sample is unknown in the field. The stability of the k parameter, in the negative binomial distribution function, between years is also an unknown factor that may reduce the efficiency and accuracy of this sampling technique.

The introduction of global positioning systems (GPS) into mainstream agriculture for management of pesticides will alter the requirements of weed scouting. The mean density of the weed species over the field and whether the value is above or below an economic threshold for a whole field, may not be important. Managers using GPS require a map of the field indicating the density of weeds at specific locations and identification of those locations where weed populations within the field exceed a threshold. Many sampling methods have been examined that can be used to generate weed maps for a field (Colliver et al. 1996). Perimeter mapping, mapping at the time of harvest and the use of real time sensing using infrared are a few of the alternatives currently being investigated.

The maps created from these methodologies do not usually contain detailed spatial information of the density within the field. Few attempts have been made to incorporate the threshold concept to further refine maps. Johnson et al. (1995) created weed maps based on traditional sampling techniques combined with spatial statistics. A total of 674 and 812 quadrats were sampled within two 4 ha fields and kriging was used to create maps. Areas where densities exceeded a threshold value were identified using kriging then converted to probability estimates using a Bayes classification rule. The classification rule was used to minimize incorrect classification. Although this methodology provides the best estimate of the threshold map, it also required intensive sampling that would not be appropriate on a large scale. Adaptive cluster sampling, often used in sampling of rare clustered populations, combined with the appropriate decision rule and geostatistical analysis may reduce the level of sampling intensity necessary to produce detailed maps on a large scale.

The objectives of this research were firstly to evaluate common sampling protocols for their effectiveness in detecting herbicide resistance in wild oat population. This was achieved using simulated sampling of artificial fields created from field data collected at three different spatial scales. The second objective was to evaluate the potential to use geostatistical techniques to reduce sampling intensity with traditional sampling strategies. The third objective was to determine if a knowledge of weed location could be used in combination with adaptive sampling strategies and geostatistical methods to further reduce the sample intensity.

Materials and Methods

Simulation Program

A computer program was written in Java to simulate the sampling of density and resistance of wild oat within a field. Five different sampling strategies were investigated; simple random sampling, sampling using transects, stratified random sampling, W-method, and adaptive sampling. Sample number collected for each method ranged from 8 to 2048 samples per field. Simulated sampling was carried out in two fields. One field contained wild oat in an aggregated spatial pattern with the level of aggregation based on previous sampling. The second field contained wild oat that were randomly dispersed throughout the field.

Field structure

Two fields, 45 ha in size, were constructed to evaluate sampling methods through simulation. One field was created using a combination of measures of the distribution of triallate-resistant and susceptible wild oat at three spatial scales (Chapter 3 and 4). The wild oat patch perimeter was based on a field (45 ha) that was mapped by perimeter mapping using an Ashtech GPS (Colliver et al. 1996). Perimeter mapping of wild oat involved outlining patch locations throughout the entire field. The edges of patches were detected visually and their location was recorded using GPS. The perimeter maps were analysed using Fragstats. Fragstats, a spatial pattern analysis program for landscapes, was used to determine the size, shape and location of patches relative to one another. Specific metrics calculated included mean patch size (ha), patch size standard deviation, patch

density (patches 100 ha^{-1}), edge density (m ha^{-1}), nearest neighbor distance (m), and the percent of the landscape occupied by wild oat.

The first stage in the construction of maps was to add density values to the patch within the field. The spatial distribution of density within patches was generated from distributions obtained from previous studies (Chapter 4). The spatial relationships were maintained by incorporating the spherical semivariogram model to generate patch densities. The second stage in the construction of the map was to allocate a resistance status to individual wild oat plants within the field. The distribution of triallate resistance within the field is often aggregated at different scales. Malchow (1995) found a pattern of resistance in the field at 7 m to 8 m and also at 25 m to 27 m. Davidson et al. (1996) found autocorrelation at one site at a scale of 1 m. To incorporate the spatial arrangement of resistance within the field, different patches were assigned as either resistant or susceptible. The decision was based on the relative position of the patches to one another and the frequency of resistant patches within previous sampled fields containing resistant wild oat.

One additional field was generated to contrast the impact of aggregation of resistance and density on sampling. The random spatial distributions of wild oat were generated using an array of uniformly distributed random variables between 0 and 1. The location of each plant was obtained by

$$\begin{aligned} X &= \text{field width} * \text{random uniform number} \\ Y &= \text{field length} * \text{random uniform number} \end{aligned}$$

The number of individuals generated in the random location field was not fixed. Individuals were generated until the mean density of the random location field equalled

the mean density from the aggregated field. If plants were located within the same 1 m² area then the plant numbers were summed. Resistance values were assigned to each plant with resistance frequency for each cell calculated by dividing the number of resistant plants within each cell by the total number of plants within the cell.

Sampling Strategies

Random Sampling A random sample was drawn from a population so that every sample has an equal possibility of being selected. The selection of sample locations within each of the fields was achieved by multiplying the maximum x and the maximum y coordinates of the field by a number generated from a random uniform distribution.

Transect Sampling Four transects were located parallel to one another within each field. The starting locations for each transect were determined by multiplying the maximum x coordinate by a number generated from a random uniform distribution. An equal number of samples were collected on each transect. The location of the samples in the y direction were determined by dividing the maximum y coordinate by the number of samples on the transect. This achieved an equal spacing of the samples along each transect.

Stratified Random Sampling A stratified random sample was obtained by dividing the field into non-overlapping groups, called strata, and then selecting a simple random sample from within each strata. The number of strata within the field equalled the sample number. Strata size was determined by the size of the field and the number

of samples to be collected. The dimensions of the strata were as close as possible to a square as calculated by an optimization routine.

W-method Sampling First, the W sample was achieved by randomly selecting the location of the first leg of a 400 m W pattern. The program rejected initial locations that did not fit all the legs of the W pattern within the field. Sample locations were divided evenly among the four legs of the W and evenly spaced along each leg.

Adaptive Sampling The adaptive sampling was conducted using a combination of random transect sampling and a simple decision rule. Four parallel transects were randomly located within the field. The number of samples to be collected from the field was evenly divided among each transect. The location of the first sample on each transect was calculated by dividing the width of the field by the number of samples to be collected along that transect (if the number of samples was greater than one). The location of the second sample was located an equal distance (first location to the edge of the field) from the first sample location. Wild oat density and resistance values were then determined for the sample location. The location of the next sample was dependent on the difference between the two samples. If the difference was large (greater than 50%) then the distance to the next sample location was halved. If the distance was small (less than 50%) then the distance to the next sample location was doubled. This basic rule enabled an increased number of samples to be collected where the density of wild oat was changing most rapidly and reduced the number of samples where there were no wild oat or where the density between sample locations was similar. The mean value of density

or resistance was calculated, not from the average of the individual samples, from the average of kriged estimates of the individual locations based on semivariogram analysis of the sample data from each field.

Sampling Method Comparison

The sample methods were compared by determining the difference in the estimated density and resistance values obtained by simulated sampling from the true values for each field. The true value for density and resistance was obtained by averaging the values contained within all cells (450,000) for each field. The coefficient of variation and standard error was calculated to determine the change in variation with increased sample number for each of the sample methods.

Results and Discussion

The analysis of field perimeter data, collected for wild oat in one Montana field, showed how little area is affected by the weed population (Table 6.1). This level of aggregation is typical of many weed species (Wiles et al. 1992). Only 16% of this 45 ha field was occupied by wild oat (Figure 6.1). The mean patch size was approximately 0.2 ha with a standard deviation of 0.6. Although most patches of wild oat were small, the range was large, with two of the 40 patches almost 3 ha in size. The location of patches within the field showed that almost half of the patches were located within 2 m of another patch.

The shape of the patches was relatively constant with only 5.9% CV calculated. The perimeter method of detecting wild oat may have reduced the real variation in this

measure. The mean density of the aggregated field was 0.6 panicles per m² (64 panicles per m² for occupied cells). The average resistance value was 11%. To achieve the same mean density in the randomly distributed field a lower density per occupied site was required.

Table 6.1. Landscape metrics for the field of wild oat sampled by simulation.

Patch Characteristics		Minimum	Maximum	Mean	Standard deviation	Coefficient of Variance
Size	(ha)	0.001	2.39	0.21	0.57	279
Nearest Neighbour	(m)	1.2	44	8.6	10.8	125
Fractal Dimensions		1.08	1.27	1.15	0.06	5.9

The aggregated field, when sampled, showed a high coefficient of variation for density (greater than 400%) even with a high sampling intensity of 45 samples per hectare. There was no downward trend as reported by Marshall (1988) for *Bromus* spp. The standard error of the mean did show a decline as sample number increased (Figure 6.2). Random sampling methodology consistently resulted in the lowest standard error. This may reflect the increased field coverage that is possible by this method compared to the restricted paths taken when using the W-Method or the transect method. The standard error increased from less than 10% of the mean at the highest sampling intensity (35 samples per hectare), to over 50% at 15 samples per hectare. The standard error for the field where wild oat were distributed randomly, was consistently lower for all sampling methods for the corresponding sample number (Figure 6.3). Weeds that have

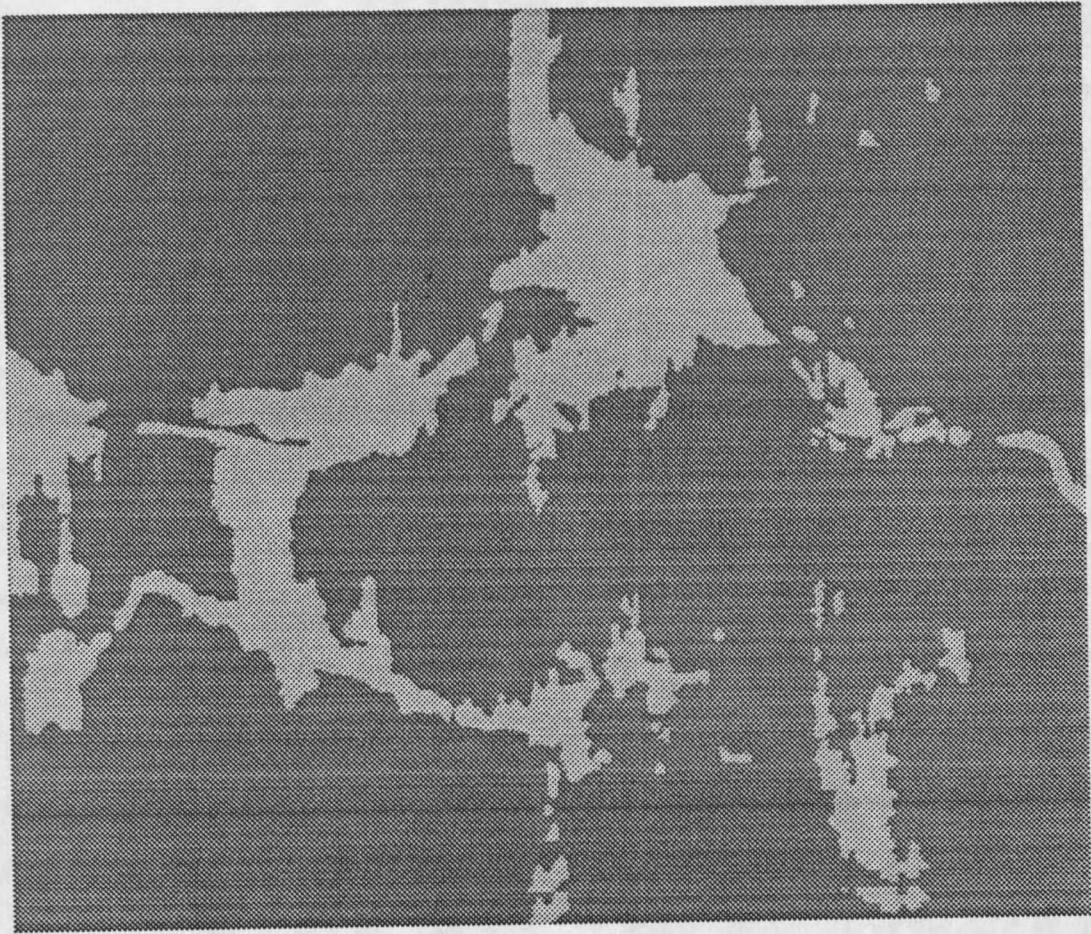


Figure 6.1. Perimeter map for wild oat in an aggregated field. ■ Wild oat patches. ■ non-infested area.

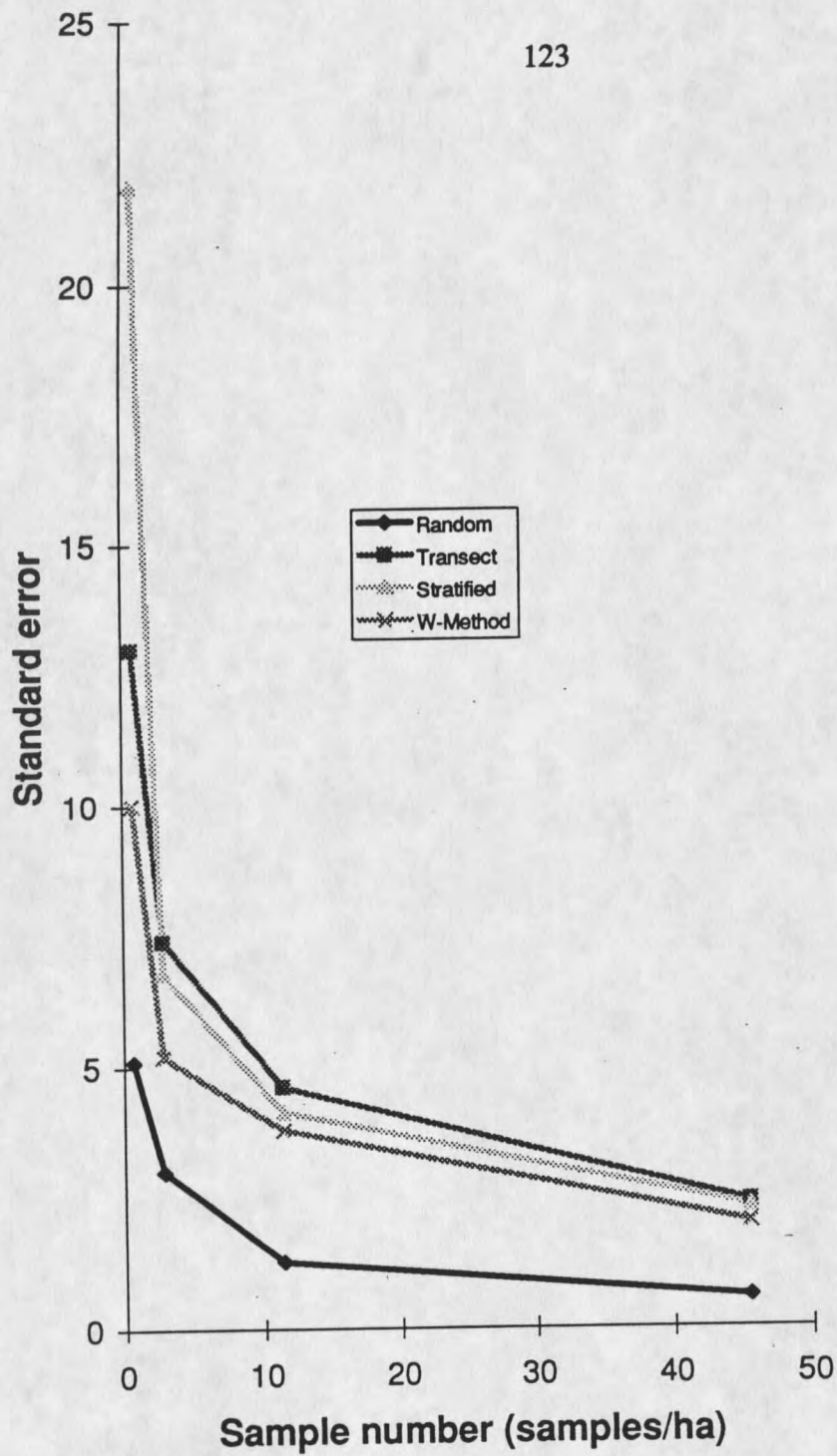


Figure 6.2 Standard error of the mean of density for each sampling method with increasing sample number. Sample number range from 8 samples to 2048 samples. Aggregated (patchy) wild oat field.

a high degree of aggregation will require a greater level of sampling to obtain the same level of precision in estimates of resistance or density.

At low sample numbers (8 or 32 samples per field) the level of detection of triallate-resistant wild oat was very low (Table 6.2). In 80% of sampling simulations, no resistance was detected despite the field containing greater than a 10% average level of resistance. The precision of the estimates when resistance was detected was also low. When just under 1 sample per hectare (32 samples in total) was simulated, resistance estimates ranged from 2% to 88%. Recommendations for the collection of resistant seed from within a field need to allow for a high level of variability when resistance is relatively low. Monitoring of fields where resistance is well established is not likely to result in the same level of variability as resistant plants would be more evenly distributed among patches.

Table 6.2. Frequency of resistance detection with increasing sample numbers.

Sample Method	Resistance not detected (8 samples)	Resistance estimate (32 samples)	
	(frequency)	Minimum	Maximum
Random	0	2	16
Transect	0	2	30
Stratified	0	3	88
W-Method	0	2	34

Semivariogram analysis adaptive sampling and transect sampling at two sampling intensities did not identify a pattern or spatial association (Figures 6.4, 6.5, 6.6 and 6.7). This may indicate that the sampling intensity is still too low to detect the spatial

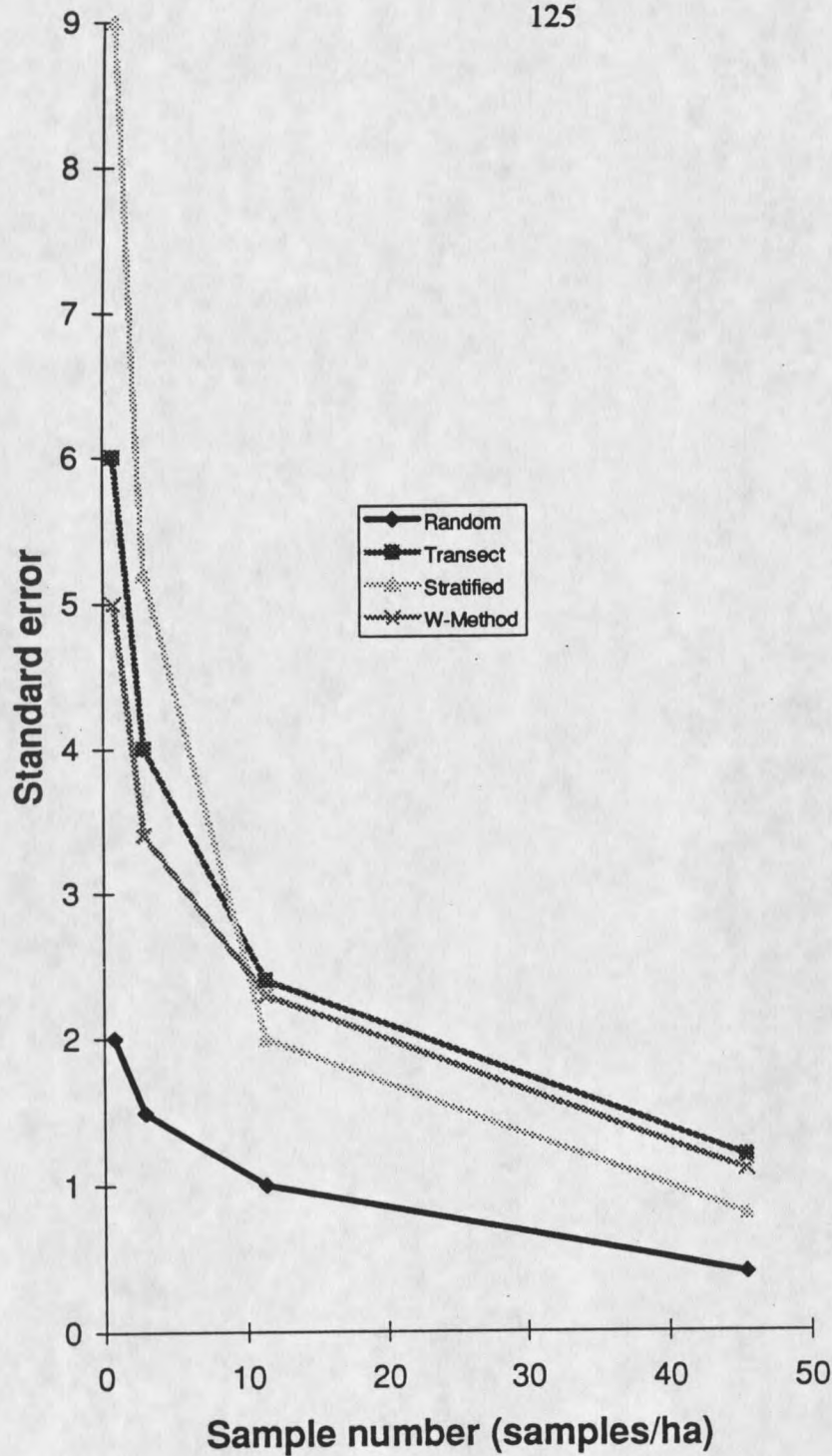


Figure 6.3 Standard error for density for each sampling method with increasing sample number. Sample number range from 8 samples to 2048 samples. Random infestation of wild oat in a wheat field.

relationship. A relationship between measures of densities has been identified in the field up to a distance of 10 m. The shortest lag distance, as indicated on Figures 6.6 and 6.7, was 20 m. A modification of the adaptive sampling rule is required to enable a sufficient number of samples to be collected at distances less than 10 m. The decision rule needs to reflect sampling that can be achieved in a true field. In a true field optimized sampling would involve few samples in regions where the weed population was zero and many samples when patch edges were encountered. The level of precision in the map obtained would still be a function of sampling intensity, but sample number should be reduced from that required with traditional sampling methods. Further investigation of decision rules that can be readily adopted in the field is required.

The analysis of the field by Fragstats indicated that considerable economic gains would be possible by using GPS technology. The wild oats were aggregated into patches that were located relatively close together enabling a large reduction in the area that would need to be sprayed by herbicide. The field could be divided into a few large blocks where either wild oat control was carried out, or not, depending on the presence of patches. The closeness of the patches would enable management on a block system without requiring a detailed map. Patch location also influences the potential expansion rate. Patches close together increase the probability that seed dispersed from one patch will land in an area already occupied by wild oat.

The optimum use of resources for management of weeds requires a method of scouting that quickly and accurately identifies the number and location of different weed species. Intensive sampling is required when using traditional sampling protocols. The

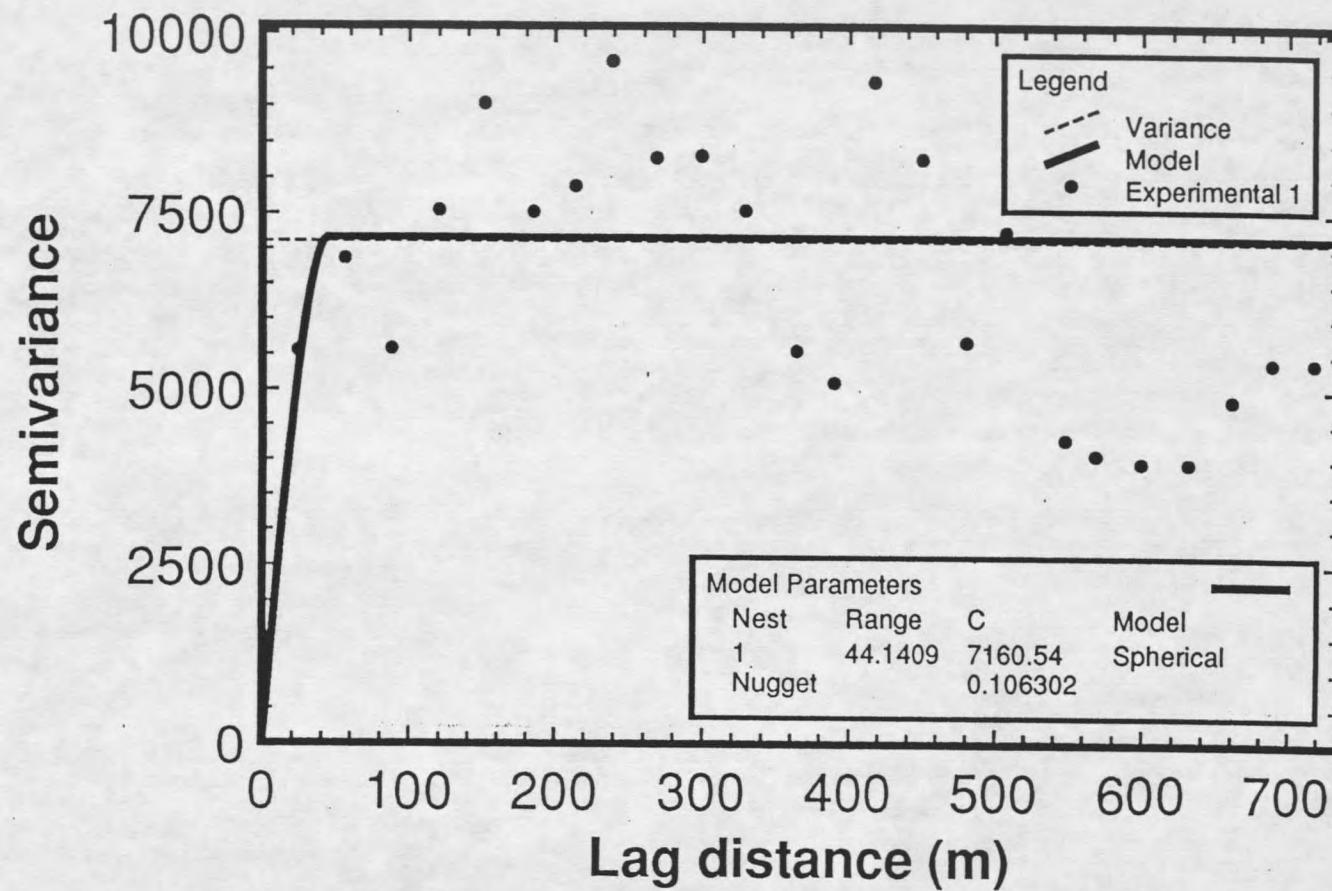


Figure 6.4. Semivariogram of wild oat density within the field sampled using the transect method. Sample number was 132 samples.

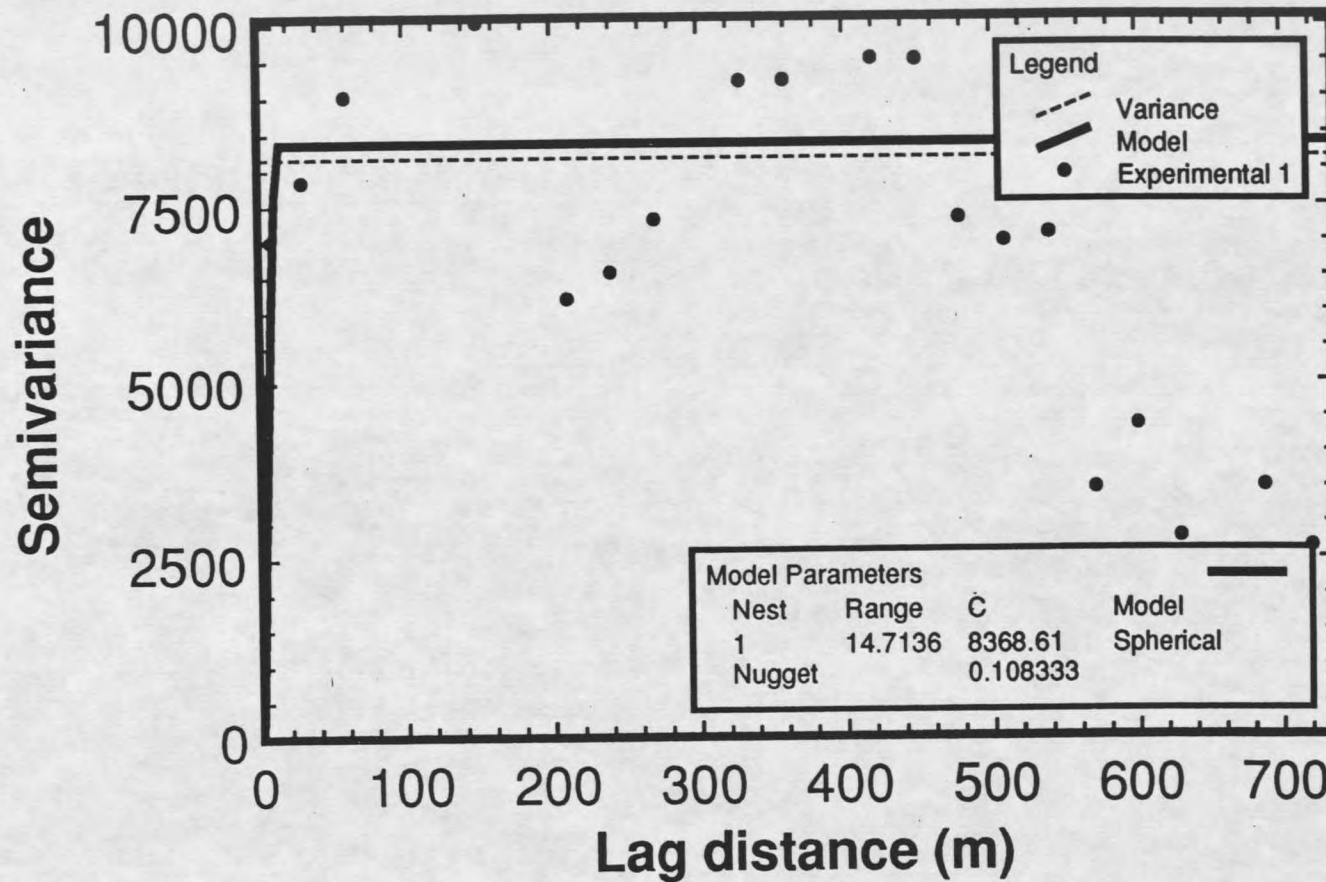


Figure 6.5. Semivariogram of triallate resistance frequency within the field sampled using the adaptive sampling method. Sample number was 132 samples.

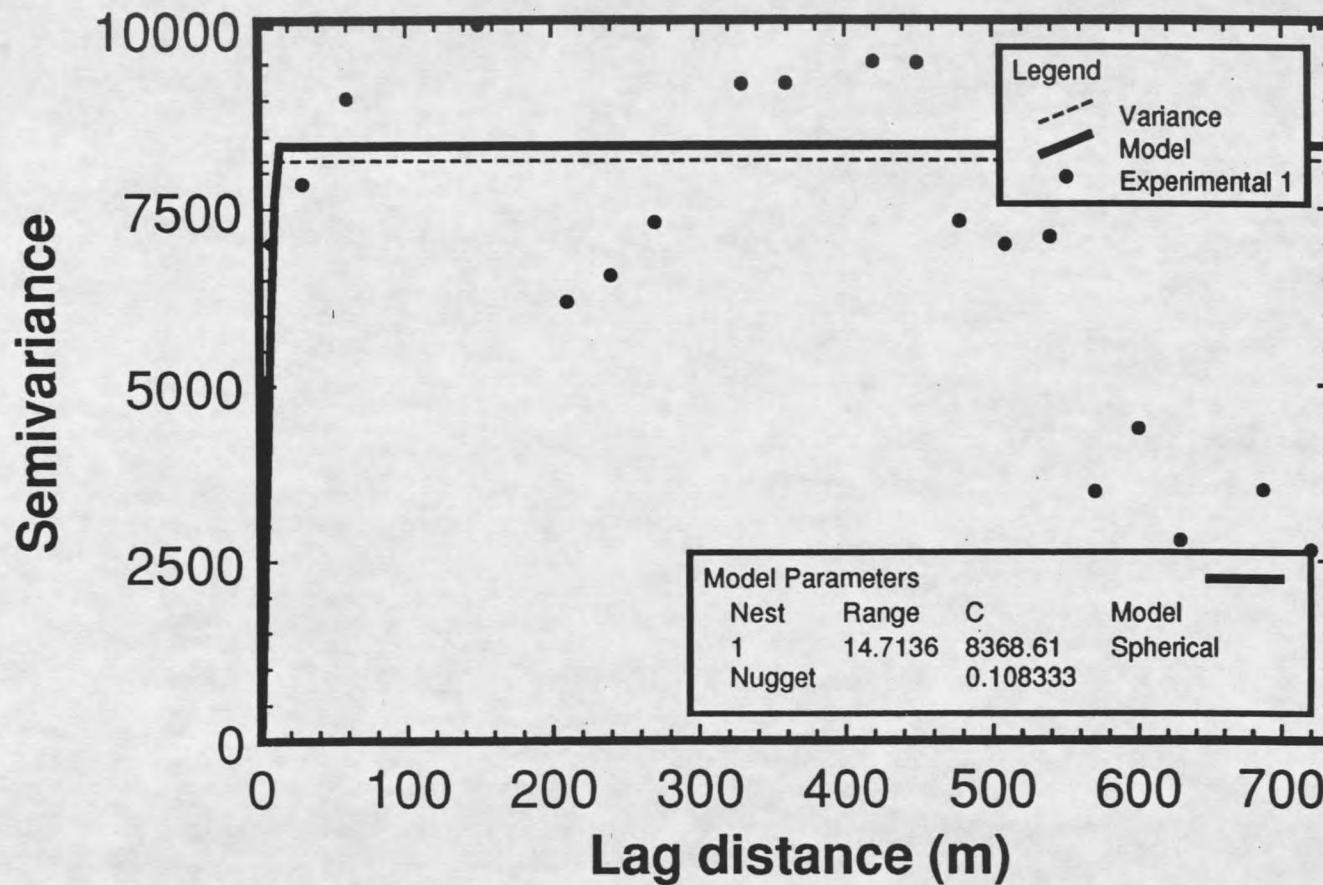


Figure 6.6. Semivariogram of wild oat density within the field sampled using the transect sampling method. Sample number was 512 samples.

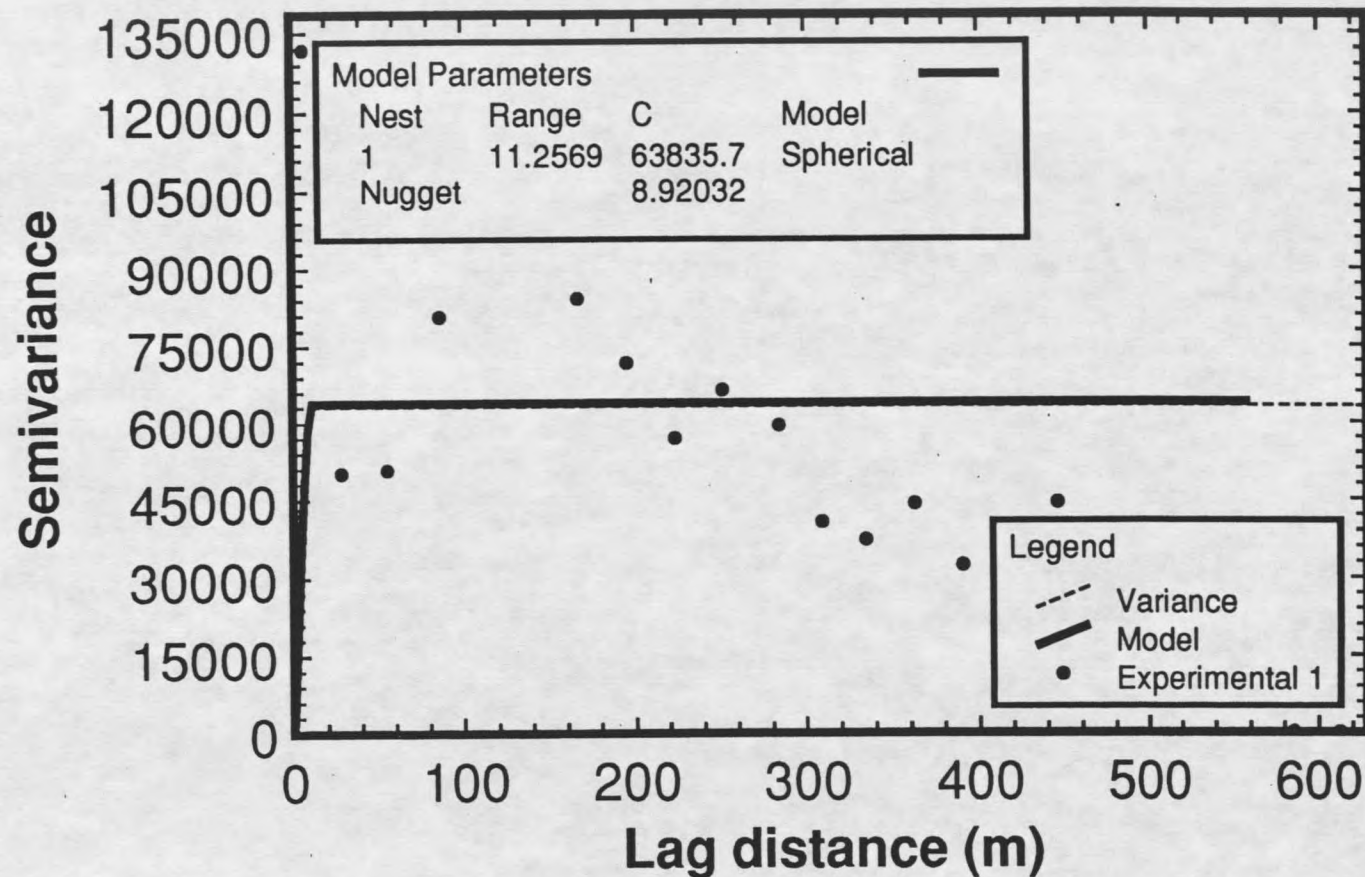


Figure 6.7. Semivariogram of triallate resistance frequency within the field sampled using the adaptive sampling method. Sample number was 512 samples.

level of sampling required was not reduced using geostatistical techniques as no spatial relationship for wild oat density could be detected at the sample intensity examined. Highly aggregated weed populations are the most difficult to obtain an accurate estimate of density or resistance by sampling. However, they will often be the same fields that give the greatest economic gains from managing with weed maps (Maxwell and Colliver, 1995). The reduction in cost associate with spatial specific application of herbicide or other weed control will offset at least partially the expense associated with sampling.

CHAPTER 7

EPILOGUE

The demands placed on modern agriculture in future years will outstrip our ability to supply food if world population demand for food continues to increase at its current rate. The demand will be, not only for increases in food quantity, but also for higher quality food. These demands will impact many of the current agricultural practices, of which management of weed populations is an integral component. Traditional weed management strategies have relied heavily on herbicide application. This approach is becoming both ineffective due to the widespread evolution of herbicide resistance in weed populations and unpopular due to both real and perceived health risks associated with the use of pesticides. In Denmark targets have been set for reductions in levels of herbicide use, with a 50% reduction in herbicide use to be achieved before the year 2000. Dramatic reductions in herbicide use have already been achieved. However, most of the reductions in herbicide use have resulted from switching to herbicide products that are able to achieve a high level of activity from a low level of active ingredient. Innovative management will be required to make further gains without sacrificing crop yield. Although no targets for herbicide use have been set in legislation in the USA, it is clear that public perception of herbicides will result in a change in their use. There has already been a substantial increase in organic produce and a reduction in chemical use in some of the more sensitive horticultural markets.

The key to meeting the demands placed on the agricultural community is to develop a system that enables producers to better understand their own system and to implement new management strategies. Only then will we be able to develop an ecologically benign agricultural system which can adapt to the increasing food demands of the future.

How can this be achieved? Many agricultural researchers are still struggling to provide much more than a recipe for crop management. A number of aspects of weed research and extension need to change. The first change required is for researchers to gain a greater appreciation of the variance of parameters within their systems and how they change over space and time. Advice on the potential of a new management technique to improve weed control can only be given with confidence if there is knowledge of how parameters change, within the region in question. Recommendations can then be given as a probability of success. For example, this study has indicated the need to consider the distribution and interactive processes between herbicide susceptible and resistant biotypes as they occur in patches throughout the field. Weed management strategies based on data collected at a field scale are therefore likely to be ineffective in the monitoring of populations, as they provide inadequate information about the weed population. If a knowledge of the distribution or variation in the population is known then the impact of the weed on the crop can be more accurately assessed.

A second change that is required is to advance the methodology of assessing population dynamics by being prepared to attempt new approaches. Examples include different sampling techniques, computer population modeling, different statistical analyses,

increased attention to genetics, and the use of model species. Innovative research will in turn provide valuable directions for advancement of weed research. In this study, a model species, *Arabidopsis thaliana*, was used to investigate changes in gene frequencies in populations due to different management strategies. It was successful in determining the effect of herbicide use on resistance evolution. Increased efficacy of herbicides increased the rate of evolution of resistance and the use of mixtures and rotations slowed the rate of evolution. However, the problems associated with the choice of this species hampered the experiments. The time required to manage population dynamics experiments using *Arabidopsis thaliana* under greenhouse conditions was equal or greater than that required for a similar number of plots in a typical field experiment. The advantage of a short generation time was outweighed by the problems of susceptibility to insect and fungal attack when grown at high plant densities. Although *Arabidopsis thaliana* plants are able to produce large quantities of seed, it is not an easy process to obtain sufficient quantities (minimum of one million seeds per treatment per generation) of seed for population experiments. Difficulties associated with this approach may be overcome by choosing a different species. The problems were identified however, which is always the first step towards developing robust techniques.

The use of geostatistical techniques to examine the spatial structure of weed populations is another example of where the adoption of innovative methods has proved to be extremely useful. This technique, which has its origins in the mining industry, enabled the identification of the genetic and demographic structure of populations at regional, field and patch scales. The importance of the spatial structure in populations in

determining the dynamics of weeds within fields was first recognized in the 1940s. However, it has only been in the past five years, with the introduction of new technology, that population structure of weed species within agricultural fields has been reported.

Understanding weed population dynamics within a field, requires that both genetic and demographic components of populations are examined. This study, examining wild oat populations at different scales, illustrates how further valuable information can be gained by observing patches within fields. The dynamics of a patch was found to be influenced by the distribution of resistant and susceptible biotypes within the patch. Herbicide-resistant biotypes were found to occur more commonly at the periphery of the patch and in areas of lower plant density. This may be due to a decreased ability of resistant biotypes to compete with susceptible biotypes. The greater presence of resistant biotypes at the patch edge is a very important observation as this will influence patch expansion and gene flow of resistant biotypes between patches. The location of resistant plants on the patch edge enables those plants to contribute a greater proportion of seed to subsequent generations through reduced competition and greater dispersal throughout the field. The dynamics of patches are complex and further work is required to understand the processes driving patch location and expansion. The implementation of radical experiments such as sowing weed seed over an entire field could be invaluable in understanding the dynamics of weed patches. Monitoring the intrinsic rate of increase in the population at different locations to detect patch expansion and establishment of new patch locations could provide parameters for models to assess the most appropriate management strategies. What are the relationships between patch expansion rates and

patch size and shape? What impact do management practices have on patch characteristics? How can we manipulate these aspects of patch dynamics?

Knowledge gained from field studies will assist in development of a robust framework for weed population dynamics. The incorporation of the theories involved in metapopulation models and resource based models such as source-sink theory based models, will provide the starting point for the next generation of predictive models. Metapopulation based models will be useful for investigating the effects of different levels of fragmentation of fields. The use of different crops and weed control methods to fragment the field is anticipated to reduce gene flow reducing the spread and rate of evolution of herbicide resistance. The long term effects of site specific weed management on weed populations could also be simulated. A better understanding of how resource availability in the field affects weed seed production may enable further manipulation of weed populations.

An important step often overlooked in research is how information is transferred to farmers. The current advances in technology are providing new avenues for the transfer of information. The development of decision aid software that is accessible by the Internet will provide the most effective means to keep information current. A decision aid program utilizing Bayesian techniques, could be designed that would enable farmers to simulate different management options and see the risks and rewards associated with their choices. Java would be an appropriate choice of programming language as it is readily adapted to the internet and ideal for collaborative research between diverse groups. Models are only as good as the information they contain so incorporating adaptive

management methodology would further enhance this approach. An ongoing database which includes individual farmer's management choices and economic responses could then be incorporated. The ability to continually update and improve the accuracy of predictions on an individual farm basis is a major advantage of adaptive management.

This study has demonstrated the need to incorporate the spatial and temporal relationships of weed populations into weed management strategies. To do so will require innovative new methodology and technology, but this is essential if we are to improve our ability to manage weed populations, an essential requirement for agriculture in the next century.

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APPENDICES

APPENDIX A - Regional Scale

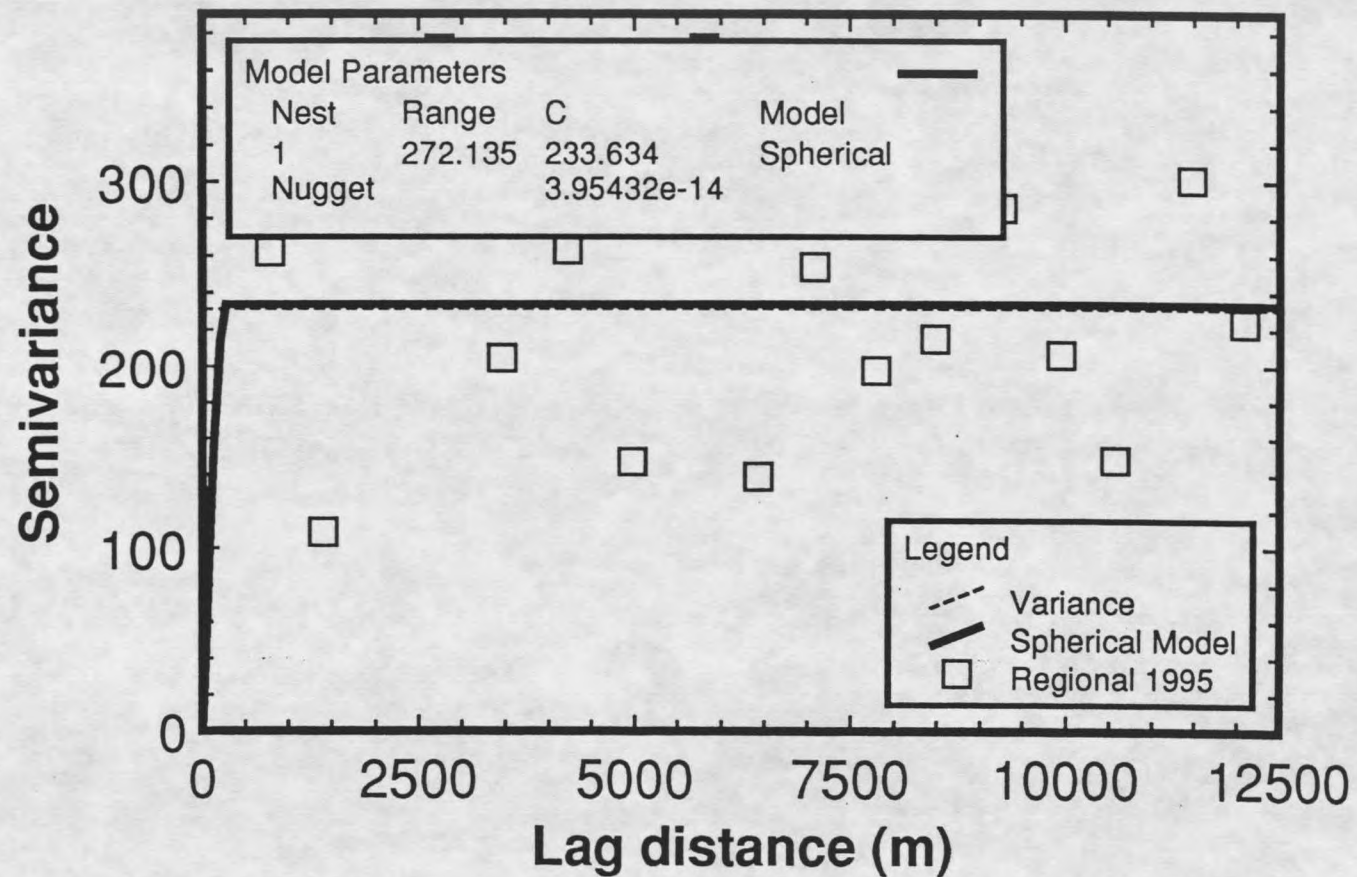


Figure A.1. Semivariogram of triallate-resistant wild oat sampled at a regional scale in 1995. Resistant wild oat were detected in 77% of fields.

APPENDIX B - Field Scale

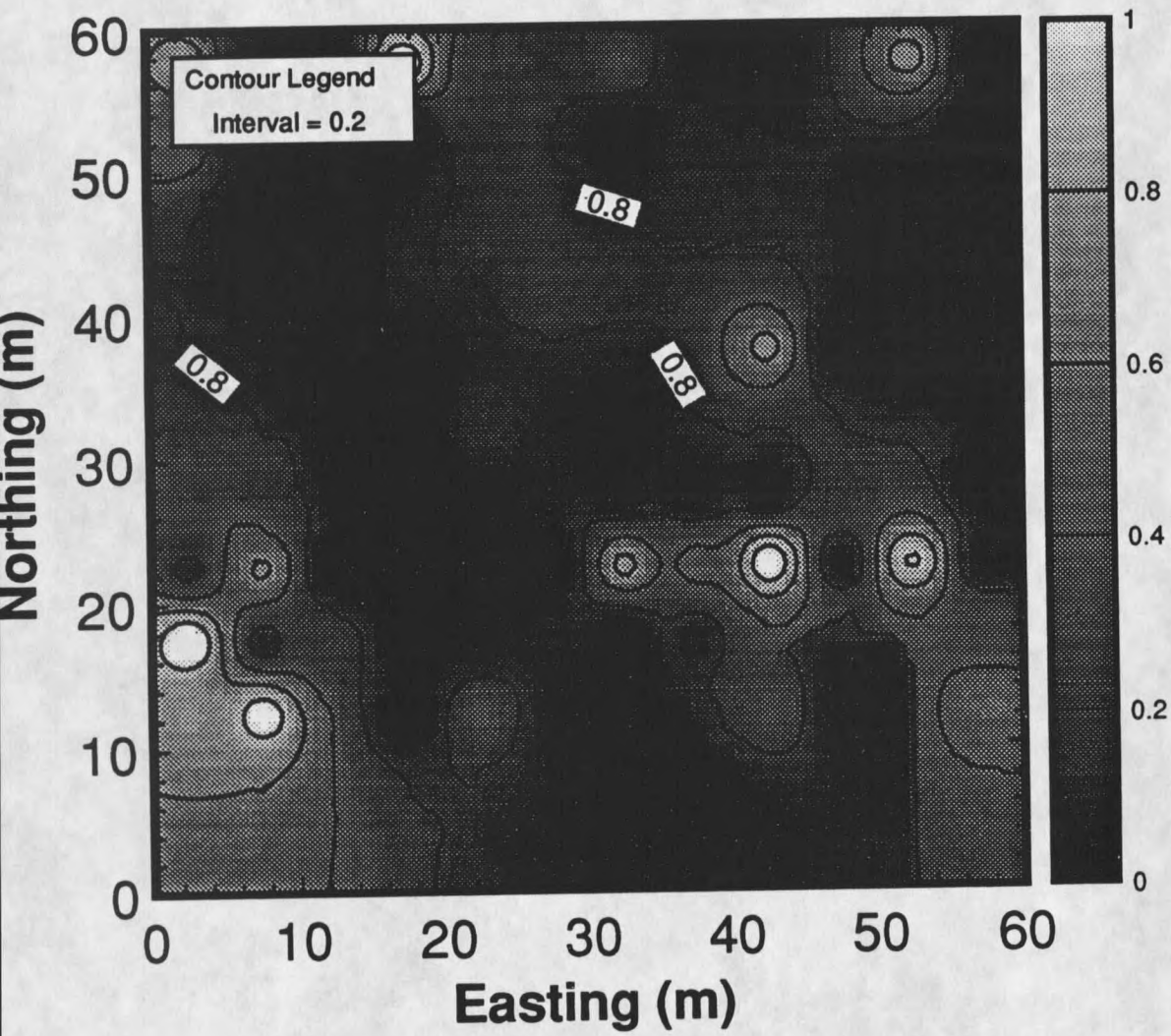


Figure B.1. Interpolation map of aggregation (C Value) at Site 3.

APPENDIX C - Patch Scale

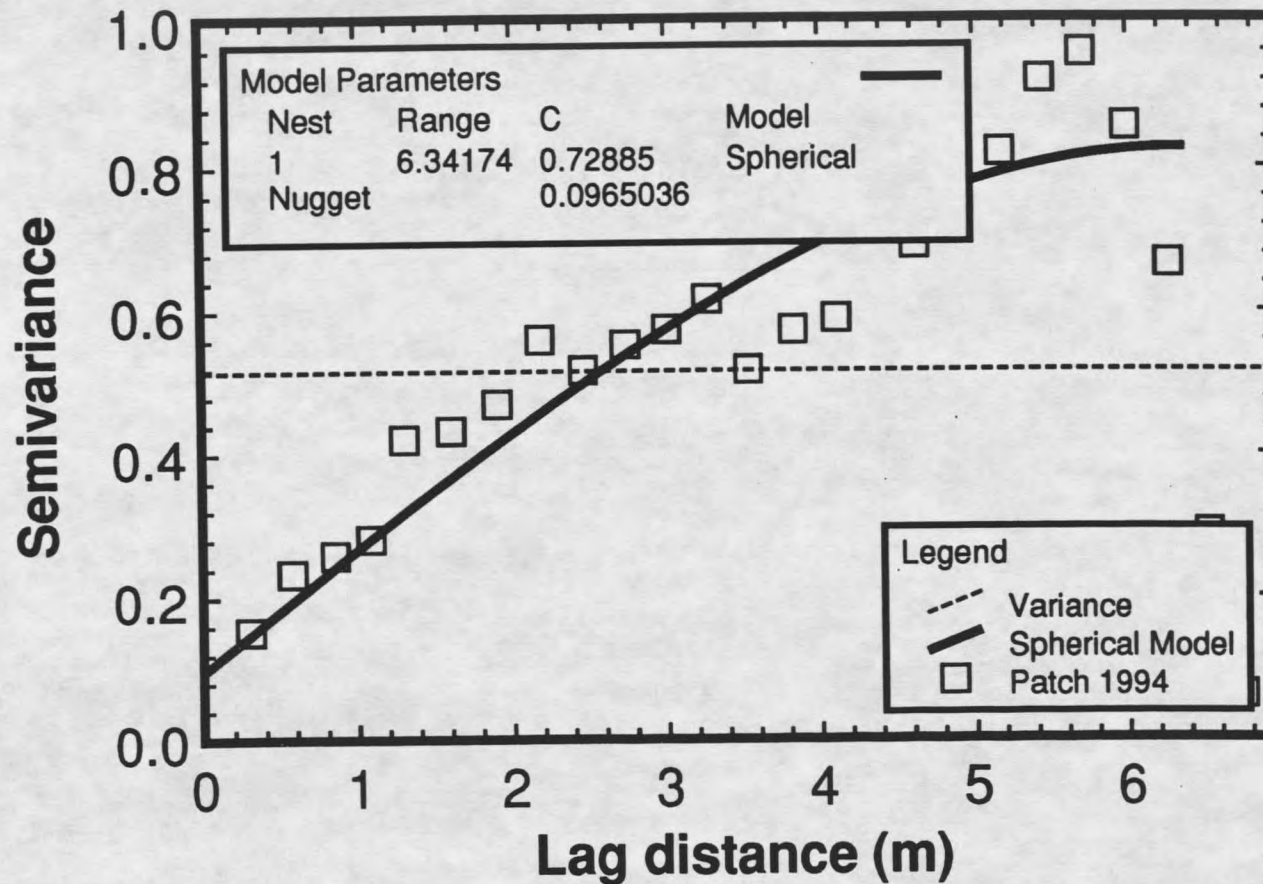


Figure C.1. Semivariogram of triallate-resistant wild oat density sampled at a patch scale at Site 1 in 1994.

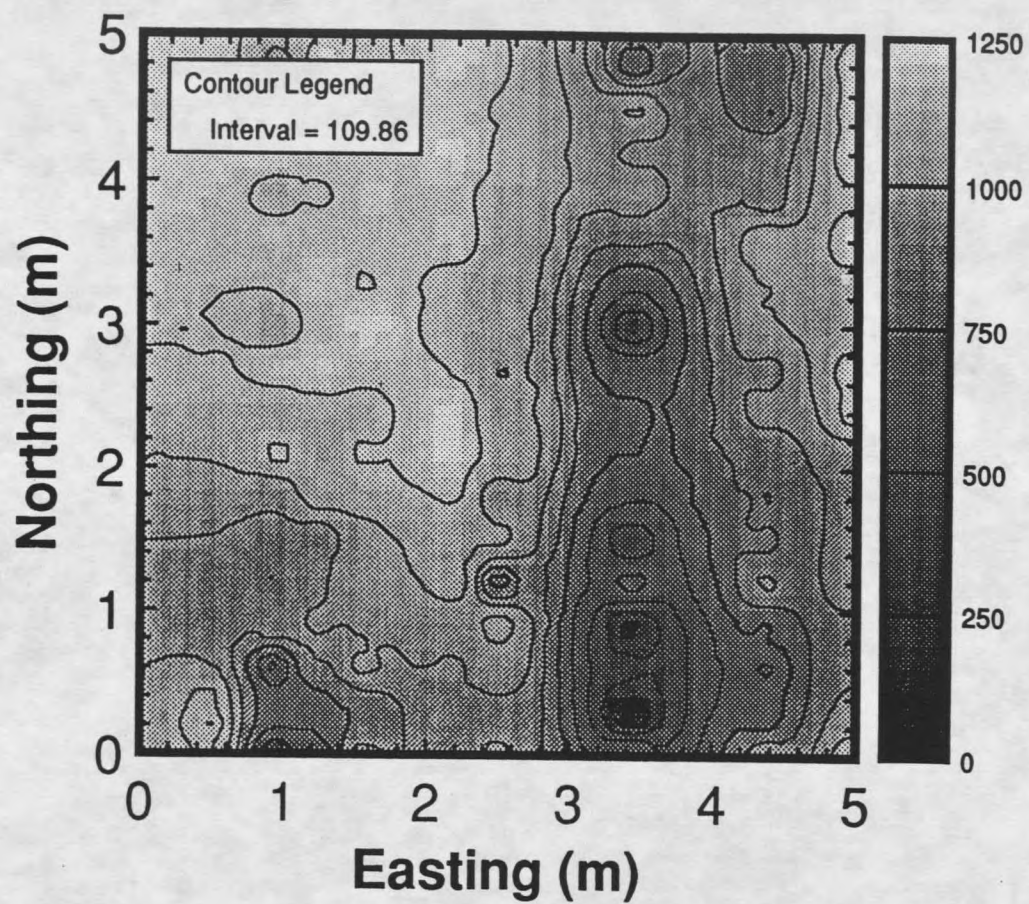


Figure C.2. Kriged map of density of wild oat sampled at a patch scale in 1994 at Site 1.

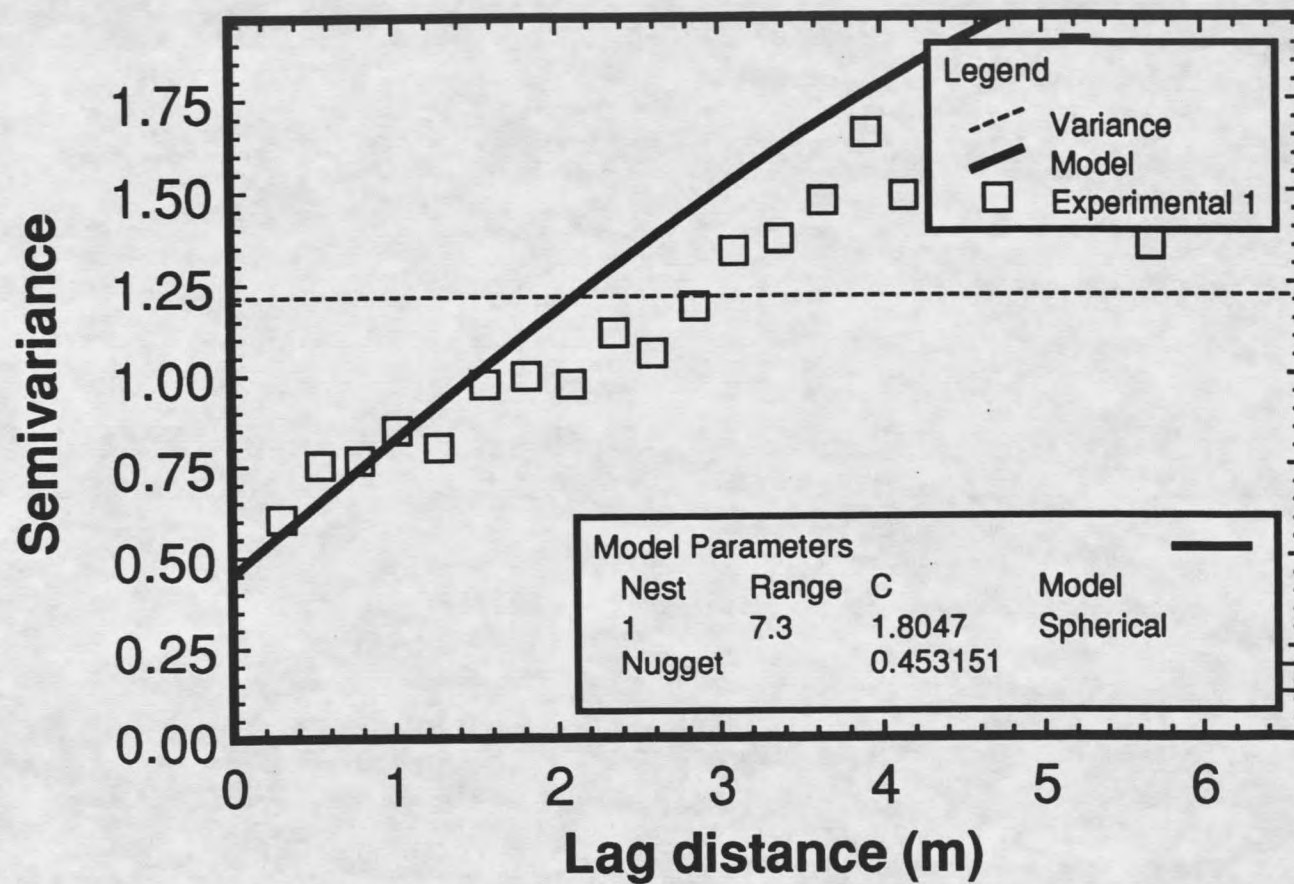


Figure C.3. Semivariogram of wild oat sampled at a patch scale in 1994 at Site 2.

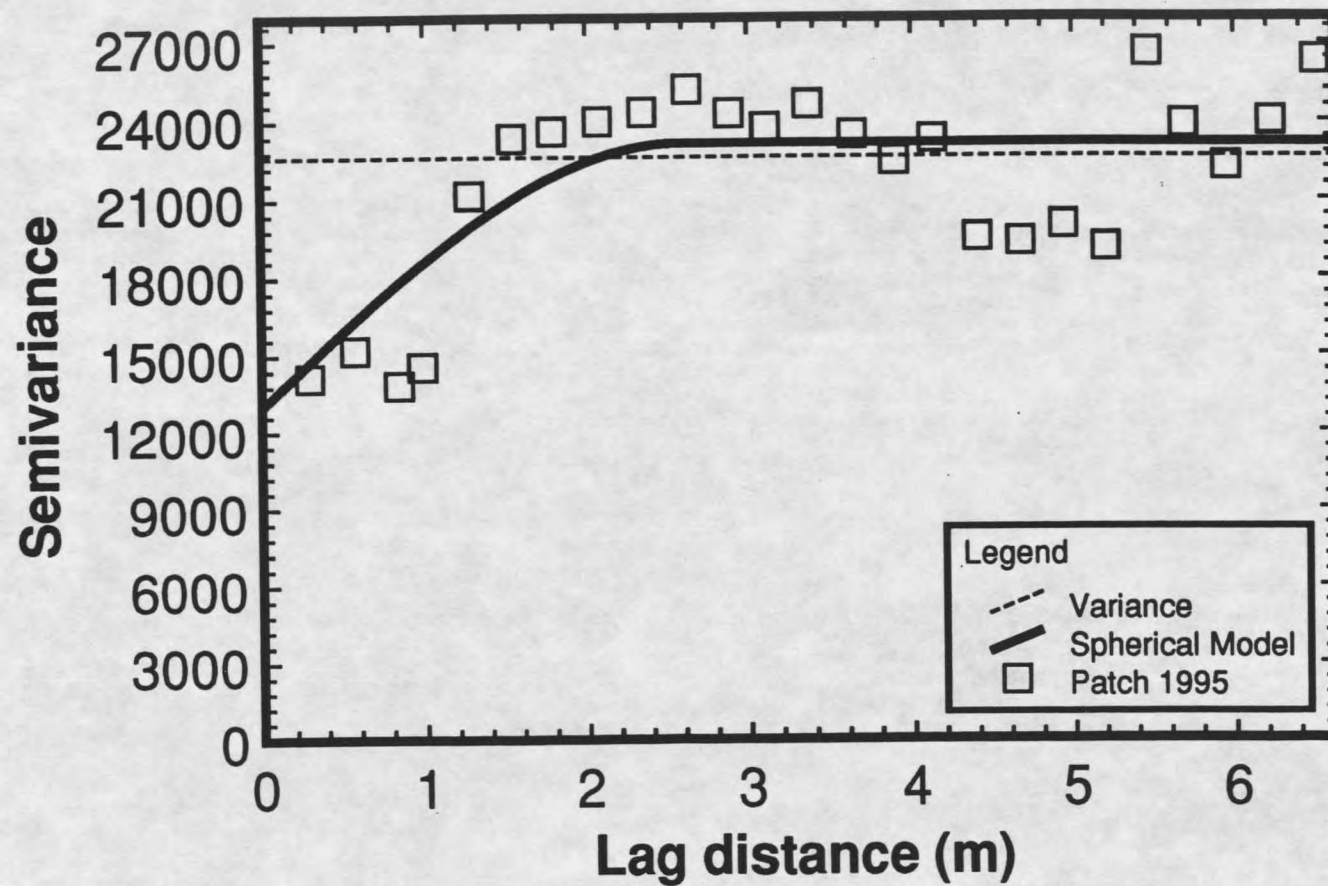


Figure C.4. Semivariogram of wild oat sampled at a patch scale in 1995 at Site 2.

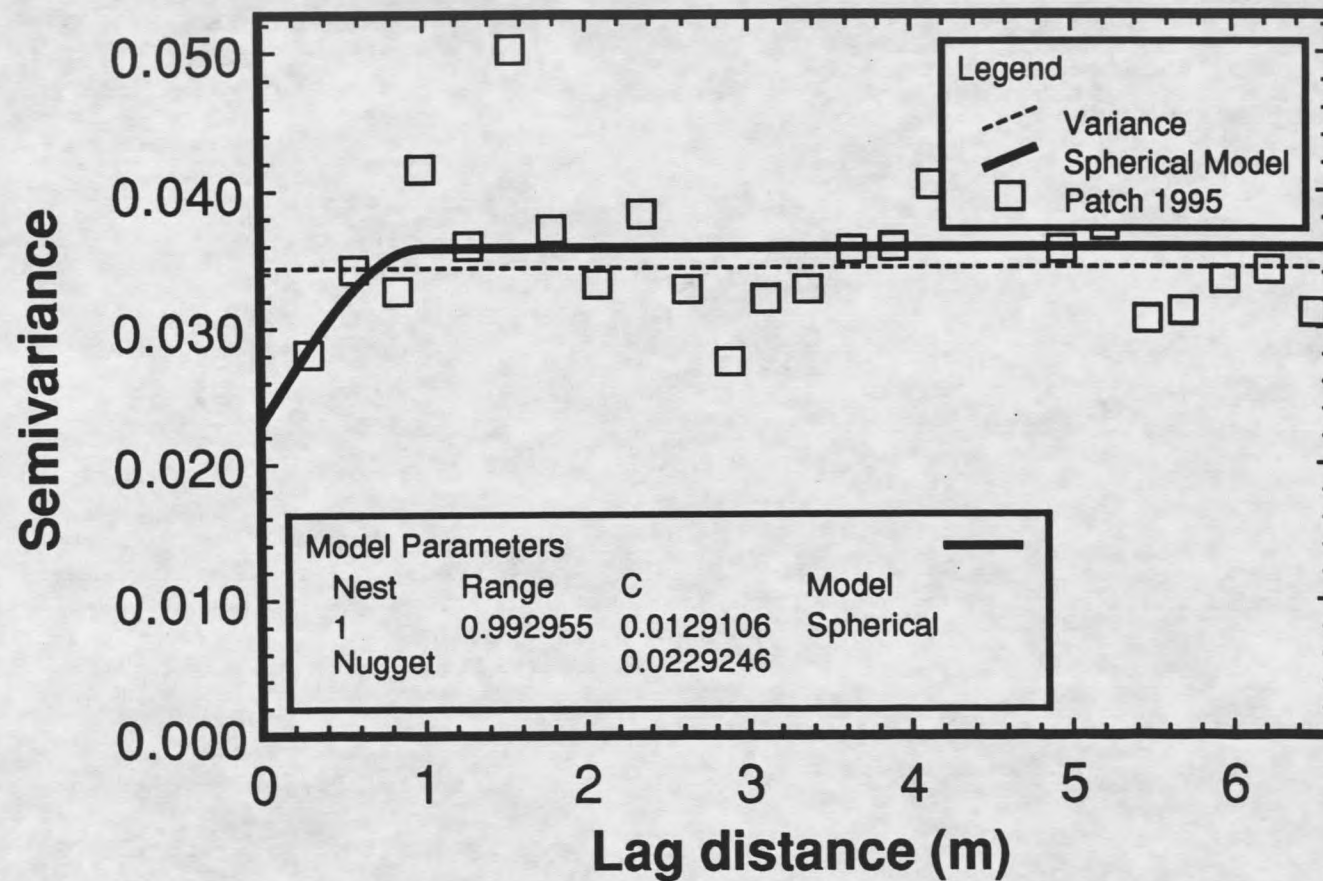


Figure C.5. Semivariogram of triallate resistance in wild oat sampled at a patch scale in 1995 at Site 2.

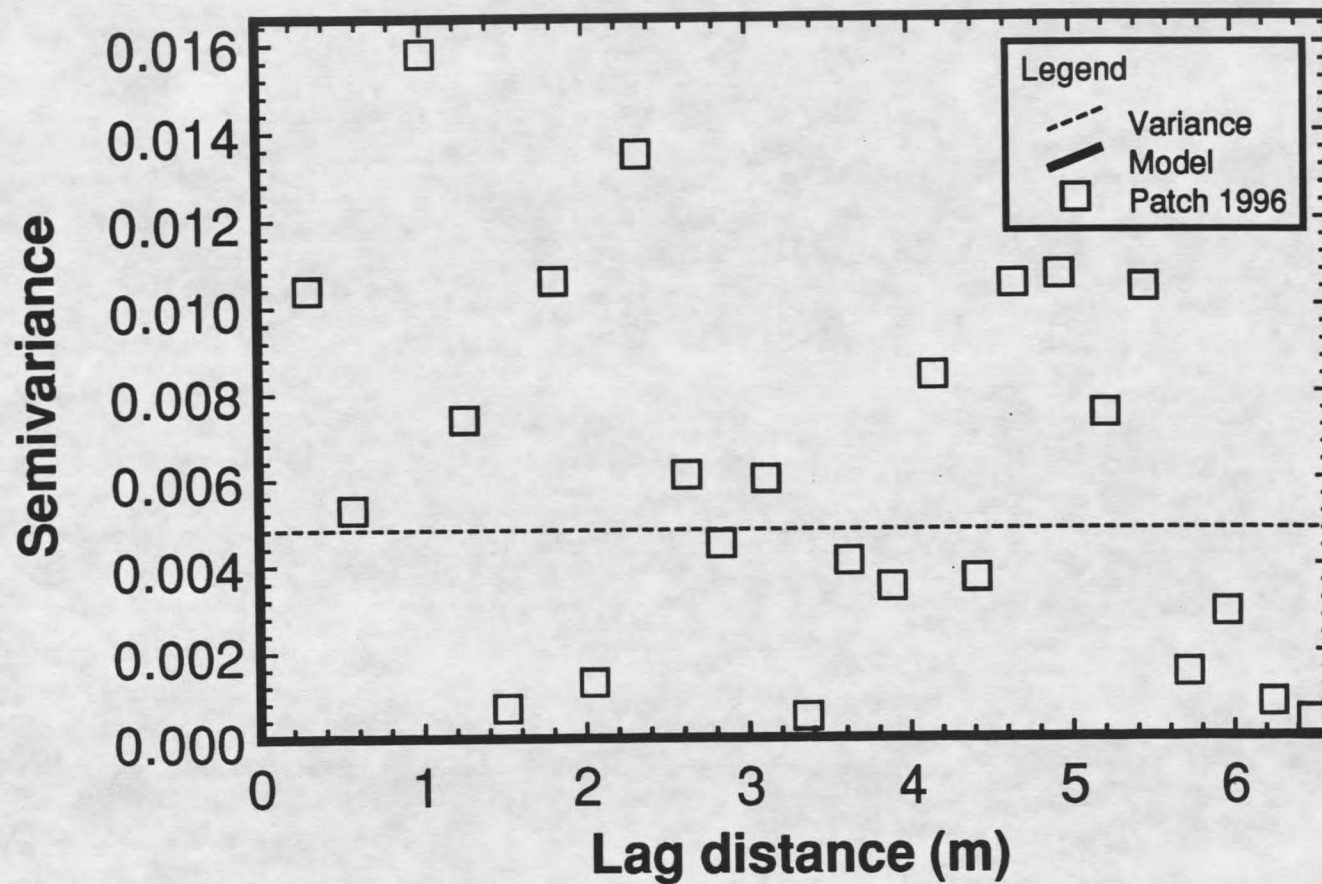


Figure C.6. Semivariogram of triallate resistance in wild oat sampled at a patch scale in 1996 at Site 2.

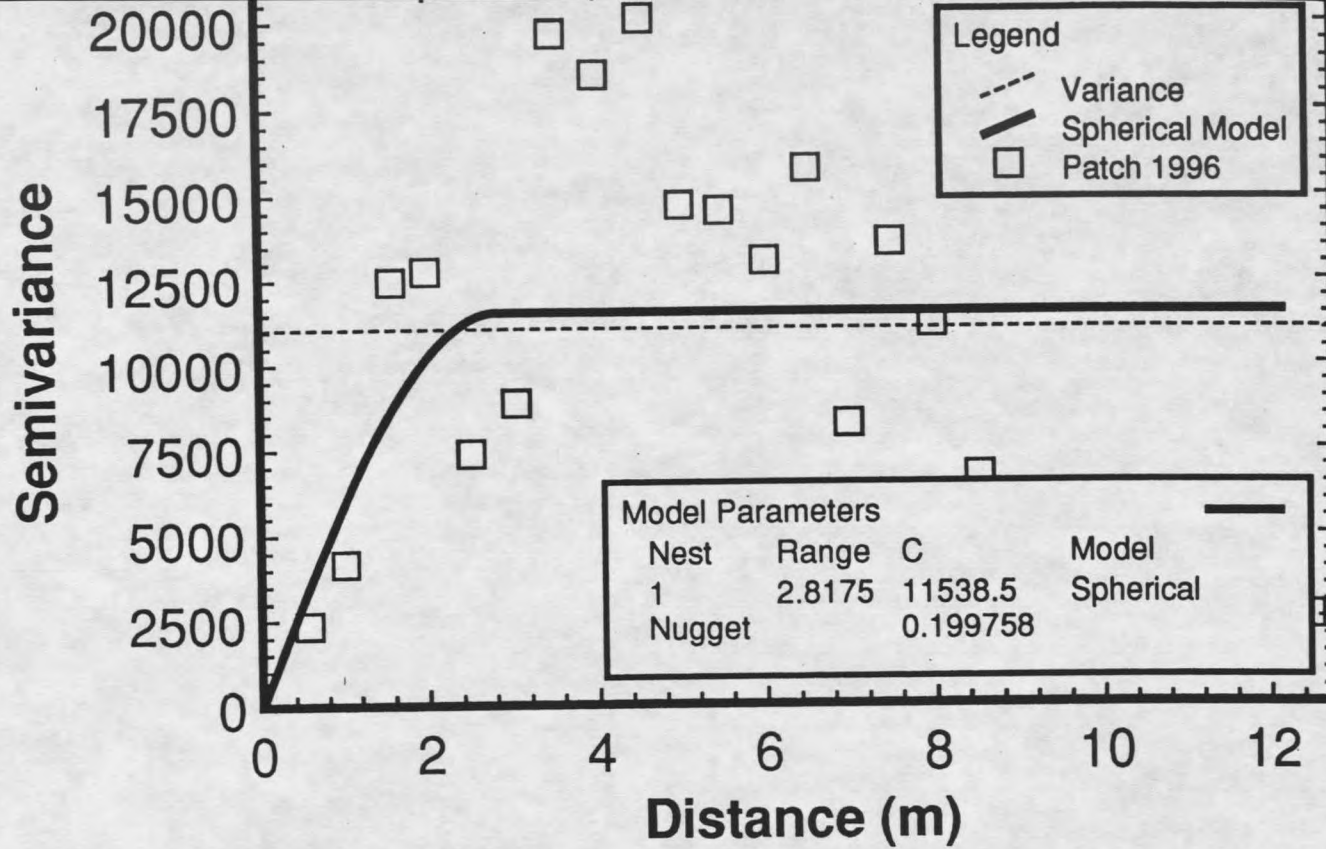


Figure C.7. Semivariogram of density of wild oat sampled at a patch scale in 1996 at Site 3.

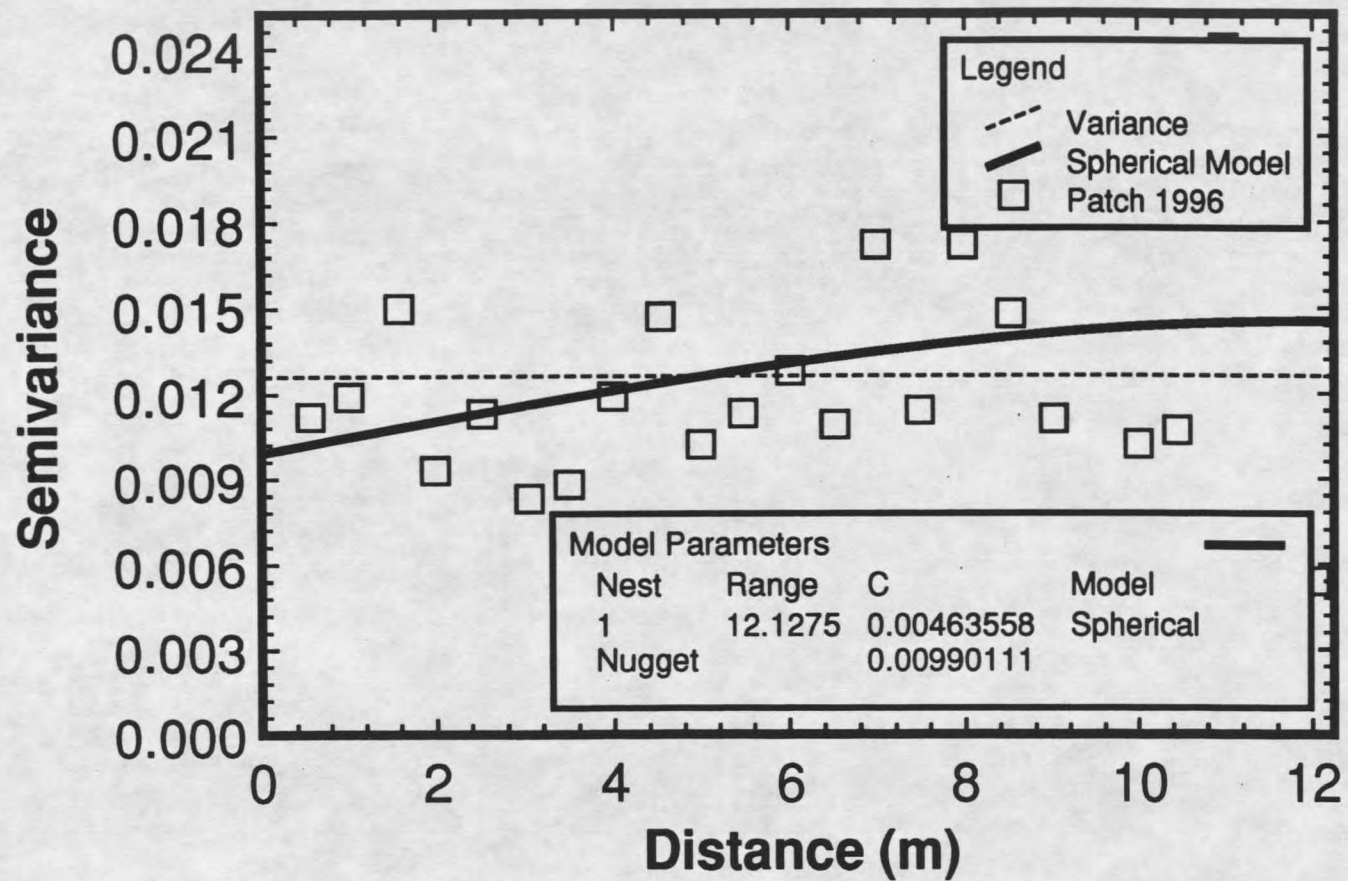


Figure C.8. Semivariogram of resistance in wild oat sampled at a patch scale in 1996 at Site 3.

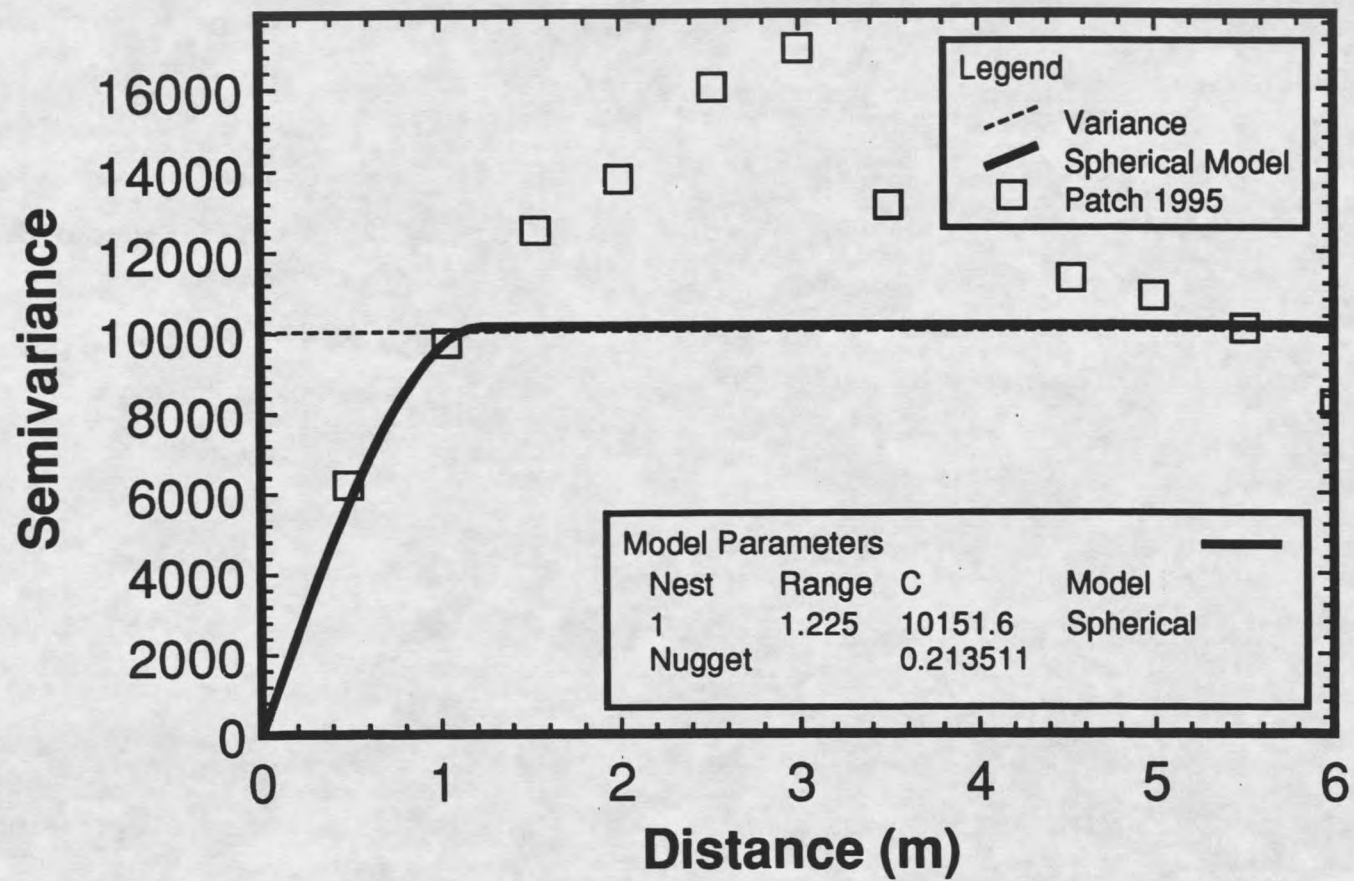


Figure C.9. Semivariogram of density of wild oat sampled at a patch scale in 1995 at Site 3.

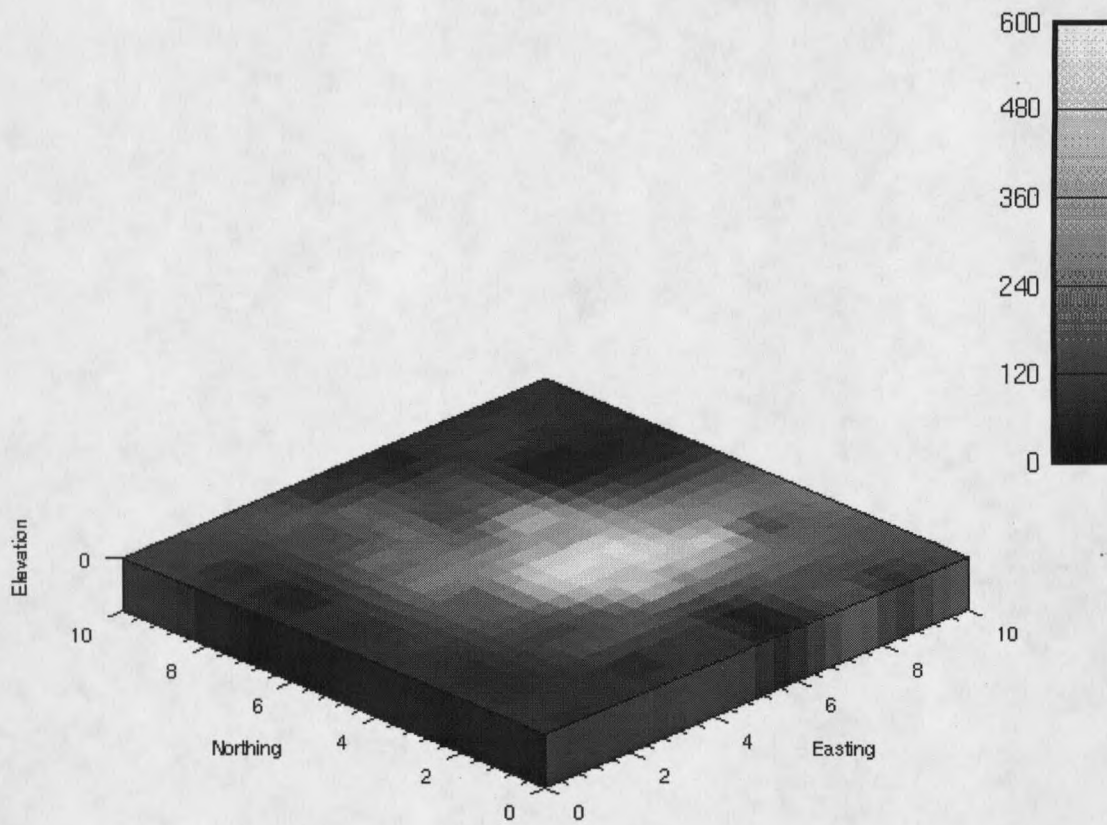


Figure C.10. Block contour map of isotropic analysis at a patch scale. Site 3 1995.

APPENDIX D - Java Class Files

Class SampleMenu

```

java.lang.Object
|
+----sunsoft.jws.visual.rt.base.AttributeManager
|
+----sunsoft.jws.visual.rt.base.Group
|
+----SampleMenu

```

```

public class SampleMenu
extends Group

```

Constructor Index

- **SampleMenu()**

All the attributes used by the group must be defined in the constructor.

Method Index

- **action**(Message, Event, Object)

- **createGroup()**

createGroup is called during group creation.

- **destroyGroup()**

destroyGroup is called during the destroy operation.

- **getOnGroup**(String)

"getOnGroup" may be overridden by sub-groups that store attribute values themselves, and do not depend on the group superclass to store them.

- **handleEvent**(Message, Event)

handleEvent may be overridden by subclasses that want to get notified when AWT events that are sent by the gui components.

- **handleMessage**(Message)

handleMessage may be overridden by subclasses that want to act on messages that are sent to the group.

- **hideGroup()**

hideGroup may be overridden by group subclasses that want to know when the group becomes non-visible.

- **initGroup()**

initGroup is called during initialization.

- **initRoot()**

initRoot must be overridden in group subclasses to initialize the shadow tree.

- **setOnGroup**(String, Object)

"setOnGroup" may be overridden by sub-groups that want notification when attributes are changed.

- **showGroup()**

showGroup may be overridden by group subclasses that want to know when the group becomes visible.

• **startGroup()**

This method may be overridden by group subclasses that want to be informed when the application is starting.

• **stopGroup()**

This method may be overridden by group subclasses that want to be informed when the application is stopping.

CONSTRUCTORS

• **SampleMenu**

```
public SampleMenu()
```

All the attributes used by the group must be defined in the constructor. setOnGroup is called at initialization for all the attributes. If the attribute has not been set prior to initialization, setOnGroup is called with the default value.

Methods

• **initRoot**

```
protected Root initRoot()
```

initRoot must be overridden in group subclasses to initialize the shadow tree. The return value must be the root of the newly initialized shadow tree.

Overrides:

initRoot in class Group

• **action**

```
public boolean action(Message msg,  
                      Event evt,  
                      Object arg)
```

Overrides:

action in class AttributeManager

• **initGroup**

```
protected void initGroup()
```

`initGroup` is called during initialization. It is called just after `initRoot` is called, but before the sub-groups are initialized and before the attributes are sent to the `setOnGroup` method. `initGroup` is only called once in the lifetime of the Group. This is because groups cannot be uninitialized. Anything that needs to be cleaned up should be created in `createGroup` instead of `initGroup`, and then can be cleaned up in `destroyGroup`. `createGroup` and `destroyGroup` may be called multiple times during the lifetime of a group.

Overrides:

`initGroup` in class Group

☉ **showGroup**

```
protected void showGroup()
```

`showGroup` may be overridden by group subclasses that want to know when the group becomes visible. It is called just before the group becomes visible. The group will already be initialized and created at this point.

Overrides:

`showGroup` in class Group

☉ **hideGroup**

```
protected void hideGroup()
```

`hideGroup` may be overridden by group subclasses that want to know when the group becomes non-visible. It is called just before the group becomes non-visible.

Overrides:

`hideGroup` in class Group

☉ **createGroup**

```
protected void createGroup()
```

`createGroup` is called during group creation. Groups can be created and destroyed multiple times during their lifetime. Anything that is created in `createGroup` should be cleaned up in `destroyGroup`. `createGroup` is called just after the group has been created. Anything that needs to be done before the group is created should be done in `initGroup`.

Overrides:

createGroup in class Group

☉ destroyGroup

`protected void destroyGroup()`

`destroyGroup` is called during the destroy operation. Groups can be created and destroyed multiple times during their lifetime. Anything that has been created in `createGroup` should be cleaned up in `destroyGroup`. `destroyGroup` is called just before the group is destroyed.

Overrides:

destroyGroup in class Group

☉ startGroup

`protected void startGroup()`

This method may be overridden by group subclasses that want to be informed when the application is starting. This method is only called after the entire application has been initialized and created. For applets, `startGroup` is called whenever `start` is called on the applet.

Overrides:

startGroup in class Group

☉ stopGroup

`protected void stopGroup()`

This method may be overridden by group subclasses that want to be informed when the application is stopping. This method will be called before a destroy is done. For applets, `stopGroup` is called whenever `stop` is called on the applet.

Overrides:

stopGroup in class Group

☉ getOnGroup

```
protected Object getOnGroup(String key)
```

"getOnGroup" may be overridden by sub-groups that store attribute values themselves, and do not depend on the group superclass to store them. This method should be overridden instead of "get". Any attributes handled in setOnGroup where super.setOnGroup is not called must also be handled in getOnGroup. The default implementation of getOnGroup retrieves the value from the attribute table. The reason that "getOnGroup" should be overridden instead of "get" is that "getOnGroup" is guaranteed not to be called until the group class is initialized. This means that initRoot will always be called before any calls to getOnGroup are made. Also, this method is only for attributes that are defined in the sub-groups. It is not called for forwarded attributes.

Overrides:

getOnGroup in class Group

⊗ setOnGroup

```
protected void setOnGroup(String key,
                          Object value)
```

"setOnGroup" may be overridden by sub-groups that want notification when attributes are changed. This method should be overridden instead of "set". Any attributes handled in setOnGroup where super.setOnGroup is not called must also be handled in getOnGroup. The default implementation of setOnGroup puts the value in the attribute table. The reason that "setOnGroup" should be overridden instead of "set" is that "setOnGroup" is guaranteed not to be called until the group class is initialized. This means that initRoot will always be called before any calls to setOnGroup are made. During initialization, "setOnGroup" will be called for all the group's attributes even if they have not be changed from the default value. But for attributes that have the DEFAULT flag set, "setOnGroup" will only be called if the value of the attribute has changed from the default. Also, this method is only called when attributes defined in the sub-groups are updated. It is not called for forwarded attributes.

Overrides:

setOnGroup in class Group

⊗ handleMessage

```
public boolean handleMessage(Message msg)
```

handleMessage may be overridden by subclasses that want to act on messages

that are sent to the group. Typically, messages are either AWT events that have been translated to messages, or they are messages that have been sent by other groups. `super.handleMessage` should be called for any messages that aren't handled. If `super.handleMessage` is not called, then `handleEvent` will not be called. The default implementation of `handleMessage` returns "true". This means that no events will be passed up the group tree, unless a subclass overrides this method to return "false". AWT events are not propagated regardless of the return value from `handleEvent`. If you want a message to go to the parent group, override `handleMessage` to return false for that message. If you want an AWT event to go to the parent group, you need to call `postMessageToParent()` with the event message.

Overrides:

`handleMessage` in class Group

● `handleEvent`

```
public boolean handleEvent(Message msg,
                          Event evt)
```

`handleEvent` may be overridden by subclasses that want to get notified when AWT events that are sent by the gui components. The return value should be true for handled events, and `super.handleEvent` should be called for unhandled events. If `super.handleEvent` is not called, then the specific event handling methods will not be called. The message's target is set to the shadow that sent the event. The event's target is set to the AWT component that sent the event. The following specific event handling methods may also be overridden: `public boolean mouseDown(Message msg, Event evt, int x, int y)`; `public boolean mouseDrag(Message msg, Event evt, int x, int y)`; `public boolean mouseUp(Message msg, Event evt, int x, int y)`; `public boolean mouseMove(Message msg, Event evt, int x, int y)`; `public boolean mouseEnter(Message msg, Event evt, int x, int y)`; `public boolean mouseExit(Message msg, Event evt, int x, int y)`; `public boolean keyDown(Message msg, Event evt, int key)`; `public boolean keyUp(Message msg, Event evt, int key)`; `public boolean action(Message msg, Event evt, Object what)`; `public boolean gotFocus(Message msg, Event evt, Object what)`; `public boolean lostFocus(Message msg, Event evt, Object what)`;

Overrides:

`handleEvent` in class Group

Class Researcher

```
java.lang.Object
|
+-----Researcher
```

```
public class Researcher
```

```
extends Object
```

Researcher - Designs and issues instructions for sampling fields with different methods, sample sizes, sample numbers and repetitions

Constructor Index

- **Researcher()**

Constructor.

Method Index

- **main(String[])**

- **runSimulation()**

The simulation is controled by the GUI no options are passed to the simulation directly.

CONSTRUCTORS

- **Researcher**

```
public Researcher()
```

Constructor.

Methods

- **runSimulation**

```
public void runSimulation()
```

The simulation is controled by the GUI no options are passed to the simulation directly. The method updateParameters is called and updates GUI information prior to the simulation run

- **main**

```
public static void main(String args[])
```

Class ParameterSet

```
java.lang.Object
|
+-----ParameterSet
```

```
public class ParameterSet
extends Object
```

Constructor Index

• **ParameterSet()**

Constructor.

Method Index

• **getField(int)**

• **getFieldSize(int)**

• **getQuadratSize(int)**

• **getSampleMethod(int)**

• **getSampleNumber(int)**

• **initializeDefaultValues()**

• **initializeFields()**

• **initializeFieldSize()**

• **initializeQuadratSizes()**

• **initializeSampleMethods()**

• **initializeSampleNumbers()**

• **main(String[])**

• **readFile(String)**

Read the parameters from a file

• **readParameters()**

Retrieves the parameters from a file stored on the server

• **saveParameters()**

Write the parameters to a file

• **updateParameters()**

Updates the parameters from the screen (GUI)

CONSTRUCTORS

• **ParameterSet**

```
public ParameterSet()
```

Constructor.

Methods

• **updateParameters**

```
public void updateParameters()
```

Updates the parameters from the screen (GUI)

• **readParameters**

```
public void readParameters()
```

Retrieves the parameters from a file stored on the server

• **saveParameters**

```
public void saveParameters()
```

Write the parameters to a file

• **readFile**

```
public void readFile(String fileName)
```

Read the parameters from a file

• **initializeDefaultValues**

```
public void initializeDefaultValues()
```

• **initializeQuadratSizes**

```
public void initializeQuadratSizes()
```

• **initializeSampleNumbers**

```
public void initializeSampleNumbers()
```

⊙ **initializeSampleMethods**

```
public void initializeSampleMethods()
```

⊙ **initializeFieldSize**

```
public void initializeFieldSize()
```

⊙ **initializeFields**

```
public void initializeFields()
```

⊙ **getField**

```
public String getField(int fieldNumber)
```

⊙ **getFieldSize**

```
public double getFieldSize(int options)
```

⊙ **getQuadratSize**

```
public double getQuadratSize(int options)
```

⊙ **getSampleNumber**

```
public int getSampleNumber(int options)
```

⊙ **getSampleMethod**

```
public int getSampleMethod(int options)
```

⊙ **main**

```
public static void main(String args[])
```

Class Field

```
java.lang.Object
```

```
  |  
  +-----Field
```

```
public class Field
```

```
extends Object
```

Constructor Index

```
• Field(String)
```

Constructor.

Method Index

```
• getFieldArea()
```

```
• getFieldLength()
```

```
• getFieldName()
```

```
• getFieldWidth()
```

```
• getResistance(int, int)
```

```
• main(String[])
```

Constructors

```
• Field
```

```
public Field(String field)
```

Constructor.

Methods

```
• getFieldLength
```

```
public int getFieldLength()
```

```
• getFieldWidth
```

```
public int getFieldWidth()
```

⊙ **getFieldArea**

```
public double getFieldArea()
```

⊙ **getResistance**

```
public double getResistance(int xvalue,  
                             int yvalue)
```

⊙ **getFieldName**

```
public String getFieldName()
```

⊙ **main**

```
public static void main(String args[])
```

Class Statistician

```
java.lang.Object
|
+----Statistician
```

```
public class Statistician
```

```
extends Object
```

```
Statistician - basic statistical analysis
```

Constructor Index

• Statistician()

Constructor.

Method Index

• calcCofV(double[])

Coefficient of Variation - relative dispersion.

• calcMean(double[])

Calculates the mean of all values in the array

• calcStdDeviation(double[])

Calculates the standard deviation and the mean of a double array

• getMean()

Returns the mean of a previously calculated sample

• getStd()

Returns the standard deviation of a previously calculated sample

• printSample(double[])

Prints an of doubles array

CONSTRUCTORS

• Statistician

```
public Statistician()
```

Constructor.

Methods

• calcMean

```
public double calcMean(double array[])
```

Calculates the mean of all values in the array

☉ calcStdDeviation

```
public double calcStdDeviation(double array[])
```

Calculates the standard deviation and the mean of a double array

☉ calcCofV

```
public double calcCofV(double array[])
```

Coefficient of Variation - relative dispersion. In order to obtain the CoV - the standard deviation and mean of the array are also calculated

☉ getMean

```
public double getMean()
```

Returns the mean of a previously calculated sample

☉ getStd

```
public double getStd()
```

Returns the standard deviation of a previously calculated sample

☉ printSample

```
public void printSample(double array[])
```

Prints an of doubles array

Class Sampler

```
java.lang.Object
|
+----Sampler
```

```
public class Sampler
```

```
extends Object
```

Sampler is responsible for collecting sample information from a single field of fixed size. Multiple simulated bulk samples can be collected with different methods and different sample numbers. Quadrat size must be changed with the changeQuad method

Constructor Index

- Sampler()

- Sampler(String, int)

Method Index

- adaptiveSample()

- main(String[])

- sampleField(int, int)

Method to obtain a bulk sample from one field using a specified sampling method

Constructors

- **Sampler**

```
public Sampler()
```

- **Sampler**

```
public Sampler(String field,
               int howManySamples)
```

Methods

- **sampleField**

```
public void sampleField(int method,
                       int samplesPerBulkSample)
```

Method to obtain a bulk sample from one field using a specified sampling method

• **adaptiveSample**

```
public void adaptiveSample()
```

• **main**

```
public static void main(String args[])
```

Class Sample

```
java.lang.Object
```

```
|
+----Sample
```

```
public class Sample
```

```
extends Object
```

```
At each sample point a measure of resistance is calculated
```

Constructor Index

```
• Sample(String, String, String, int)
```

```
Constructor.
```

Method Index

```
• addSample(int, int, double)
```

```
Adds a sample to bag (Bulk Sample)
```

```
• outPutSample()
```

```
Prints to screen the sample information
```

```
• writeSample(PrintStream)
```

```
Saves sample information to file
```

CONSTRUCTORS

```
• Sample
```

```
public Sample(String field,
               String measurement,
               String method,
               int numberOfSamples)
```

```
Constructor.
```

Methods

```
• addSample
```

```
public void addSample(int xLocation,
                      int yLocation,
                      double measurementValue)
```

```
Adds a sample to bag (Bulk Sample)
```

```
• writeSample
```

```
public void writeSample(PrintStream os) throws IOException
```

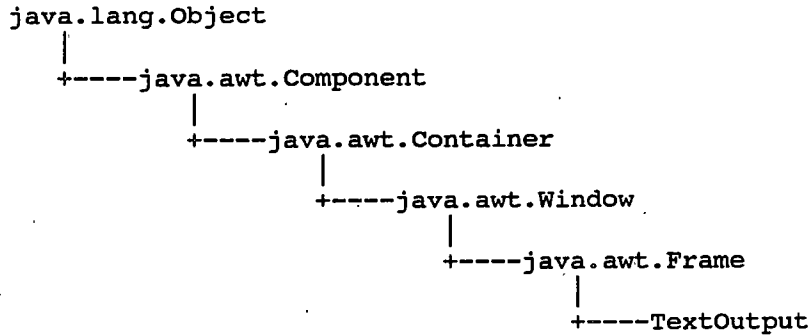
Saves sample information to file

● **outPutSample**

```
public void outPutSample()
```

Prints to screen the sample information

Class TextOutput



```
public class TextOutput
```

```
extends Frame
```

Constructor Index

- **TextOutput**(String)

Method Index

- **action**(Event, Object)

- **handleEvent**(Event)

- **main**(String[])

Constructors

- **TextOutput**

```
public TextOutput(String label)
```

Methods

- **handleEvent**

```
public boolean handleEvent(Event evt)
```

Overrides:

handleEvent in class Component

⊙ action

```
public boolean action(Event evt,  
                      Object arg)
```

Overrides:

action in class Component

⊙ main

```
public static void main(String args[])
```

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