ACCASE INHIBITOR RESISTANT WILD OAT IN MANITOBA: PREDICTION, IDENTIFICATION, AND CHARACTERIZATION

BY

LUC BOURGEOIS

A Thesis
Submitted to the Faculty of Graduate Studies
in partial Fulfilment of the Requirements
for the Degree of

Doctor of Philosophy

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ABSTRACT

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ACCase Inhibitor Resistant Wild Oat in Manitoba: Prediction, Identification, and Characterization

Supervisor: Dr. Ian N. Morrison, Department of Plant Science.

High use of aryloxyphenoxypropionate (APP) and cyclohexanedione (CHD), collectively referred to as acetyl coenzyme A carboxylase (ACCase) inhibiting or Group 1 herbicides, has led to the selection of resistant wild oat (*Avena fatua*) populations in some regions of Manitoba. The first objective of this thesis was to determine areas of Manitoba at low, medium, and high risk of developing Group 1 resistance. This objective was achieved by using data included in the Manitoba Crop Insurance Corporation (MCIC) database. Low, medium, and high risk areas were those in which Group 1 herbicides were sprayed on less than 30%, 30 to 50%, and over 50% of the sprayed fields, respectively. Results showed that Group 1 herbicide use increased from 15 to 50% of the sprayed fields between 1981 and 1993. Almost 40% of the townships were at high risk between 1989 and 1993. Wild oat seeds were collected in fields from different risk areas with the second objective of determining the proportion of fields infested with resistant wild oat. In a high risk township, resistant wild oat occurred in 20 out of 30 surveyed fields. In general, Group 1 resistant wild oat were more common in high risk townships than in medium or low risk townships. Finally, the third objective was to characterize resistant wild oat lines according to cross-resistance patterns and to determine which one, if any, is the most common. Cross-resistance levels were based on seed-bioassay, and more specifically on the coleoptile length. Three types of cross-resistance were established. The first type included wild oat lines with high levels of resistance to the APP and CHD chemical families. The second type included lines with low levels of resistance to both chemical families, while the third type included lines with high levels of resistance to APP's but almost no resistance to CHD's. More than one type was found in some fields which indicates independent selection of several mutants within a field. Overall wild oat types with high levels of resistance to both chemical families were the most common.

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This thesis has been written in manuscript style. All manuscripts were prepared in accordance with the style requirements of Canadian Journal of Plant Science. However, a subnumbering system was used for main section headings and subsection headings.

1. INTRODUCTION

Acetyl coenzyme A carboxylase (ACCase) inhibitors are post-emergence herbicides used to control grassy weeds in cereals and broadleaf crops. These herbicides are also referred to as Group 1 herbicides and include the aryloxy-phenoxypropionate (APP) and cyclohexanedione (CHD) chemical families. Despite substantial differences between these two chemical families, there is evidence that APP and CHD herbicides bind in the same region of the ACCase enzyme (Rendina et al. 1991) and share a common mechanism of action (Devine and Shimabukuro 1994).

In 1973, diclofop, an APP herbicide, was the first Group 1 herbicide to be registered in western Canada (Morrison et al. 1992). Ever since, farmers have relied increasingly on Group 1 herbicides, mainly because they controlled both wild oat (*Avena sativa* L.) and green foxtail (*Setaria viridis* L.), and because of a reduction in use of soil applied herbicides (Goodwin 1994). In 1996 Manitoba farmers had the choice of eight Group 1 active ingredients, available in 14 different commercial formulations[1]. However, the heavy reliance on Group 1 herbicides has led to the selection of herbicide resistant wild oat and green foxtail in Manitoba. In 1990, Heap et al. (1993) identified the first Group 1 resistant wild oat in north-western Manitoba. Over the next three years, more than one hundred additional resistant populations were identified (Morrison and Devine 1993). Group 1 resistant green foxtail was identified in Manitoba in 1991 (Heap and Morrison 1996)

Since 1989, weed scientists around the world have reported Group 1 herbicide resistance in weeds such as Italian ryegrass (*Lolium multiflorum* Lam.) (Stanger and Appleby 1989), blackgrass (*Alopecurus myosuroides* L.) (Moss

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[1] Guide to Crop Protection, Manitoba Agriculture Box 1149, Carman MB R0G 0J0.

1990), oat species (*Avena* spp.) (Mansooji et al. 1992; Seefeldt et al. 1994), goosegrass (*Eleusine indica* L.) (Marshall et al. 1994), large crabgrass (*Digitaria sanguinalis* L.) (Wiederholt and Stoltenberg 1995), and giant foxtail (*Setaria.faberi* Herrm.) (Stoltenberg and Wiederholt 1995). In all instances development of Group 1 herbicide resistance was linked to the heavy use of APP and CHD herbicides in the previous 4 to 10 years.

However, differences in cross-resistance patterns and resistance levels characterize ACCase resistant weeds. In wild oat, resistance to a specific Group 1 herbicide can vary from 2- to 3000-fold between populations (Heap et al 1993, Seefeldt et al. 1994). While some populations are resistant to all Group 1 herbicides to some degree, others are resistant only to APP herbicides (Heap et al. 1993). In maize, differences in cross-resistance patterns were related to different mutations of the ACCase enzyme (Marshall et al. 1992). Genetic studies also indicated that two alleles at the same gene locus induced two different cross-resistance patterns in wild oat (Murray et al. 1995).

Based on herbicide use history data collected by the Manitoba Crop Insurance Corporation (MCIC), the first objective of this study was to determine risk areas of Group 1 resistance development based on Group 1 herbicide use frequency. The MCIC database is unique in that herbicide use history can be established as far back as 1981. As a direct follow-up, wild oat seeds were collected in fields in townships rated as being either at high, medium and low risk of Group 1 herbicide resistance development. The purpose of this collection was to determine the occurence of Group 1 herbicide resistant wild oat in relation to herbicide use histories. The diverse origin of the resistant wild oat collected in the survey provided a base for the study of cross-resistance patterns to Group 1 herbicides in wild oat. Samples with similar cross-resistance patterns may or may not originate from a common source while samples with discrete cross-resistance patterns have built up from different mutations.

The rapid emergence of herbicide resistant weeds in the 1990's has brought to light weaknesses in our understanding of evolutionary processes in weed populations. Consequently, researchers have focused on studying weed resistance “after the fact”. The overall objective of this study is to enhance knowledge on the development and characterization of Group 1 resistant wild oat. Furthermore, weed resistance risk maps are drawn to enhance practices of proactive weed resistance management in those areas at greatest risk.

2. LITERATURE REVIEW

2.1. Introduction

This chapter reviews the literature relevant to the objectives of the study. It is divided into four main sections. The first one provides an overview of techniques to measure the physical characteristics of a weed population. Mapping techniques are also discussed as they relate to new applications of geographic information systems used to study evolution of weed populations. The second section relates to techniques used in the measure of genetic variation within and among weed populations. This section focuses only on research relating directly to *Avena* species. The third section describes the fate of genes in a population. This section provides an overview of the role of genetic variation in the survival of weed species under a strong selection pressure. Finally, the last section provides an overview of genetic modifications of weed population caused by management techniques. Emphasis is placed on the role of herbicides in the evolution of herbicide resistant weeds.

2.2. Sampling and mapping weed populations

2.2.1. Definition of a population

"A population is a collection of individuals belonging to the same species, living in the same area at the same time" (Silvertown and Lovett-Doust 1993). Examples of populations provided by Silvertown and Lovett-Doust (1993) are water hyacinth, *Eichhornia crassipes*, in a ditch, perennial ryegrass, *Lolium perenne*, in a lawn, and Norway spruce, *Picea abies*, in a forest. These examples illustrate that each population is studied within a defined area and has little interaction with populations outside of that area. Therefore, a weed species in a field can be considered a population since all individuals share the same space at the same time. Isolated groups of individuals within a field are referred to as sub-populations. Rai (1985) sampled sub-populations of *Avena barbata* in orchards where inter-row cultivation created artificial "islands" of weeds. Each island constituted a sub-population of *Avena barbata* because they grew within the same field but were isolated by distance.

The genetic variation within a population is dependent on the population gene pool, with the frequency of specific genes highly variable over space and time (Primack and Kang 1989). Evolution of a population is driven by the extent of initial genetic variability, reproductive biology of the species, gene flow, and selective forces (May and Dobson 1986).

The definition of a population also indicates that individuals share the same space at the same time (Silvertown and Lovett-Doust 1993). In the case of plants, individuals compete for space, water, light, and nutrients, when these resources are in short supply (Primack and Kang 1989). The degree of competition will depend on the density and distribution of individuals from the same population and with individuals from other populations (Silvertown and Lovett-Doust 1993).

Density is the number of plants per unit area expressed as the number of plants per hectare, per meter, or per pot (Silvertown and Lovett-Doust 1993). Distribution characterizes the geographic location of individuals within the space occupied by the population. Distribution can be random, where individuals occupy the entire space in no specific arrangement. The presence of the weed in one area of the field provides no clue as to the presence of the weed in another area of the field (Wiles et al. 1992b). Distribution can also be uniform, meaning the distance between individuals is equal over the entire area. Uniform distribution seldomly occurs in weeds due to the nature of their dispersal mechanisms (Van Groenendael 1988). Finally, a clumped or patchy distribution is characterized by areas in a field with a high density of plants and other areas with a low density. In a clumped distribution, the presence of one individual increases the likelihood of finding another individual in the proximity of the first one (Van Groenendael 1988; Wiles et al. 1992c).

Weed distribution is often assumed to be either random or uniform because of the difficulty in measuring clumped weed distribution (Van Groenendael 1988; Hughes 1990; Wiles et al. 1992c). This assumption often results in an overestimation of weed presence since weeds are generally distributed in patches (Johnson et al. 1995). The patchy distribution of weeds is related to the fact that the majority of offspring of plants are spread within meters of the parental plants (Howard et al. 1991; Levin and Kerster 1994). Patchy distribution is common not only for species relying on vegetative reproduction such as Canada thistle, *Cirsium arvense* (Donald 1994) and field bindweed, *Convolvulus arvense* (Duncan and Weller 1987), but also for annual broadleaf (Wiles et al. 1992a; Johnson et al. 1995) and grassy (Marshall 1996; Johnson et al. 1995) weeds.

2.2.2. Measuring density and distribution of weed populations

Density and distribution of weed populations are measured by inference from values obtained from population samples (Cochran 1977). The accuracy of the density and distribution estimates will depend on the scale of sampling and the sampling method (Kenkel et al. 1989). The scale of sampling refers to the number of samples per unit area. In general, an increase in the number of samples augments the accuracy of the estimate (Marshall 1996). According to

Marshall (1996), at least 18 samples ha^{-1} of 1 m^2 each are required to establish precise density and distribution estimates for grassy weeds in a 11.5 ha cereal field. Such a sampling intensity resulted in the collection of 205 samples in 8.6 hours. Clearly, a sampling intensity of 18 samples ha^{-1} is not practical for large fields. Therefore, the scale of sampling is also related to the time, money, and manpower available (Kenkel et al. 1989; Marshall 1996).

Common methods of sampling weeds in fields include random and systematic sampling. Random sampling is preferred to other methods, since statistical inference should always be based on estimates from a random sample of the population (Cochran 1977). This method consists of selecting samples at random locations. The major limitation of random sampling is the difficulty of locating the sampling locations in the field. Systematic sampling simplifies the collection procedure since samples are taken at regular intervals. When choosing a systematic sampling method, it is important to have general knowledge of the population to avoid biased inference (Cochran 1977).

Sampling parameters such as sample size and number should be dictated by the experimental objectives (Kenkel et al. 1989). Transect methods are commonly used in surveys of large areas (Jacobsohn and Andersen 1968; Phillipson 1974; Elliott et al. 1979). Transect methods consist of sampling any individual along a predetermined path (Phillipson 1974; Elliott et al. 1979). Sampling at random locations (Jacobsohn and Andersen 1968) or at regular intervals (Thomas and Wise 1988) along a predetermined path can also be considered as transect methods. This method was used to survey blackgrass *Alopecurus myosuroides* and wild oat *Avena spp* in England. The procedure consisted of walking along a square with 120 pace sides, and counting the number of weeds in a 2 pace wide swath around the square (Phillipson 1974;

Elliott et al. 1979). The survey method used by Thomas and Wise (1988) consisted of sampling at regular intervals (20 paces) along a W shape pattern. Both methods provide a reasonable likelihood of a weed being recorded if present, but does not prove the absence of a weed (Elliott et al. 1979). Therefore, surveys such as these often underestimate the number of weeds present. However, transect methods provide a compromise between the probability of recording the presence of a weed in a field and the convenience of collecting a comparatively small number of samples (Elliott et al. 1979).

Where fields are sampled to determine weed distribution, a large number of samples are required. As well, the sampling pattern must cover the entire field area (Marshall 1996). In such cases, fields are almost always sampled along a grid pattern with regular distances between samples (Johnson et al. 1995; Eberlein et al. 1992; Marshall 1996). The results are greatly affected by the number of sample sites (Marshall 1996). Grid sampling proved to be an excellent tool to develop weed control strategies only in areas of the fields with high weed densities (Johnson et al. 1995). For example, a Nebraska study (Johnson et al. 1995) determined that as much as 70% of a field area was free of grassy weeds and therefore would not require herbicide treatment. Their conclusions were based on a grid sampling procedure.

Improvements in computer technologies such as database management systems, geographic positioning systems (GPS), and geographic information systems (GIS) have greatly improved the possibilities for weed distribution studies (Donald 1994; Nordmeyer et al 1996). These techniques provide a means of locating weeds over large areas. GPS supplies the geographical coordinates of weeds in the field through radio-communication wheareas GIS reproduces a map of the field with the coordinates of the weeds (Lass and

Callihan 1993). Recent applications of GIS in weed science are discussed in the following section.

2.2.3. Mapping techniques

A GIS is a computer-based software program which graphically positions map features in relation to known locations, and relates these positions to other cartographic features (Lass and Callihan 1993). Two types of maps are generated by GIS. Raster- or pixel-based images associate information with colour schemes, where each colour represents specific information. Vector-based images associate information by connecting dots, where all geographic locations with similar information are connected. GIS techniques can be used to characterize and study weed distribution at various scales, and to relate weed density with other biological or geographical variables (Donald 1994). While GIS has become a common tool in soil science, examples in weed science are scarce because of the perceived lack of statistical methods for analysing maps (Donald 1994).

Although the technique is in its infancy, GIS weed maps are used in order to reduce herbicide use in fields. The technique has been developed to address the patchy distribution of weeds in chemical-control decisions (Brown et al. 1994; Nordmeyer et al. 1996; Christensen et al. 1996). In Denmark, Christensen et al. (1996) used a GIS weed map to calculate potential crop yield losses in 130 locations of a 5.3 ha field. Herbicides were applied only in those area of the fields with weed densities above the economical threshold. In this experiment, patch spraying saved up to 50% of the cost of spraying the entire field. However, these techniques remain costly and time consuming because of the necessity of intensive weed sampling (Walter 1996). Progress in remote sensing techniques based on differential light absorption between plants could simplify sampling. Remote sensing coupled with GIS maps successfully identified weeds in a no-till corn (Brown et al. 1994). Walter (1996) suggested that sampling was not necessary every year because weed patches are stable, especially in fields with spring-sown crops.

GIS applications in weed science also were used to study the evolution of Canada thistle patches (Donald 1994), to derive models for reducing herbicide use (Johnson et al. 1995), and to support weed eradication programs in rangelands (Prather and Callihan 1993). These examples are not exhaustive, but they represent different aspects of GIS use in weed science. In Donald's study, GIS was used to construct models to simulate Canada thistle growth over time as well as to build root biomass maps according to shoot biomass. In contrast, Johnson et al. (1995) used GIS to map out weed distribution and to establish areas of fields that required herbicide treatment. Finally, GIS helped establish methods of control for specific areas according to health concerns (proximity of inhabited areas or water streams) and physical access (presence of road or trail)(Prather and Callihan 1993).

2.3. Measuring population variation in weeds; the example of oat *Avena* spp

The study of genetic variation of weed populations can be segregated into two areas. Firstly, genetic variation can be based on phenotypic differences, including physiological responses such as seed dormancy or response to herbicides. However, phenotype is the result of the interaction of the environment and the genetype. Therefore, phenotypic differences may not always provide accurate genotypic variation. Secondly, new technologies in molecular biology have provided researchers with tools to study population variation at the molecular level (Dyer 1991).

2.3.1. Phenotypic variations

Lemma colour and leaf sheath hairiness were used to study polymorphisms in a natural population of *Avena barbata* (Rai 1985). Based on these two markers, the authors found four major climatic regions with different degrees of polymorphism for both characters. The differences among plants from different regions were related to precipitation and temperature. Lemma colour and leaf sheath hairiness were also used to characterize polymorphisms in small wild oat, *Avena fatua*, populations (Jain and Rai 1974). It was observed that genetic drift created a mosaic of monomorphic colonies (Rai 1985; Heywood 1991). However, genetic drift is more likely to occur in autogameous species as opposed to allogameous species (Heywood 1991). In autogameous species, gene flow is limited to seed while in allogameous species gene flow can occur through seed and pollen.

Genetic variability of weed populations can be greatly altered by agricultural practices (Barrett 1983). A change toward increased winterhardiness was observed in wild oat, *Avena sterilis*, after switching from spring-seeded cereals to fall-seeded cereals (Darmency and Aujas 1987). The effect of summer-fallow on wild oat, *Avena fatua*, genetic variability was observed using seed dormancy and seed characters as markers (Jana and Thai 1987). The frequency of wild oat with low dormancy rapidly decreased in favour of wild oat with high dormancy in a summer-fallow rotation. The reduction in genetic variability caused by summer-fallow could result can impede evolution. Rare genes present in non-dormant lines are lost resulting in a genetic bottleneck.

Jana and Thai (1987) predicted a slow evolution of herbicide resistant wild oat in Saskatchewan because of a reduction of the genetic variability resulting from frequent summer fallowing in that province (Jana and Thai 1987).

Response to herbicides can also be used as a genetic marker in the study of population polymorphisms. In 1950, Blackman said that "repeated spraying with one type of herbicide will sort out resistant strains within the weed populations". Jacobsohn and Andersen (1968), Jana and Naylor (1982) and Price et al. (1983) confirmed Blackman's statement by finding a great variation in the response of wild oat, *Avena fatua*, populations to herbicides. For example, Jacobson and Anderson (1968) isolated wild oat lines from field populations with up to a 10-fold difference in tolerance to the herbicide barban. Although detrimental for producers, herbicide resistant weeds have provided researchers with a genetic marker to study pollen-flow in natural weed populations (Stallings et al. 1995; Murray and Morrison 1996).

2.3.2. Molecular variations

Dyer (1991) provides a review of the new applications of molecular biology in weed science. Finch (1994) also provides an overview of the theory and applications of molecular biology applications. These applications include methods to characterize polymorphisms among plant populations at the enzyme and DNA level. Enzyme polymorphism or isozyme variation was the first application of molecular science in population variation studies (Dyer 1991). This technique consists of determining changes in amino-acid composition of the enzyme or part of the enzyme. Individuals with a high degree of similarity indicate that they are closely related while those with substantial differences indicate less genetic relation. Isozyme analyses were consistent and added strength to phenotypic measurements showing differences among *Avena* populations (Darmency and Aujas 1987; Jain and Rai 1974). However, small genetic variations are not accurately differentiated by isoenzymes (Heun et al. 1994).

More recently, random amplified polymorphic DNA (RAPD) and restricted fragment length polymorphism (RFLP) have provided researchers with new tools to identify genetic differences at the DNA level (Finch 1994). RAPD methods consist of multiplying random segments of the DNA with primers of arbitrary nucleotide sequences (Williams et al. 1990). Polymorphisms among individuals are detected by the presence or absence of segments. RAPD methods were used by Goffreda et al. (1992), Phillips et al. (1993), and Heun et al. (1994) to determine the relationship among *Avena sterilis* populations. Multivariate analyses techniques such as cluster analysis provide a ranking of the populations based on the minimum differences in the nucleotide sequences between populations. Multivariate analyses are used to interpret relationships among several variables (James 1990). These techniques offer an interpretation of the relationship of objects according to their response to a set of variable. Techniques such as principal component or principal coordinate analyses maximize the variance among all variables into two axes for two-dimentional representation. Using cluster analysis and principal coordinate analysis, Goffreda et al. (1992) demonstrated that molecular techniques segregated wild oat populations according to their places of origin. The order of the populations produced by cluster analysis can also be used to compare molecular techniques (Heun et al. 1994). In this study, RAPD and isoenzyme resulted in similar segregation among populations.

RFLP is similar to RAPD with the exception of the primers. With RFLP, the primers associated with a chemical marker bind to a specific sequence of

DNA which is present, or not, from one plant to another. Moser and Lee (1994) estimated genetic divergence among tame oat cultivars using RFLP techniques.

2.4. Gene fate in a weed population

2.4.1. Genetic variation

Genetic variation is an essential ingredient for the adaptation of a species to a stress; high genetic variability increases the probability and speed of adaptation to a new stress (Jana and Thai 1987). Harper (1956) reported research conducted in Sweden by Sylven stating that heterozygous strains of clover, *Trifolium pratense*, adapted to climatic changes and pests while homozygous strains did not. Therefore, low genetic variability can lead to the extinction of a population by preventing adaptation to new stresses. Such a condition is also referred to a “bottleneck” (Silvertown and Lovett-Doust 1993).

Small populations are likely to be in a bottleneck situation because of limited gene resources (Barrett 1983). The random fluctuation of gene frequency in small populations, also called genetic drift, can further reduce the genetic variability (Silvertown and Lovett-Doust 1993). The reduction of genetic variability was demonstrated in a wild oat population from a California orchard (Jain and Rai 1974). Inter-row cultivation segregated small wild oat sub-populations around trees. Genetic analyses demonstrated heterogeneity among sub-populations resulting from random drift (Jain and Rai 1974).

Genetic heterogeneity in a population can increase by mutation, which is a transmissible change in gene structure (Silvertown and Lovett-Doust 1993). Postulated frequencies for natural mutations range from 10^{-5} to 10^{-11} per locus per generation (Warwick 1991). The perpetuation of a mutation through successive generations depends on the selective advantage of the mutant compared to the other individuals in the population (Silvertown and Lovett-Doust 1993). The selective advantage represents the benefit of the mutation for survival in an environment. Therefore, the chance of a mutant successfully establishing in a population increases with increasing stress or selection pressure (Macnair 1991).

2.4.2. Weed strategies

Rapid adaptation to stress is a character of an ideal weed because it enhances the survival chances of the weeds in diverse environments (Young and Evans 1976). Different reproductive systems occur in plants which can greatly affect the movement of genes among individuals within a population or from one population to another (Silvertown and Lovett-Doust 1993). The movement of genes, or gene-flow, can occur via plant propagules (roots, rhizomes, and stolons), pollen, or seed. Low gene flow within a population results in a mosaic of homogeneous sub-populations (Snaydon and Davies 1976; Jain and Rai 1974). Such mosaic genetic patterns are common in plants that rely mostly on vegetative propagules for reproduction such as Canada thistle (Donald 1994) and field bindweed (Duncan and Weller 1987).

Gene flow via pollen is limited in selfing species (Silvertown and Lovett-Doust 1993). Selfing results in a reduction of genetic variability, which further impedes adaptation to stress (Mortimer 1991). However, selfing increases the successful establishment of recessive mutations within a population (Jasieniuk et al. 1996). A single mutation of a recessive gene was responsible for inducing resistance to dinitroaniline herbicides in green foxtail, *Setaria viridis*, a highly selfed species (Jasieniuk et al. 1994).

In contrast to selfing, out-crossing species have a relatively high frequency of gene flow via pollen (Mortimer 1991). Out-crossing in kochia of 13.1% per plant spaced 1.5 m apart was reported by Stallings et al. (1995). In the same study, out-crossing was found as far as 30 m from the pollen donor. Out-crossing populations benefit from large genetic variability and little loss of genetic variation due to selection because most of the surviving plants are heterozygous (Chauvel and Gasquez 1994). However, heterozygosity reduces the chances of recessive gene mutations reaching high frequencies in a population (Jasieniuk et al. 1996). Most of the resistance cases in weeds are conferred by single dominant or semi-dominant gene mutations (Macnair 1991; Jasieniuk et al. 1996).

Several weeds such as wild oat combine the flexibility of out-crossers with the ability of inbreeders to maintain specific genotypes (Imam and Allard 1965). Despite a low outcrossing rate of less than 5% (Murray 1996), wild oat populations maintain high genetic variability even under intense selection pressure (Darmency and Aujas 1987; Davidson et al. 1996). Davidson et al. (1996) reported fields treated annually with triallate for 20 years had wild oat patches made up of a mixture of susceptible and resistant plants. The maintenance of a high genetic variability enables the population to adapt rapidly to shifts in cropping practices (Somody et al. 1984). This characteristic of wild oat is probably the reason why it has been a problematic weed for centuries (Imam and Allard 1965).

Gene flow via seed can occur by natural causes such as wind or water, but also by human-related activities such as combine-harvesting or seeding (Silvertown and Lovett-Doust 1993). Some weed species have developed efficient means of seed dispersal. One of these is Russian thistle, *Salsola* *iberica* (Stallings et al. 1994). Mature Russian thistle plants break off at the soil surface and can tumble over several kilometers by the force of wind, dispersing seeds along the way. This efficient means of transportation is of great concern in trying to restrain herbicide resistant populations from spreading (Stallings et al. 1994).

Human-related spread of weeds has often resulted in severe weed problems (Barrett 1983). The majority of the weeds in North America were imported with crop seeds from Europe. More recently, the rapid spread in eastern Ontario of weed species resistant to triazine herbicides was related to broadcasting manure of silage-fed cattle to cultivated fields (Stephenson et al. 1990). A similar observation was made in Idaho where a population of triazine resistant powell amaranth, *Amaranthus powellii*, is believed to have spread through manure purchased from a feedlot (Eberlein et al. 1992). However, the exact origin of the initial resistant population could not be determined because of the numerous sources of corn used for silage by the feedlot.

Other agricultural activities such as combine-harvesting also contribute to the spread of weeds. In a corn field, a combine driving through a proso millet, *Panicum miliaceum*, patch produced an average seed rain of 10 seeds m^{-2} within 50 m of the patch (McCanny and Cavers 1988). It is believed that combine-harvesting is responsible for the spread of proso millet in southern Ontario (McCanny et al. 1988). Howard et al. (1991) reported seed dispersal by combine-harvester of brome species to a maximum distance of 20 m. In the same study, Howard et al. (1991) indicated spread of weed seed of 2 m by cultivation. More significantly, the use of uncleaned seedlots can disperse weed seed over entire fields in a single season. Jensen (1962 in Walker and Buchanan 1982) reported that over half of wheat seedlots in Utah contained weed seeds. In this survey, the worst sample would have resulted in the spread of 50 weed seeds m^{-2}. In 1981, 30% of crop seedlots from Manitoba contained wild oat seeds (Anon. 1982). Out of a total of 534 seedlots, 64 of them had weed seed contamination above requirements set by the Canadian Seed Act.

Some weed species have adapted to improve their chances of being planted with the crop. This is the case for weeds which have evolved seeds that closely resemble the seed of the crop in which they usually grow. Examples of such an adaptation are vetch, *Vicia sativa*, and false flax, *Camelina sativa*, populations that evolved into perfect seed mimics of lentils and flax seeds, respectively (Barrett 1983).

Evolutionary changes in weed populations may result in a loss of gene variability which reduces the chances for adaptation to a new stress (Young and Evans 1976). Loss of genetic variation was documented in wild oat populations after the implementation of summer-fallow (Jana and Thai 1987) and repetitive applications of herbicides (Jana and Naylor 1982; Price et al. 1983). Therefore a survival characteristic of weeds is to maintain genetic variability even under high selection pressure (Simpson 1992). In part, this is accomplished by dormancy mechanisms which preserve a reserve of seeds in the soil seed bank (Gressel and Segel 1978). The soil seed bank buffers population genetic changes by storing a large range of genetic variability as dormant seed (Darmency and Aujas 1987). Weed populations with long seed dormancy periods have a slow rate of change because of a slow seed bank turn-over (Gressel and Segel 1978).

While dormancy has a role in survival of weeds in agriculture, several agricultural practices can modify the dormancy characteristics of weed populations (Peters 1991). Therefore, characteristics of the seed in the soil seed bank are often related to past cropping history (Wilson et al. 1985). For example, cultivation during summer-fallow periods is known to select for individuals with a long seed dormancy (Jana and Thai 1987). Cultivation during the fallow year encourages growth of non-dormant types while dormant types germinate in sequence with the crop (Barrett 1983; Jana and Thai 1987).

2.4.3. Selection pressure

Gressel and Segel (1990a) defined selection pressure of a herbicide on a weed species as the proportion of resistant plants surviving treatments divided by the proportion of susceptible plants surviving treatment. The intensity of selection pressure on weed populations has increased greatly with the development of mechanization and chemical control (Barrett 1983). More efficacious herbicides (which mean higher selection pressure among weeds) has allowed farm size to increase.

New adaptive responses of weeds seem to emerge every time a new agricultural technology is applied (Gould 1991). The evolution of weed resistance is an example of such an adaptation. Weed resistance reduces long-term control options because it becomes more and more difficult to produce new herbicides for both technological and social reasons (Barrett 1983; Ruscoe 1987).

Long-term integrated weed control strategies focus on varying the type and strength of selection pressure, which ultimately impede adaptation of the weed to control methods (Gressel 1991). Over-reliance on a single control strategy using highly effective herbicides is doomed in the long term because of the evolution of weed resistance (Tardif and Powles 1993). In order to slow the rate of appearance of weed species adapted to control strategies, Maxwell (1992) suggests that weed control decision models include strength and type of selection pressure to prevent resistance evolution.

Selection pressure acts on a population of weeds whenever growing conditions are altered to promote crop growth and suppress weeds. Therefore, a weed that can adapt to resemble a particular crop has a better chance of survival, since little room for selective control remains (Barrett 1987; Walker and Buchanan 1982). Continuous cropping provides an ideal environment for such weed adaptation because of the consistency of the selection pressure over time (Edwards and Regnier 1989; Walker and Buchanan 1982). Weed adaptation in continuous cropping systems are numerous (Walker and Buchanan 1982; Barrett 1983). The majority of new cases of herbicide resistance are also related to several years of continuous use of a particular herbicide or chemically-related herbicides (Holt 1992). For example, the first case of herbicide resistance, triazine resistance in common grounsel, *Senecio vulgaris*, was selected after 10 years of consecutive applications of simazine (Ryan 1970). Other examples are provided in Table 2-1, which summarizes different durations of selection by herbicides that were necessary for evolving resistance in weeds. This aspect of selection pressure with repeated application of herbicides is discussed in more details in chapter 2.5.3.

Selection can only act on populations which harbour some genotypes more adapted to a stress than others (Gressel and Segel 1978). In the case of herbicides, substantial variability in resistance is documented in the literature. Variability in response to triallate in Canadian wild oat populations was described as early as 1982 (Jana and Naylor 1982; Thai et al. 1985). Unselected populations were more variable, but on average less resistant, than selected populations (Thai et al. 1985). Similar findings were reported from studies in California (Price et al. 1983) and North Dakota (Jacobsohn and Andersen 1968).

Table 2-1. Number of years with a similar herbicide prior to the development of resistant weeds.

Herbicide	Species	No. years	Reference
Aryloxyphenoxypropionates and cyclohexanedione	*Avena fatua*	5-10	(Heap *et al.*1993)
	Setaria faberi	<9	(Stoltenberg and Wiederholt, 1995)
	Digitaria sanguinalis	<8	(Wiederholt and Stoltenberg, 1995)
	Setaria viridis	<10	(Heap, 1996)
Sulfonylurea (SU)	*Kochia scoparia*	3	(Sivakumaran *et al.*1993)
	Salsola iberica	<10	(Stallings *et al.*1994)
Dinitroaniline	*Eleusine indica*	10	(Mudge *et al.* 1984)
	Setaria viridis	<20	(Morrison *et al.*1989)
	Amaranthus palmeri	24	(Gossett *et al.* 1992)
Phenoxy	*Ranunculus acris*	>35	(Bourdôt *et al.*1990)
	Brassica kaber	20-25	(Heap and Morrison 1992)
Triallate	*Avena fatua*	20-25	(O'Donovan *et al.*1993)
Paraquat	*Erigeron philadelphicus*	5-6[z]	(Itoh and Matsunaka, 1990)
	Hordeum glaucum	24	(Powles and Howat, 1990)
Triazine	*Senecio vulgaris*	10[z]	(Ryan, 1970)

[z] more than one application per year

A reduction in genetic variation occurs rapidly as a result of a strong selection pressure imposed on a population (Gressel and Segel 1978). Triazine resistant populations of lamb's quarter, *Chenopodium album*, were more homogeneous than susceptible populations (Warwick and Marriage 1982).

Despite the reduction in genetic variation, populations adapted to a specific stress can have difficulties surviving in the absence of that stress (Harper 1956). Furthermore, some populations revert to their original composition in the absence of stress (Maxwell et al. 1990; Mortimer 1991). However, the speed at which they do this depends on the difference in relative fitness between the selected and the original individuals in the absence of selection pressure (Jasieniuk et al. 1996). In some cases of herbicide resistance in weeds, resistant individuals are less fit than susceptible individuals because they need to maintain a resistance mechanism with an associated biological cost (Mortimer 1991). However, in many cases there is no apparent reduction in fitness associated with resistance (Holt 1996; Wiederholt and Stoltenberg 1996). It is assumed that the “cost” of resistance is low in most mechanism of resistance.

2.5. The effects of management practices on genetic and phenotypic variations of weed species

2.5.1. Adaptation to cultural practices

There is evidence that many weed populations associated with particular cultural systems have evolved to optimize survival by growing during the most favourable conditions (Barrett 1983). Survival strategies, also called avoidance strategies, include precocious reproduction, delayed germination, or increased dormancy. Populations of teosinte, *Zea mexicana*, a common weed in corn-fallow rotations in Mexico, illustrate such an adaptation. Teosinte avoids destruction by cultivation or grazing during the fallow period by increasing its seed dormancy period so that germination of the weeds coincides with planting of the crop (Barrett 1983). A similar adaptation was observed in wild oat where populations collected from fields which were summer-fallowed had greater dormancy than those from fields which were continuously cropped (Jana and Thai 1987). Also in wild oat, a change from spring- to winter-sown cereals in a rotation rapidly increased the frost tolerance of the wild oat population (Darmency and Aujas 1987).

Weed populations have also evolved by modifying their growth habit or form to avoid destruction by cultural practices. In California, Schoner et al. (1978) studied a population of yellow foxtail with a prostrate growth habit. This particular population was selected in an alfalfa field which was cut frequently. The prostrate growth form prevented the seed heads from being cut with the alfalfa.

2.5.2. Adaptation by crop mimicry

Crop mimicry involves survival strategies whereby the weed resembles the crop at a specific stage (Barrett 1983). Most cases of crop mimicry involve weeds and crops from similar families, and even from the same species. Mimicry involving weeds and crops of the same species develop rapidly because of gene flow (Barrett 1987). Exchange of genes from the crop to the weed was documented between wild and tame rice (Gould 1991). Unfortunately, the hybrids resulting from the crop-weed cross retained the traits of the weed in terms of seed quality. In India, researchers included phenotypic markers such as red pigments in tame rice varieties but wild rice populations rapidly aquired the trait (Barrett 1983).

Crop mimicry has been documented particularly in seedling or heading stages (Barrett 1983; Gould 1991). Mimicry at the seedling stage is common in areas of the world where hand-weeding prevail such as in rice paddies. Mimicry between tame rice and wild rice or barnyard grass, *Echinochloa crus-gali*, populations have been documented (Barrett 1987; Gould 1991). In the case of barnyard grass, the resemblance is such that this weed has become a threat in all rice growing areas. Although hand-weeding has been replaced by chemical control in areas such as the USA, barnyard grass is often mistaken for rice resulting in herbicide misapplications (Barrett 1987).

Some weed species have developed a survival scheme whereby instead of risking predation or death during off-seasons, weed seeds are stored with the seed of the associated crop (Barrett 1983; Barrett 1987). In this case, the weed seed resembles the crop seed. In eastern Europe, vetch found in lentils, *Lens culinaris*, has evolved from a round seed-shape to a lenticular seed-shape almost identical to the lentil seed (Gould 1991). The change in seed shape was attributed to a single recessive mutation selected by winnowing practices. It is believed that crops such as oat and rye were also selected by winnowing wheat and barley (Barrett 1983).

2.5.3. Herbicide resistance

Although herbicide resistance in weeds appeared later than pesticide resistance in insects and fungi, the number of new resistant weed occurrences is increasing at a fast rate (Holt and LeBaron 1990; Heap 1996). In 1970, Ryan reported that a population of common groundsel evolved resistance to triazine herbicides. This was the first documented instance of herbicide resistance in weeds. Twenty years later, populations of 99 weed species were reported to be resistant to herbicides around the world (Holt and LeBaron 1990). Of these 99 species, 55 were resistant to the triazine herbicides. Recently, an updated survey identified 150 unique instances of herbicide resistant weeds around the world (Heap 1996).

Instances of weeds resistant to a number of unrelated herbicides have been reported in Australia (Heap and Knight 1990; Heap 1991), England (Moss and Cussans 1991), and Canada (Morrison and Bourgeois 1995). In these cases, selection with one herbicide leads to the selection of plants resistant to several unrelated herbicides. For example, blackgrass sprayed repeatedly with chlorotoluron evolved resistance to herbicides in different chemical classes including sulfonyl-ureas, aryloxyphenoxypropionates, and dinitroanilines (Moss 1990). Although the mechanism is not known, such resistance is referred to as multiple resistance. Multiple resistance has evolved successively in other species as well. In dinitroaniline resistant green foxtail, repeated application of ACCase inhibitor herbicides selected for plants with resistance to both chemical groups (Heap unpublished data). In the case of outcrossing species such as rigid ryegrass and blackgrass, strong selection pressure from various herbicides can also result in multiple resistance because of intense exchange of resistant gene through gene flow (Powles and Preston 1995).

In the late seventies, Gressel and Segel (1978) proposed a mathematical equation to model the rate of appearance of resistant individuals in weed populations. Factors included in the equation are the initial frequency of resistant individuals in the population, the selection pressure, the fitness of the resistant individuals compared to the susceptible ones, and the soil seed reservoir. As much as these factors affect the rate of herbicide resistance evolution, the number of herbicide applications over time is essential for selecting herbicide resistant weeds (Harper 1956; Gressel and Segel 1978).

Initial frequency. Initial frequency of resistant individuals is often assumed to correspond to the mutation rate, which is in the order of one in a million. However, several studies have shown that the initial frequency of resistant individuals in some species is in the order of one in a thousand (Matthews and Powles 1992). A higher initial frequency of resistant individuals results in a faster appearance of resistance (Price et al.1983; Jasieniuk et al.1996). The possibility of a high frequency of resistant individuals in an untreated population cannot be ruled out (Price et al.1983).

The majority of the known herbicide resistant cases are conferred by major gene mutations (Jasieniuk et al.1996). Major gene mutations usually lead to resistance not only to the herbicide that was used on the population, but also to herbicides from the same chemical family or herbicides with the same mode of action (Tardif and Powles 1993). For example, aryloxyphenoxypropionate and cyclohexanedione herbicides are ACCase inhibitors, and bind at the same region of the enzyme (Rendina et al.1989). Resistance was conferred by a mutation of the ACCase enzyme in *Setaria viridis* (Marles et al.1993) and *Eleusine indica* (Leach et al.1995). The level of resistance to chemically related herbicides can vary from one mutation to another (Seefeldt et al.1994; Murray et al.1995; Sivakumaran et al.1993). Mazur and Falco (1989) identified 24 amino acid substitutions at 10 different sites of the acetolactate synthase (ALS) enzyme in bacteria, resulting in different levels of resistance to sulfonylurea herbicides. The diversity of resistance patterns and the coevolution of resistant individuals at several locations adds difficulty to the management of resistance in weeds (Seefeldt et al.1994).

Selection pressure. The selection pressure is represented in the Gressel and Segel equation as the proportion of resistant plants divided by the proportion of susceptible plants surviving the herbicide treatment (Gressel and Segel 1978). The speed of selection of herbicide resistance is proportional to herbicide efficacy (Jasieniuk et al.1996). Strong selection pressure also increases the chances of selecting for major gene mutations which allow for high resistance levels (Macnair 1991).

Refered to as 'creeping resistance' by Gressel (1995), polygenic resistance is likely to occur under low selection pressure. Weak selection pressure by applying sub-lethal herbicide doses on a weed population can affect the selection of traits such as cuticle thickness, stomate size, and efficiency of respiration, translocation, and photosynthesis (Price et al.1983). The use of sub-lethal dose of herbicide only postpone occurences of resistance by major gene mutation by diluting rare resistant individuals (Morrison and Friesen 1996). Moreover, the weed density is likely to increase because of a large number of weeds reaching maturity after recovering from sub-lethal dose of herbicides.

Fitness. It is often assumed that the fitness of resistant plants in the absence of herbicide treatment will be less than that of susceptible ones because of the initial biological cost associated with resistance (Jasieniuk et al.1996). This assumption held true for triazine resistance, but has seldom been observed in non-triazine resistance cases (Jasieniuk et al.1996). Low fitness in resistant plants when no herbicides are applied results in a reduction in their frequency within a population (Gressel and Segel 1990b, 1990c).

Soil seed reservoir. The soil seed reservoir buffers the changes in gene frequency in a population (Gressel and Segel 1978). Mansooji et al. (1992) partly attributed a slower evolution of resistance in wild oat than in ryegrass to a slower seed bank turnover and an extended germination period for wild oat compared to ryegrass. A slow seed bank turnover results in a slow depletion of susceptible plants from the population. An extended germination period provides chances for susceptible individuals to escape control by not being sprayed.

Number of herbicide applications. Harper (1956) predicted that "rotation of herbicides differing fundamentally in toxic action would minimise the chance of resistant strains becoming established". Harper appears to have been correct, since lack of herbicide rotation characterizes all new cases of resistance. Selection due to historical exposure to MCPA was demonstrated in *Ranunculus acris* with a 0.9 correlation between exposure and resistance level (Bourdôt et al. 1990). Table 2-1 summarizes the number of applications of herbicide required for the evolution of resistance in different weed species.

In contrast with the other factors indicated in Gressel and Segel's equation, the number of herbicide applications is the only one that can be controlled by producers. In order to impede resistance evolution it is essential to use a variety of control methods. The implementation of a "1 in 3 rotation" of herbicide was first advocated in Manitoba (Goodwin 1994), where producers are encouraged to use specific herbicide groups only one year in three. In addition to the rotation of herbicides, Manitoba Agriculture encourages producers to use non-chemical weed control practices as part of an integrated weed management system. Scientists agree that integrated weed management reduces the probability of selecting for herbicide resistant weeds (Harper 1956; Maxwell et al. 1990; Thill et al. 1994). Facing a lack of chemical weed control options, integrated weed management systems are also implemented to control existing weed resistance cases (De Prado and Menendez 1996; Powles and Matthews 1996).

2.6. Summary

Weed populations include individuals sharing a similar space at the same time. Density and distribution of individuals describe the likelihood of finding an individual from a population. The measurement of density and distribution is complex since most weeds occur in clusters or patches. Therefore, the density and distribution of individuals from one population can not be inferred to another population. Recent techniques such as GPS and GIS simplify sampling and surveying for characterization of distribution and densities of weed populations.

Weed populations are generally polymorphic implying a diversity of genes in the population. This diversity can be determined by phenotypic analyses or molecular analyses. A large gene variation in a weed population increases the chances of survival of some individuals following the introduction of a new stress. Strong selection pressure tends to reduce the genetic variation of a population which will weaken the capacity of the population to adapt to a new stress. Therefore, weed selected under a specific cropping system often are unable to grow under another cropping system.

Herbicide resistance is an example of adaptation and evolution of a weed population subjected to selection pressure imposed by continuous use of a strong selective agent. Several components such as the initial gene frequency of resistant individuals, the fitness of resistant versus susceptible individuals, the size of the seed bank, and the selection pressure interfere with the speed at which resistant individuals increase in a population.

3. MAPPING RISK AREAS FOR RESISTANCE TO ACCase INHIBITOR HERBICIDES IN MANITOBA.

Abstract: Since 1976, seven acetyl coenzyme-A carboxylase (ACCase) inhibitors (referred to as Group 1 herbicides) have been registered in western Canada for wild oat (*Avena fatua* L.) and green foxtail (*Setaria viridis* L.) control. In 1990, Group 1 resistant wild oat populations were identified from fields in Manitoba which had been repeatedly sprayed with these products over the previous 10 years. Since the occurrence of resistance is directly related to the frequency of herbicide use, the purpose of this study was to compile herbicide use histories on a province-wide basis using data included in the Manitoba Crop Insurance Corporation (MCIC) database. The database was used to determine the relative importance of Group 1 herbicide use in major crops compared to other products, and to identify individual townships at low, medium, and high risk for developing Group 1 resistance. Low, medium and high risk townships were arbitrarily defined as those in which Group 1 products were used on less than 30%, 30 to 50% and over 50% of the sprayed fields, respectively. From 1981 to 1993, Group 1 herbicide use increased from 15 to 50% of the sprayed area, and since 1990, these products were used on one out of two sprayed fields on an annual basis, with the most intensive use in flax. In the early 1980's, fewer than 5% of the townships were at high risk. These were located near the towns of Swan River, Dauphin and Treherne, and the city of Winnipeg, which were the locations where Group 1 resistant wild oat and green foxtail were first identified. Between 1989 and 1993, over 40% of the townships were considered to be at high risk. Should the trend toward increased use of Group 1 herbicides continue through the 1990's, the resistance problem will inevitably worsen.

Résumé: Depuis 1976, sept herbicides inhibiteurs de l'acétyl coenzyme-A carboxylase (ACCase), communément appelés les herbicides du Groupe 1, ont été enregistrés dans l'ouest canadien pour le contrôle de la folle avoine (*Avena fatua* L.) et de la sétaire verte (*Setaria viridis* L.). En 1990, on a identifié de la folle avoine résistante aux herbicides du Groupe 1 dans des champs au Manitoba où l'on avait fréquemment utilisé ces produits dans les 10 dernières années. Puisque la résistance est intimement liée à la fréquence d'utilisation des herbicides, le but de la présente étude était de compiler des antécédents d'utilisation des herbicides dans l'ensemble de la province au moyen de données tirées de la base de données de la Direction d'assurance-récolte du Manitoba (MCIC). On a premièrement utilisé la base de données afin de déterminer l'utilisation relative des herbicides du Groupe 1 en grande culture comparativement à d'autres produits. En outre, la base de données a permis d'identifier les townships à faible risque, à risque moyen et à risque élevé de développer de la résistance aux herbicides du Groupe 1. Dans un township à faible risque, moins de 30 % des champs sont traités annuellement avec un herbicide du Groupe 1. Les townships à moyen risque et à risque élevé sont ceux dont les champs sont traités de 30 à 50 % et à plus de 50 % avec des herbicides du Groupe 1, respectivement. Entre 1981 et 1993, l'utilisation des produits du Groupe 1 sur les terres traitées au Manitoba a augmenté de 15 à 50 %. Depuis 1990, un champ sur deux est traité chaque année avec un herbicide du Groupe 1, surtout en ce qui concerne les champs de lin. Au début des années 1980, moins de 5 % des townships étaient considérés à risque élevé de résistance. Il s'agit de townships situés près des agglomérations de Swan River, Dauphin, Treherne et Winnipeg, régions qui correspondent aux endroits où l'on a premièrement identifié de la folle avoine et de la sétaire verte résistantes aux herbicides du Groupe 1. Entre 1989 et 1993, plus de 40 % des

townships étaient évalués à risque élevé de résistance. Si l'utilisation des herbicides du Groupe 1 continue d'augmenter d'ici la fin des années 1990, le problème de résistance ne pourra que s'aggraver.

3.1. Introduction

Diclofop-methyl, an aryloxyphenoxypropionate (APP), and sethoxydim, a cyclohexanedione (CHD) were the first two ACCase inhibitor herbicides recommended for use in Manitoba. These were registered as post-emergence herbicides in 1976 and 1983, respectively, and were readily accepted by producers for three reasons (Goodwin 1994). Firstly, an increase in minimum-tillage and concerns over soil erosion prompted wider use of post-emergence herbicides in place of pre-emergence, soil incorporated herbicides (PEI). Secondly, diclofop-methyl and sethoxydim provided excellent control of both wild oat (*Avena fatua* L.) and green foxtail (*Setaria viridis* L.) in numerous crops; and thirdly, they could be tank-mixed with certain other herbicides, including bromoxynil, to control grasses as well as broadleaf weeds in one application.

ACCase inhibitor resistance was discovered in the fall of 1990 in three populations of wild oat near Swan River in north-western Manitoba (Heap et al. 1993), and subsequently, five resistant green foxtail populations were identified in Manitoba in 1991 (Heap et al. 1996). By 1993, hundreds of ACCase resistant wild oat populations were reported throughout the province (Morrison and Devine 1994). In all cases, resistant weeds occured in fields that were sprayed repeatedly with ACCase inhibitor herbicides over a 5 to 10 year period.

Since 1991, Manitoba Agriculture has promoted ways to delay or avoid development of resistant weeds (Goodwin 1994). As part of the extension message, the "Group" concept was established to simplify discussion about resistance with farmers. Herbicides were grouped according to their mode of action or where there was evidence that resistance to one herbicide also confered resistance to another. The development of the "Group" concept was a cornerstone in promoting a "1 in 3" herbicide rotation strategy to delay the onset of resistance (Morrison and Bourgeois 1995). Farmers were advised to use a herbicide from the same Group no more than once in three years. This strategy was adopted on the basis of mathematical simulations derived from a predictive model (Gressel and Segel 1990).

Since the risk of selection for herbicide resistance is related to herbicide use, the objective of this study was to identify areas at risk of developing Group 1 resistance in Manitoba based on herbicide use history data collected by the Manitoba Crop Insurance Corporation (MCIC).

3.2. Materials and methods

The MCIC has collected agronomic data on insured fields since 1960. The database was initiated to identify areas with different production risks, namely "risk areas". Insurance premiums and coverage were adjusted for each "risk area". In 1981 a research questionnaire was included and a number of management practices were added to the database, including fertilizer use, date of planting, and herbicide use practices.

The data used in this study consisted of a subset of the MCIC database from 1981 to 1993, representing reports on three quarters of a million fields throughout Manitoba. Annual representation of reported fields varied from 20 to 60% of the provincial cropped area (data not shown). Between 1981 and 1989 the annual representation averaged 35% of the cropped area but decreased to 20% of the cropped area from 1990 to 1992. This decrease in representation occurred consequently to the implementation of the Gross Revenue Insurance Program (GRIP). In order to qualify for GRIP, crops had to be insured with MCIC and the corporation could not handle the subsequent increase in the number of questionnaires. To reduce the number of questionnaires, only a random sample consisting of a third of respondents were requested to file a management questionnaire from 1990 to 1992. Finally, in 1993 the survey questionnaire included all insured fields, representing 60% of the provincial cropped area. Despite the year to year variation in the number of survey questionnaires, the large sample size of the MCIC database provides a reliable representation of agricultural practices in all areas of the province for the whole of the time period under consideration.

The analysis of the database was conducted with SYBASE[1], a database management program. Averages and sums were calculated over years, regions, and/or crops. Average herbicide use was calculated as a percentage of fields sprayed with at least one herbicide. Query programs used with Sybase are listed in Appendix 1.

An average annual use of common herbicides registered for wild oat control was calculated for the whole of the province. Herbicides were grouped according to the scheme adopted by Manitoba Agriculture, namely Group 1, Group 2, Group 3, and Group 8 (Table 3-1).

Group 1 includes both aryloxyphenoxypropionate and cyclohexanedione chemical families, both of which are ACCase inhibitors (Table 3-1). Group 2 includes both imidazolinones and sulfonylureas which inhibit acetolactate synthase (ALS), also known as acetohydroxyacid synthase (AHAS). Prior to 1993, imazamethabenz (from the imidazolinone chemical family) was the only Group 2 herbicide registered for the control of wild oat (Table 3-1). Group 3 includes the dinitroaniline herbicides, trifluralin and ethalfluralin, which are mitotic disruptors. Triallate and difenzoquat are classed together in Group 8 (Table 3-

[1] SYBASE Release 5.0, SYBASE Inc., 6475 Christie Av., Emeryville CA 94608, USA

1), since wild oat populations resistant to triallate are also resistant to difenzoquat (O'Donovan et al. 1994). Flamprop-methyl has a unique mode of action and is grouped on its own under the designation "other". Flamprop-methyl is not widely used in Manitoba with no more than 4% of the treated area being sprayed with this chemical in 1993 (data not shown).

Table 3-1. Wild oat herbicides used commercially in Manitoba between 1981 and 1993[y]

Herbicide Group	Site of action	Family	Herbicide	Registration year
1	ACCase inhibitors	aryloxyphenoxypropionate	diclofop-methyl	1976
			fluazifop-p-butyl	1984
			fenoxaprop-p-ethyl	1989
			quizalofop-ethyl	1991
		cyclohexanedione	sethoxydim	1983
			tralkoxydim	1992
			clethodim	1992
2	ALS/AHAS inhibitors	imidazolinone	imazamethabenz	1989
3	tubulin disrupters	dinitroaniline	trifluralin[z]	1970
			ethalfluralin[z]	1988
8	unknown	thiocarbamate	triallate[z]	1961
		unclassified	difenzoquat	1974
other	unknown	aminopropionate	flamprop-methyl	1978

[y] modified from Heap and Morrison (1991)
[z] Pre-Emergence Incorporated herbicides

Group 3 and Group 8 averages do not include data from fields where triallate and trifluralin were pre-plant incorporated as a formulated mixture (Fortress[2]) for control of wild oat and green foxtail in wheat and barley. An average use was calculated for the triallate and trifluralin mixture for the whole of the province.

A second analysis focused on regional use of Group 1 herbicides within specific townships. Each township was identified by legal land description and consisted of 36 sections of one square mile (256 ha). Townships with less than 100 records for the period between 1981 and 1993 were not considered for the study. Group 1 herbicide use frequencies were calculated on the basis of total number of sprayed fields for 729 townships. The number of sprayed fields included any fields where at least one herbicide was applied for any specific year. The number of sprayed fields was preferred to the total number of fields in order to focus the analyses on field crops rather than forages since less than 10% of the forage crops were sprayed annually. In contrast, over 90% of the insured wheat crops were sprayed with at least one herbicide annually.

A Geographic Information System program[3] was used to generate herbicide use maps broken down by township. Low, medium, and high use of Group 1 herbicides transcribed into low, medium, and high risk of developing herbicide resistance to these herbicides, as the frequency of herbicide use is closely related to the occurrence of herbicide resistance. While categorization of individual townships was based on average use figures for the whole of the township it is recognized that there may be wide variation in herbicide usage among fields or from one farm to the next. Hence there is a possibility that resistant weeds might well occur in low or medium risk townships in specific

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[2] 4% trifluralin and 10% triallate formulated as a granule. Manufactured by Monsanto Canada Inc., Streetsville PO Box 787, Mississauga, ON, L5M 2G4

[3] IDRISI, Grad. School of Geography, Clark University, Worcester MA, 01610, USA

fields where Group 1 herbicides have been used frequently even though the area average is less than in a high risk township. Notwithstanding the fact that the chances of finding resistant weeds is related to the intensity of herbicide use on particular fields, the overall probability of identifying resistant weeds on a regional basis will be greater in high risk townships than in low or medium risk ones. The fact that variations in Group 1 herbicide use may have occurred within townships within a particular risk category does not detract from the broader conclusions that can be drawn from such an analysis.

A township was arbitrarily classified as low risk if less than 30% of all sprayed fields within the township were treated with a Group 1 herbicide for the period between 1981 and 1993. Therefore, on average fields in low risk townships were sprayed with a Group 1 herbicide less than once every three years. This low frequency of treatment corresponds to the practice recommended by Manitoba Agriculture for delaying resistance (Morrison and Bourgeois 1995). Medium and high risk townships were those in which Group 1 herbicides were used on 30 to 50% and over 50% of the fields, respectively, from 1981 to 1993. In both medium and high risk areas Group 1 herbicides were used more frequently than currently recommended by Manitoba Agriculture[4]. Producers in high risk townships used Group 1 herbicides more than once every two years on average whereas producers in medium risk townships used Group 1 herbicides more than once but less than twice in three years.

Additionally, herbicide use frequencies were calculated for five year periods to highlight trends in Group 1 use over time within specific townships. Maps were generated to represent Group 1 risk areas for 1981 to 1985, 1985 to 1989, and 1989 to 1993. For each period, risk classification was as determined previously.

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[4] Guide to crop protection, Manitoba Agriculture Box 1149, Carman MB R0G 0J0.

3.3. Results and discussion

In 1981, PEI herbicides made up the largest proportion of the wild oat herbicide market (Figure 3-1). Dinitroanilines (Group 3), triallate (Group 8), and Group 3 and 8 mixture were used on 44% of the sprayed area. Between 1981 and 1993, Group 3 herbicide use remained fairly constant with approximately 15% of fields sprayed annually in Manitoba. The Group 3 and 8 mixture and Group 8 herbicide use declined to less than 5% of the sprayed fields in 1993. Part of this decline is related to an increasing concern about fall tillage and its effect on soil erosion. This prompted many farmers to move away from PEI herbicides. Also, PEI herbicides were less attractive than the post emergence products in terms of weed control efficiency and the spectrum of weeds controlled (Goodwin 1994).

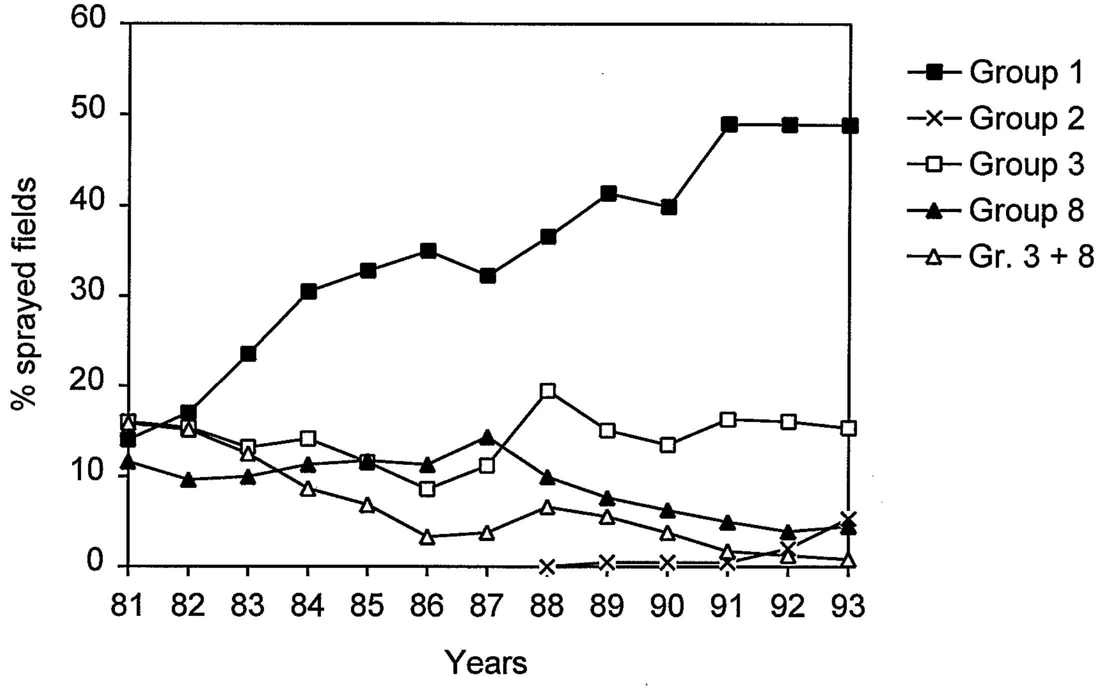

Figure 3-1. Relative importance of five wild oat herbicide Groups in Manitoba expressed as a percentage of total sprayed area from 1981 to 1993.

Imazamethabenz, a post-emergence herbicide, was the only Group 2 herbicide used for wild oat control from 1988 to 1993. Imazamethabenz use increased to a maximum of 7% of sprayed fields (Figure 3-1).

The largest increase in herbicide use for grassy weed control occurred with Group 1 herbicides. Use of these herbicides increased from 15% to 50% of the sprayed fields from 1981 to 1991 (Figure 3-1). Since 1991, Group 1 herbicides were used on approximately 50% of the sprayed fields indicating that from 1991 to 1993 on average most fields would have been sprayed once out of every two years with these herbicides.

The increase in Group 1 usage since 1981 stemmed from the rapid adoption by farmers of diclofop-methyl and sethoxydim. No less than three fields out of four treated with a Group 1 herbicide were sprayed with diclofop-methyl or sethoxydim between 1981 and 1993 (data not shown). Registration of several new ACCase inhibitors has sustained the dominance of Group 1 herbicides in the provincial market through to the present (Morrison and Bourgeois 1995).

Throughout the 1980's and early 1990's, Group 1 herbicides were more widely used in Manitoba than all other wild oat herbicides combined. This was particularly so in flax where 98% of the fields were sprayed with Group 1 herbicides in 1993 (Figure 3-2). The heavy dependence on Group 1 herbicides in flax results from their superior performance for wild oat control compared to other herbicides in this crop, which is less competitive than crops such as canola and cereals.

In Canadian Prairie Spring (CPS) wheat and Hard Red Spring (HRS) wheat, Group 1 herbicide use peaked at 60% of the sprayed fields in 1991 and 1992 (Figure 2). In 1993, their use declined to 50% of the sprayed fields. This decline may have resulted from producers adopting herbicide rotation to manage resistance since several options for wild oat control are available in cereals.

Moreover, low wheat prices in 1993 may have prompted farmers to reduce overall herbicide expenditures, thereby resulting in a drop in Group 1 herbicide use (Honey, 1995).

Canola treated with Group 1 herbicides increased from 18% of the acreage in 1988 to 58% in 1993 (Figure 3-2). The popular tank-mix of Group 1 herbicides with ethametsulfuron (Group 2) for wild mustard (*Sinapis arvensis* L.) control, and the increased economic return of canola, promoted greater Group 1 herbicide use in this crop.

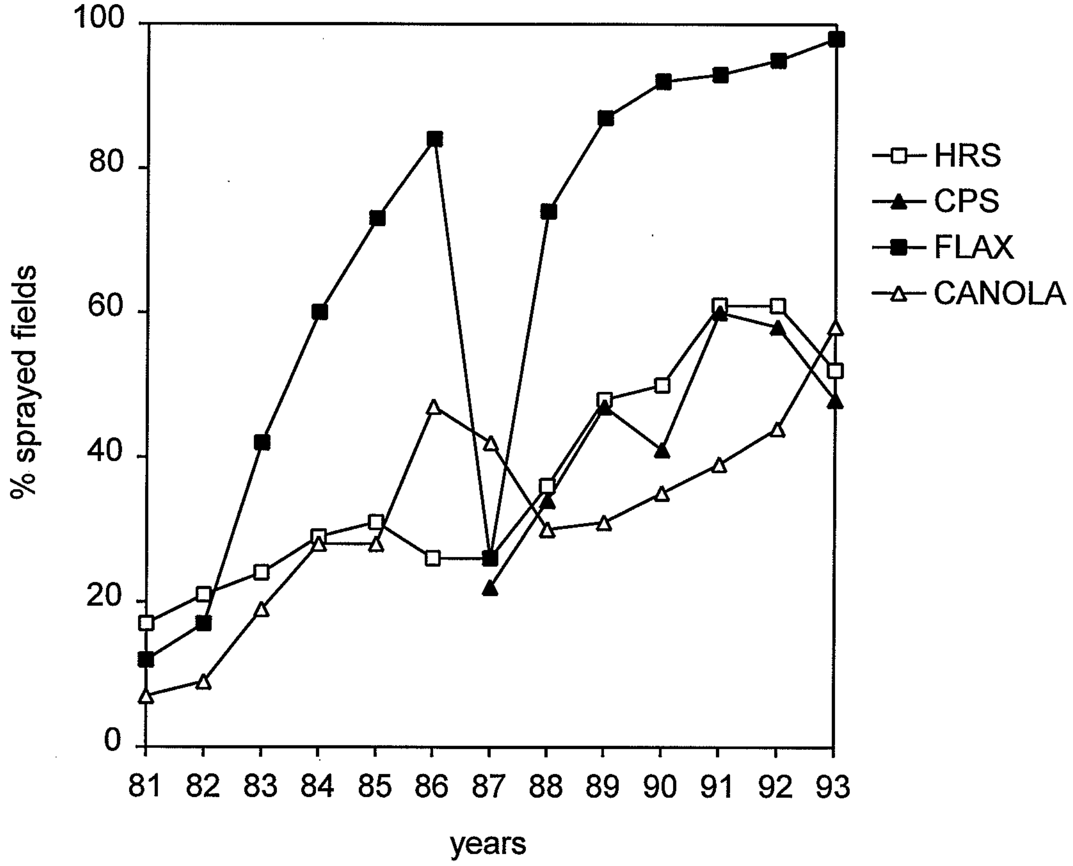

Figure 3-2. Relative importance of Group 1 herbicide use in four crops in Manitoba expressed as a percentage of total sprayed area for each crop from 1981 to 1993.

While Group 1 herbicides have been widely used throughout Manitoba since 1990, some areas of the province have had a longer history of high use of Group 1 herbicides than others. Figure 3-3 illustrates the relative use of Group 1 herbicide in each township for the period between 1981 and 1993. Generally, Group 1 use was least in the dry southwestern townships of the province, and in the townships nearby the towns of Morden and Morris. Because the major grass weed in the southwestern part of Manitoba was green foxtail, producers from this area relied heavily on dinitroaniline herbicides for weed control (Morrison et al. 1989). In addition, crops other than cereals and oilseeds (e.g. corn, beans, and sugar beets) are produced more commonly in the Morden-Morris area than in other regions of the province. Alternatives to Group 1 herbicides are available to control wild oat in these crops.

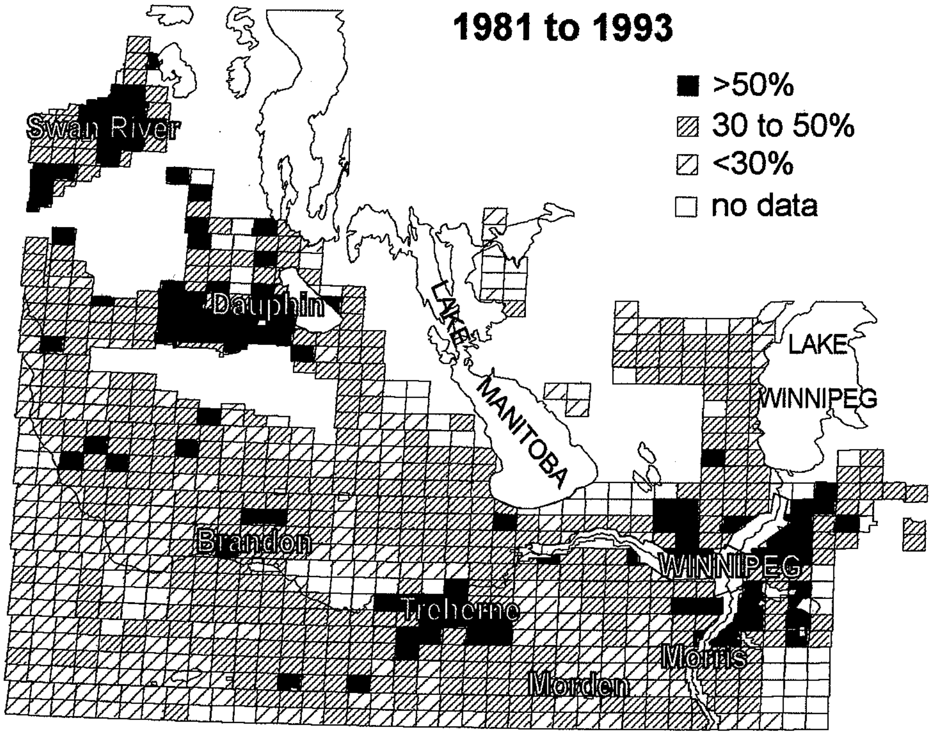

Figure 3-3. Average Group 1 herbicide use by township for the period 1981 to 1993.

The townships close to the towns of Swan River, Dauphin, Treherne, and Winnipeg had the highest Group 1 herbicide use (Figure 3-3). These areas correspond to the locations of the first documented cases of Group 1 resistant wild oat in 1990 and green foxtail in 1991 (Heap et al. 1993; Heap and Morrison 1996). This finding emphasizes that resistance is more likely to develop in areas where Group 1 herbicides are used frequently.

Group 1 use in each township, calculated on a five year moving average underscores the constant increase in Group 1 use (Figure 3-4). The proportion of township at high and medium risk increased from one five year period to the next. Between 1981 and 1985, over 80% of the townships were in a low risk category, and just 5% in a high risk category (Figure 3-4 and Figure 3-5a). Between 1985 and 1989, the proportion of townships at high risk for the development of Group 1 resistance increased to 20% while the proportion of townships at low risk dropped to 40% (Figure 3-5b). Between 1989 and 1993, 40% of the townships were at high risk while only 12% were at low risk (Figure 3-4 and Figure 3-5c).

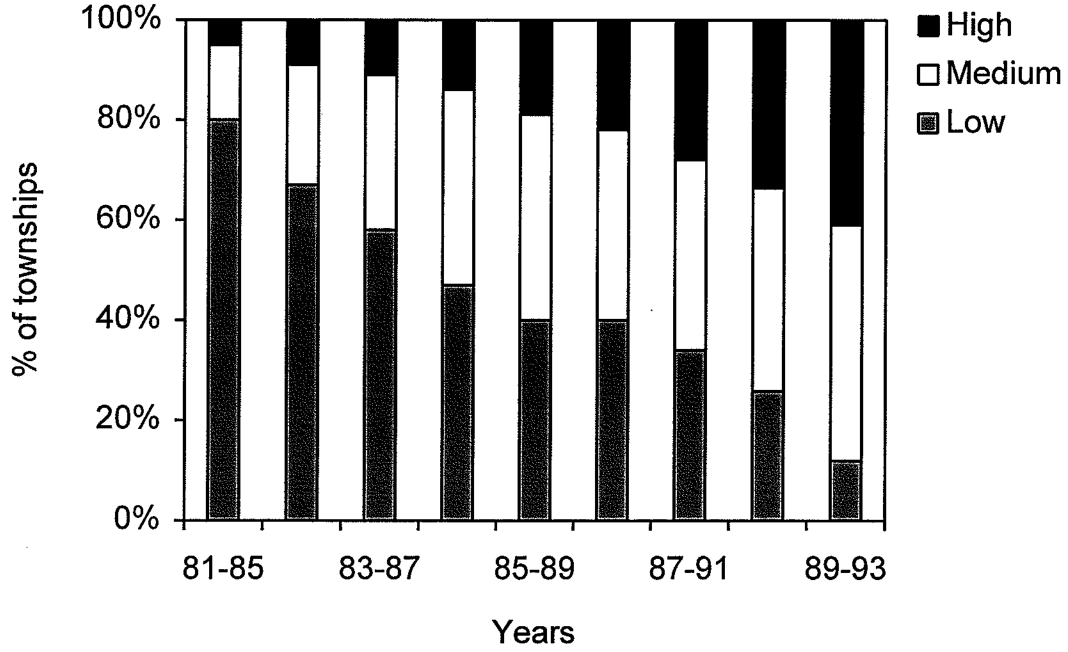

Figure 3-4. Distribution of the percentage of townships in each risk category based on five year moving averages of Group 1 herbicide use.

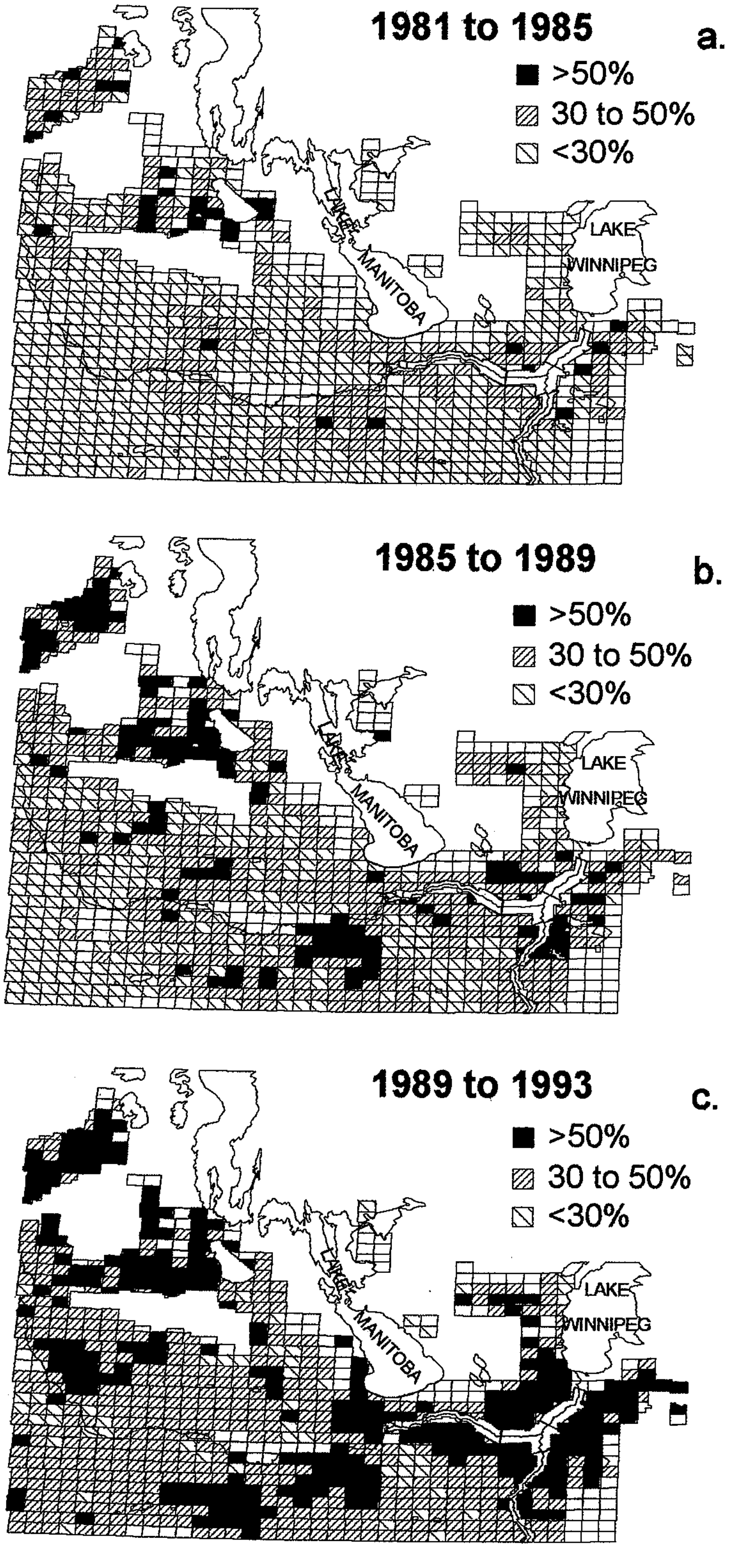

Figure 3-5. Average Group 1 herbicide use by township for three 5 year intervals begining in 1981, 1985, and 1989.

Townships close to the towns of Swan River, Dauphin, Treherne, and Winnipeg had high Group 1 herbicide use in all 5 year time periods (Figure 3-5). As indicated previously, these areas also correspond to the location of the first identification of Group 1 resistant wild oat and green foxtail. The first areas affected with Group 1 resistant weeds not only had a high use of Group 1 use between 1981 and 1993 (Figure 3-3), but were also the first areas to adopt Group 1 herbicides (Figure 3-5a). Areas north-west and south of Brandon, and south of Lake Manitoba only moved into a high risk category after 1989 (Figure 3-5c).

From 1989 to 1993, Group 1 use increased throughout the south-western part of the province (Figure 3-5). This increase followed the identification of a number of sites with trifluralin-resistant green foxtail in this region (Morrison et al. 1989). Widespread occurrence of trifluralin-resistant green foxtail compelled producers to use only Group 1 herbicides for the control of this weed. In doing so, producers are now selecting for populations with resistance to Group 1 herbicides in addition to Group 3 resistance. Already two green foxtail population are resistant to both Group 1 and Group 3 herbicides (Friesen 1994).

The introduction of new Group 1 herbicides has also exacerbated the rapid evolution of the resistance problem in Manitoba (Table 3-1). New active ingredients, as well as improved formulations, have increased the efficacy of control for the Group 1 herbicides (Tardif and Powles 1995). An increase in efficacy accelerates the change from a susceptible to a resistant population by enhancing the selection pressure (Gressel and Segel 1990).

In an attempt to predict future trends in the spread of resistant wild oat in the province, Group 1 herbicide use in each township between 1990 and 1993 was compared with use prior to 1990. Table 3-2 summarizes the Group 1 use trends between 1990 and 1993 in townships classified as high, medium or low risk areas between 1981 and 1989. Of the 74 townships considered to be at high risk between 1981 and 1989, 22 showed an increase in Group 1 herbicide use by at least 5% for the 1990 to 1993 period. In the early 1990's, 12 townships had in excess of 75% of the fields sprayed with Group 1 herbicides annually (data not shown). However, 32 (40%) and 20 (30%) of the townships showed the same or decreased use of Group 1 herbicides, respectively. The majority of the townships where Group 1 use declined between 1990 and 1993 are located near the towns of Swan River, Dauphin, Treherne, and Winnipeg (Figure 3-6). This reduction in Group 1 herbicide use is probably related to the fact that several producers in these areas had first hand experience with herbicide resistance since 1990 and realized the necessity of switching to other products with different modes of action.

Table 3-2. Group 1 herbicide use trends between 1990 and 1993 in townships classified as high, medium, or low risk areas for Group 1 resistance development for the period between 1981 and 1989.

1981 to 1989 (no. of townships)	Use trend	1990 to 1993
High Risk (74)	increase	22
	same	32
	decrease	20
Medium Risk (336)	increase	278[z]
	same	48
	decrease	10
Low Risk (347)	increase	324[y]
	same	19
	decrease	4

[z] 184 townships rated at high risk in 1990-93

[y] 95 and 208 townships rated as high and medium risk, respectively, in 1990-93

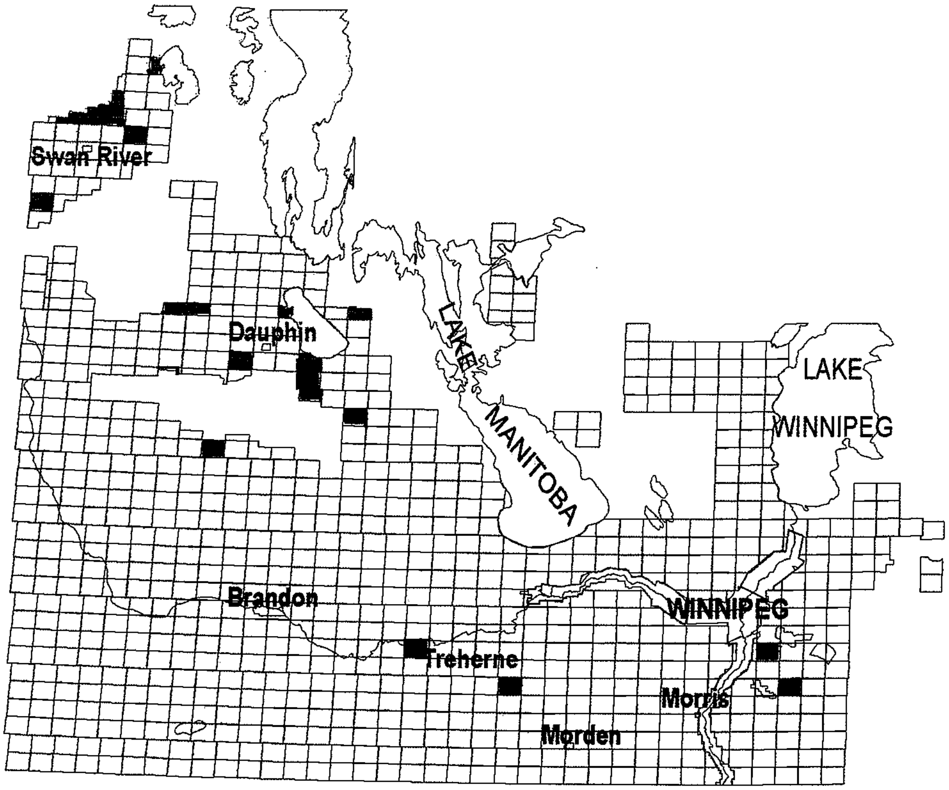

Figure 3-6. Townships having a decrease in Group 1 use of at least a 5% between 1990 and 1993 compared to 1981 and 1989.

Increased Group 1 herbicide use during the early 1990's was particularly high in the townships classified as being at medium risk between 1981 and 1989. Of 336 townships at medium risk, 278 (83%) had at least a 5% increase in Group 1 herbicide use between 1990 and 1993. Over half (184) would now qualify as being high risk townships with more than half of the fields sprayed annually with a Group 1 herbicide. A similar tendency was observed for the 347 townships classified at low risk between 1981 and 1989; in 324 (93%) of the townships, Group 1 use increased by at least 5% in the 1990's, with 95 and 208 being designated as high and medium risk, respectively.

3.4. Conclusion

Areas with a high Group 1 use history corresponded with the locations of the first known resistant populations of wild oat and green foxtail. The data corroborate the fact that repeated application of herbicides with a single mode of action will result in the development of herbicide resistant weeds.

Despite an increase in the number of cases of Group 1 herbicide-resistant weeds, a majority of Manitoba producers have continued to rely on these herbicides to control wild oat and green foxtail in the early 1990's. In 1993, 98% of the flax fields were sprayed with a Group 1 herbicide. One in two sprayed fields received a Group 1 herbicide annually between 1991 and 1993.

A slight reduction in Group 1 use occurred between 1990 and 1993 in townships that were classified as being at high risk from 1981 to 1989, probably in response to the developing problems of resistant weeds. Nevertheless, Group 1 herbicide use in the 1990's has increased significantly in areas rated as low and medium risk between 1981 and 1989. Over time, the heavy reliance on Group 1 products throughout the province may seriously hinder the usefulness of these products in Manitoba. Based on Group 1 use histories, it is inevitable that the problem of weed resistance will become increasingly troublesome in Manitoba. The active promotion of herbicide rotation and a reduction in herbicide use through adoption of integrated weed management techniques, including varied crop rotations and cultural practices, will be essential to delay further occurences of resistance.

4. A SURVEY OF ACCase INHIBITOR RESISTANT WILD OAT IN A HIGH RISK TOWNSHIP IN MANITOBA

Abstract. In the previous chapter, townships in Manitoba where ACCase inhibitors (Group 1 herbicides) had been used more than once every two years were identified as being at high risk for developing Group 1 herbicide resistant wild oat. In this study, a survey was conducted to determine the frequency of Group 1 resistant wild oat in one of these high risk townships. The selected township, 0810W, was located near Treherne in south-central Manitoba. The survey was conducted on 30 randomly selected cereal fields. On average, Group 1 herbicides had been sprayed on 61% of the 30 fields annually from 1983 to 1993. Nevertheless, none of the producers suspected a resistance problem in these fields. Wild oat were sampled at 80 m intervals on a predefined grid pattern across whole fields. Wild oat densities were recorded and seeds were collected from 0.25 m^{-2} quadrats. Seeds were also collected from conspicuous wild oat patches occurring outside the spaced quadrats. Plants were determined to be susceptible or resistant to fenoxaprop-P and/or sethoxydim using a seed bioassay procedure. Results from the structured survey indicated that resistant wild oat occurred in nine fields. Densities in quadrats containing resistant wild oat were generally higher than in quadrats with susceptible wild oat. By combining the results of the structured survey with the patch collection, resistance was detected in 20 out of the 30 fields. While resistant weeds generally occurred in small patches, in two of the fields resistant plants occurred over much larger areas. The evidence suggests that as many as two fields in three in high risk townships in Manitoba may harbour Group 1 resistant wild oat.

Résumé. Dans une étude antérieure, les townships au Manitoba traités plus d'une année sur deux avec des produits inhibiteurs de l'ACCase (herbicides du Groupe 1) ont été évalués à risque élevé de résistance de la folle avoine aux herbicides du Groupe 1. Dans la présente étude, un inventaire dans un des townships à risque élevé de résistance a permis de déterminer la fréquence de la folle avoine résistante aux produits du Groupe 1. Le township 0810 à l'ouest du méridien principal, situé près de Treherne au centre-sud du Manitoba, a été choisi pour l'étude en question. Trente champs de céréales ont été sélectionnés au hasard pour effectuer l'inventaire. En moyenne, on avait traité 61 % des 30 champs annuellement avec des herbicides du Groupe 1 entre 1983 et 1993. Malgré ce fait, aucun des agriculteurs ne soupçonnait de la résistance dans ces champs. Des échantillons de folle avoine ont été récoltés à des intervalles de 80 m selon une grille déterminée au préalable sur toute la surface des champs. Après avoir enregistré la densité de la folle avoine, on procédait à la collecte de graines à l'intérieur de quadrats de 0,25 m^{-2}. On récoltait également des échantillons de tous les regroupements denses de folle avoine situés à l'extérieur des quadrats. Au moyen d'un essai biologique, les plantes étaient ensuite évaluées comme susceptibles ou résistantes au fénoxaprop-p et/ou au séthoxydime. Les résultats de l'inventaire à intervalles réguliers ont indiqué que neuf champs contenaient de la folle avoine résistante. La densité dans les quadrats où se trouvait de la folle avoine résistante était généralement plus élevée que dans les quadrats contenant de la folle avoine susceptible. La mise en commun des résultats de l'inventaire à intervalles réguliers et de l'échantillonage de regroupements denses a signalé la présence de résistance dans 20 des 30 champs. Même si la folle avoine résistante apparaît généralement en petits regroupements denses, on a trouvé de la folle avoine résistante éparpillée un peu partout dans deux des champs selectionnés. Dans

les townships à risque élevé de résistance au Manitoba, les résultats indiquent qu'il pourrait donc y avoir de la folle avoine résistante aux produits du Groupe 1 dans deux champs sur trois.

4.1. Introduction

Wild oat populations resistant to acetyl coenzyme carboxylase (ACCase) inhibiting herbicides, also referred to as Group 1 herbicides in Canada, were first discovered in Manitoba in the fall of 1990 (Heap et al. 1993). These first cases of resistance evolved in fields that were repeatedly sprayed with Group 1 herbicides during the previous decade.

Even with the discovery of resistant weeds, producers in the 1990's have become increasingly reliant on Group 1 herbicides for grass weed control in broadleaf and cereal crops (Chapter 3). In 1993, over half the sprayed area in Manitoba was treated with a Group 1 product. This heavy reliance on Group 1 herbicides is a consequence of an ever-broadening choice of new, effective products and formulations, coupled with a reduction in use of soil-applied herbicides in response to increasing concerns about soil conservation (Goodwin, 1994; Morrison and Bourgeois 1995).

In the preceding chapter, Group 1 herbicide use histories were compiled from the Manitoba Crop Insurance Corporation (MCIC) database for 739 townships in Manitoba from 1981 to 1993 (Chapter 3). The townships were designated as being at low, medium, or high risk for Group 1 resistance development, in relation to the frequency of Group 1 use. The objective of this survey was to determine the frequency of occurrence of herbicide resistance in a high risk township as a follow-up to the analysis of the MCIC database (Chapter 3).

4.2. Materials and methods

In Manitoba, townships are 6 miles by 6 miles forming 36 square miles or sections, each of which is further divided into four quarters. Legal land descriptions define properties by quarter, section, range, and township. For example, SE010810W refers to the south-east quarter of section 1 in range 8 and township 10 west of the prime meridian.

For this study, high Group 1 use was the principal criterion for selecting a township to be surveyed. Group 1 herbicide use was determined from field histories compiled in the MCIC database (Chapter 3). The township was selected out of a total of 96 that were classified as being at high risk of developing Group 1 herbicide resistant weeds. The high risk classification was based on annual Group 1 herbicide use on greater than 50% of sprayed fields between 1981 and 1993. Other criteria in selecting a suitable township to survey included a high proportion of cultivated fields, ready access to most fields by car, and a convenient location within 2 hours driving time of the University campus.

The township selected for the survey was 0810W in south-central Manitoba. The township includes the town of Treherne, 110 km south-west of Winnipeg on Provincial Highway #2. An average of 55% of the sprayed fields in this township were treated with a Group 1 herbicide between 1981 and 1993. For this period, 0810 W ranked in 43rd position among 729 townships in Manitoba in terms of Group 1 herbicide use.

The survey was conducted on 30 randomly selected fields of cereal grains (except tame oat). The 144 quarters (4 quarters x 36 sections) in the township were randomly selected, and the first 30 on which a cereal crop other than tame oat was planted were selected for the survey. Upon choosing the fields, producers were contacted to obtain field histories.These field histories are compiled in Appendix 2. For all fields, except 4, 12, and 17, herbicide use and cropping histories were obtained for the previous 11 years. Producers were asked in person about known cases of resistant weeds in the region and if any action had been taken to prevent resistance evolution on their farm.

The survey was conducted between August 20 and 29, 1993. Two sampling procedures were followed. Firstly, a systematic sampling on a regular grid system was used. This consisted of sampling at regular intervals along equally spaced, parallel transects. Samples were collected every 100 paces (approximately 80 m) (Figure 4-1). A distance of 10 to 100 paces was randomly selected from the field border to the first transect (distance x on Figure 4-1) using a table of random numbers (Gomez and Gomez 1984). The distance from the field border to the first sampling site on the first transect was also randomly set between 10 and 100 paces (distance y on Figure 4-1). Thereafter, sampling sites on a transect were equally spaced every 100 paces. In order to achieve sample distribution in staggered rows, the distance from the field border to the first sampling site on the second transect was 50 paces less than on the first transect (distance y/2 on Figure 4-1). Sampling was not conducted within 10 paces of a field border. Field maps are included in Appendix 3.

The second procedure was less structured and entailed sampling of suspicious wild oat patches in the field whether or not they occurred along a transect. Suspicious patches refer to conspicuous, irregularly shaped patches of wild oat, possibly indicative of resistance (Heap and Morrison, 1990). The locations of these patches were established relative to the nearest systematic sampling site (Appendix 3).

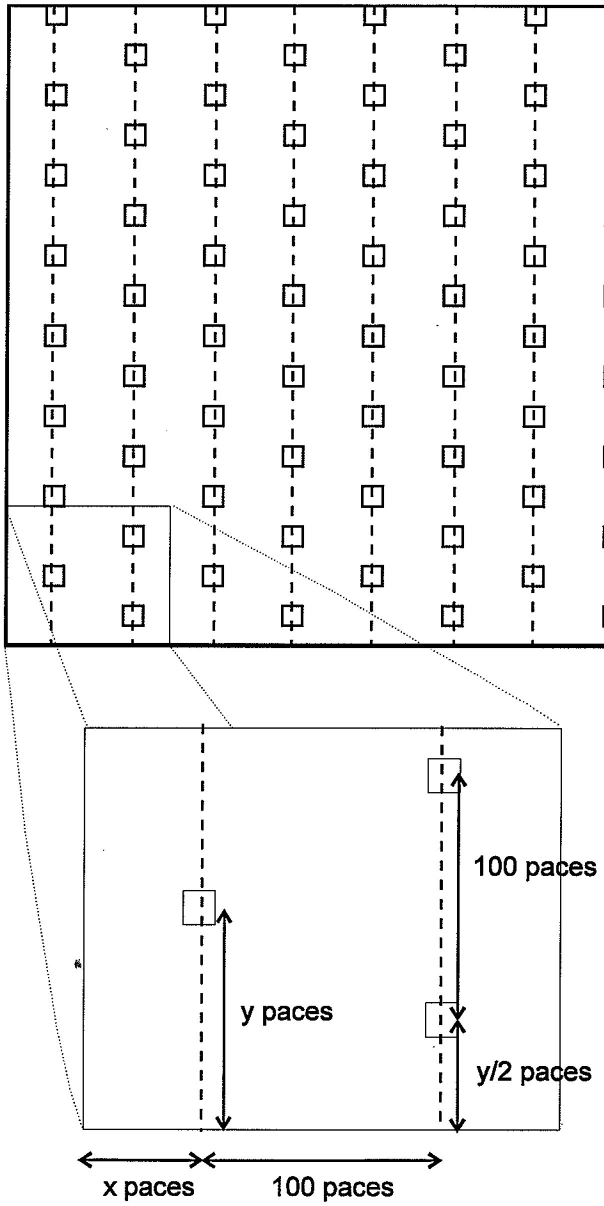

Figure 4-1. Description of the systematic survey procedure. Note: x and y are random numbers, between 10 and 100 paces.

At each sampling site, wild oat seeds were collected from plants occurring within a 0.5 x 0.5 m quadrat, and stored separately in paper bags including a label identifying the site location. Wild oat densities were recorded from the systematic sampling sites by counting the number of plants per quadrat. Exact densities were not recorded in the suspicious patches but in many instances they exceeded 100 plants m^{-2}. Field uniformity was calculated from the systematic survey as the number of sample sites in which wild oat occurred, expressed as the percentage of the total number of sites per field (Thomas and Wise 1989). A low field uniformity value is indicative of the patchy distribution in the field which reduces the chance of finding wild oat in any specific quadrat. Mean site density per field represented the average number of wild oat per systematic sampling sites in which wild oat was present (Thomas and Wise 1989). Mean site density was calculated for the total number of wild oat sites and for the number of resistant wild oat sites in a field. The mean field density, or the average number of wild oat per site over the total number of sites, was not estimated because the number of sampling sites per ha was too low. Marshall (1978) suggested a minimum of 18 samples per ha were necessary to calculate a precise mean field density.

After collection, the seed samples were stored for approximately one year at room temperature in order for the seeds to break dormancy. Screening for Group 1 herbicide resistance was conducted following the methodology developed by Murray et al. (1996). Samples were screened with fenoxaprop-P ethyl, an aryloxyphenoxy-propionate, and sethoxydim, a cyclohexanedione. Concentrations used for screening were 10μm for fenoxaprop and 5μm for sethoxydim. Agar[1] medium containing one of the two herbicides was poured into plexiglass germination boxes 13.5 by 12.5 by 3.5 cm in size.

The wild oat seeds were dehulled by hand and soaked in distilled water at 5°C for 24 hours prior to placing them on the agar. Ten seeds per sample were placed crease-down on a slit in the agar. By placing the seeds on a slit, radicle penetration into the agar and consistency of the results were improved compared to placing them on a smooth surface (data not shown). The germination boxes were left at 25°C in the dark for five days at which time the plants were scored as being either susceptible or resistant based on observations of coleoptile and root development. Samples were considered susceptible whenever the roots and shoots of all plants were inhibited by both herbicides. Samples were deemed resistant if five or more seedlings produced roots and shoots when exposed to either herbicide. Intermediate results were labeled as unknown.

To provide a definite classification of the unknowns, living seedlings were transplanted from the germination boxes into labeled rows in wooden flats (46 by 56 by 14 cm deep) filled with the soil mixture described by Murray et al. (1995). The flats were placed in a growth-room at 21/15 C with a 16/8 h day/night regime and an irradiance of 480 μE $m^{-2}s^{-1}$ PPFD. Two weeks after transplanting, the seedlings were at the 3-leaf stage and were sprayed in a cabinet sprayer. The sprayer was equipped with a flat fan nozzle that delivered 117 l ha^{-1} at 275 kPa in a single pass. Discriminatory rates were 150 g ha^{-1} of fenoxaprop and 110 g ha^{-1} of sethoxydim. Flats sprayed with fenoxaprop contained only seedlings that were transplanted from fenoxaprop-treated agar. Likewise, sethoxydim was used on seedlings rescued from sethoxydim-treated agar. Finally, three weeks

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

after spraying, the samples were confirmed to be resistant when at least one plant survived either chemical treatment.

4.3. Results and discussion

Since 1984, Group 1 herbicide use in the study area was well above the provincial average (Figure 4-2). The average annual Group 1 herbicide use on the 30 surveyed fields was 61% from 1983 to 1993. Analysis of the MCIC database indicated a similar use average (62%) on the whole township for the same period. In other words, fields were sprayed with a Group 1 herbicide almost two years out three, on average, for 11 years prior to the study. Group 1 herbicide use frequency varied from as few as 4 years out of 11 (field 2) to as many as 11 years out of 11 (field 5). A formulated mixture of triallate and trifluralin was the most common non-Group 1 wild oat herbicide used in the area. Flamprop, difenzoquat, and triallate were rarely used because these herbicides do not control green foxtail. Since both wild oat and green foxtail are a problem in this area of the province, producers tended to rely primarily on Group 1 herbicides year after year. However, a sharp decline of Group 1 herbicide use in 1992 and 1993 may be indicative of producers experiencing resistance problem and initiating herbicide rotations.

Although all producers knew of at least one case of herbicide resistance in the area, only three acknowledged having resistant weeds on their farm during the interviews. Of these three, only one reported wild oat resistant to Group 1 herbicides. The two others reported resistant green foxtail to be the problem; one to dinitroanilines and the other to Group 1 herbicides. No one was aware of any resistance problem on the 30 surveyed fields. While the majority of farmers knew about the benefits of herbicide rotation to delay resistance evolution, the lack of herbicide options to control both wild oat and green foxtail was a major impediment to them practicing herbicide rotation. A minority indicated that they were satisfied with the performance of Group 1 herbicides and would deal with the resistance problem when it happened. These attitudes are similar to those reported in the results of a survey conducted by Goodwin (1993) which identified why farmers did not adopt herbicide rotation practices.

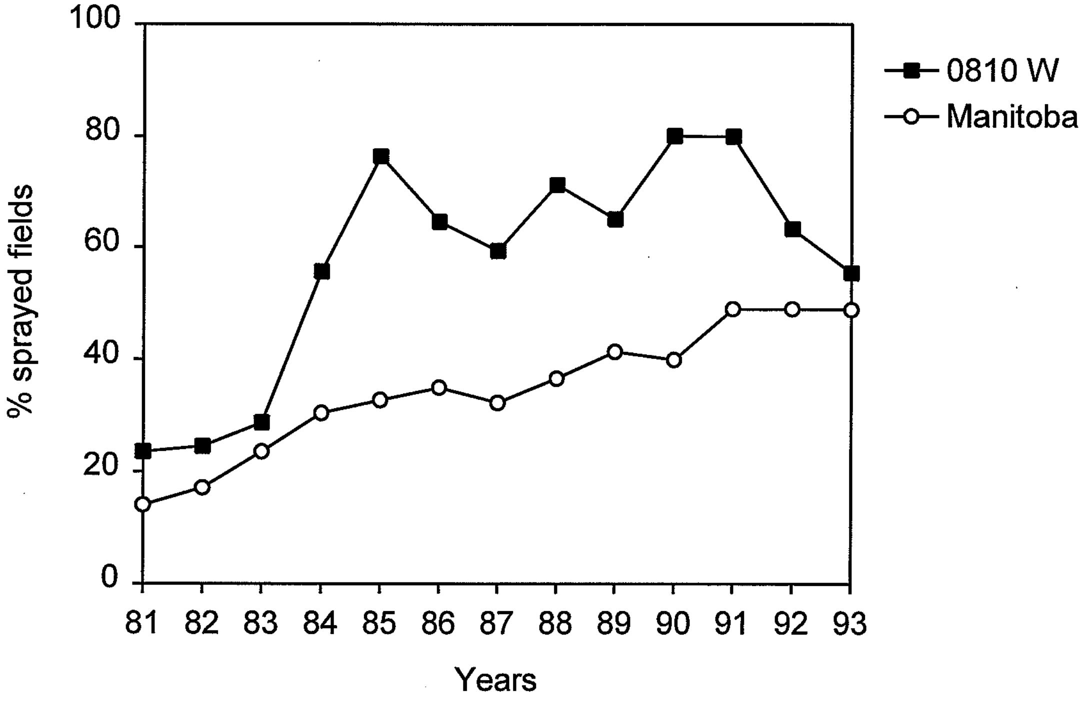

Figure 4-2. Average Group 1 herbicide use in the township 0810W and Manitoba as a proportion of sprayed fields from 1981 to 1993.

The cool and wet conditions that prevailed during the spring and summer of 1993 (data not shown) provided ideal growing conditions for wild oat (Sharma and Vanden Born 1978). Wild oat plants occurred in 339 of the 1655 sampling sites in the systematic survey of the 30 fields, corresponding to an overall uniformity of 23%. In other words, there was a 23% chance of finding wild oat in one of the systematic sampling sites. The uniformity in individual fields varied from 0 to 56.7% (Table 4-1). From the results of the systematic survey, only fields 8, 9, and 20 were free of wild oat. The average wild oat density per sampling site with wild oat present was 16.9 plants m^{-2}. The average density was higher in fields that were not sprayed in 1993 in comparison to the fields that were sprayed. The overall average densities per site where wild oat was present were 14.2 and 21.3 plants m^{-2} for fields sprayed with Group 1 herbicides and with no wild oat herbicides, respectively. On the other hand, there was no difference in wild oat uniformity between those fields which were treated in 1993 and those which were not treated (Table 4-1). In fact, wild oat occurred in more than half the sampled sites in fields 4, 13, 18, and 22, even though the fields had been sprayed with Group 1 herbicides. Overall, herbicide application in 1993 reduced wild oat populations but did not eliminate them completely in parts of the fields. Generally, the use of herbicide reduces weed densities and uniformity by increasing spatial aggregation (Johnson et al. 1995). The absence of a relationship between herbicide use and wild oat uniformity in this study can be related to the presence of resistant wild oat that were not affected by the herbicide application. In addition, a cool spring may have promoted flushes of susceptible wild oat after the application of the herbicide.

Table 4-1. Summary by field of the results of the Group 1 resistant wild oat survey in 0810W in 1993.

			Systematic survey						
Field	1993 herbicide[z]	Freq. Group 1[y] (*n*)	wild oat present[x] (*n*)	U[w] %	D[v] (all) plant m^{-2}	R[u] (*n*)	D[t] (R) plant m^{-2}	Total samples[s] (*n*)	Total R[r] (*n*)
1	none	5	15	37.5	14.9	0		21	0
2	none	4	27	61.4	26.2	0		31	0
3	none	5	3	13.0	13.3	0		10	0
4	G1	-	24	53.3	7.8	1	12.0	35	1
5	G1	11	19	22.9	11.2	0		43	1
6	no	4	35	43.8	35.6	0		42	1
7	G1	9	11	18.3	20.4	2	28.0	27	8
8	G1	7	0	0		0		3	1
9	G1	7	0	0		0		8	1
10	G1	5	1	3.3	8.0	0		3	0
11	none	7	1	3.3	4.0	0		2	0
12	G3+G8	-	6	7.6	6.7	3	6.7	22	8
13	G1	5	16	53.3	22.0	3	53.3	35	4
14	G1	6	10	12.5	10.8	0		31	0
15	flamprop	7	8	26.7	15.0	0		14	1
16	G1	8	7	9.2	8.6	0		23	2
17	G1	-	3	4.8	13.3	0		15	3
18	G1	7	37	52.9	12.8	5	33.6	68	15
19	none	6	19	36.5	15.4	1	44.0	35	2
20	G2	5	0	0		0		12	0
21	G3+G8	8	4	6.6	6.0	0		15	0
22	G1	7	17	56.7	11.3	0		30	0
23	none	7	19	24.7	7.4	0		60	1
24	none	5	5	8.8	15.2	0		21	3
25	G1	9	15	44.1	31.2	0		26	1
26	G1	9	13	18.6	8.3	7	9.1	36	19
27	G1	5	7	20.6	18.8	0		15	2
28	none	7	14	35.0	15.1	2	24.0	19	3
29	G3+G8	7	1	1.3	8.0	0		3	0
30	G1	8	2	2.5	12.0	1	16.0	7	5
All		6.7	339	22.6		25		712	82

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[z] wild oat herbicides used in 1993; G1=Group 1 (ACCase inhibitors); G2=Group 2 (acetolactate syntase inhibitors); and G3+G8=Group 3 (dinitroanilines) plus Group 8 (triallate and diphenzoquat)

[y] number of years with Group 1 herbicides used from 1983 to 1993 (11 years)

[x] number of sampling sites with wild oat present

[w] U = uniformity is the percentage of sites with wild oat in each field

[v] D(all) = density average per site with wild oat in plant m^{-2}

[u] R = number of resistant sites

[t] D(R) = density average per site with resistant wild oat in plant m^{-2}

[s] total samples = samples from the systematic survey plus samples from the patches

[r] number of resistant samples from the total samples

In addition to the 339 samples collected from the systematic survey, 372 samples were collected from distinct patches. Whereas 25 (7%) of the samples collected from the systematic survey proved to be resistant, 57 (14%) of the samples from patches were resistant. The higher number of resistant samples found during the patch survey can be explained by the greater chance of spotting small developing patches of resistant wild oat using this method as compared to the systematic survey. Maxwell et al. (1995) also noticed year to year variability in frequencies of triallate resistant wild oat in Montana in surveys resulting from a structured sampling methodology. Hence, in the early stages of resistance evolution, visual identification of resistant weed patches is more effective in discerning the problem than using a structured survey method.

Based on the systematic survey, resistant wild oat was identified in 9 of the 30 fields. However, when the results of the two survey procedures were combined, resistant wild oat occurred in 20 of 30 fields tested. Based on the systematic survey, wild oat was seemingly absent in field 8 and 9 with a 0% field uniformity. In these fields, the resistant patches were less than 10 m^2 in size (data not shown). The distance between samples was probably too large for these small patches to coincide with a sampling site in the systematic survey. However, they were detected while walking through the field and could have been destroyed by mowing. In contrast, resistance management in fields 18 and 26 would be difficult because of the widespread occurence of resistant wild oat across much of their area (Figures 4-3a and b).

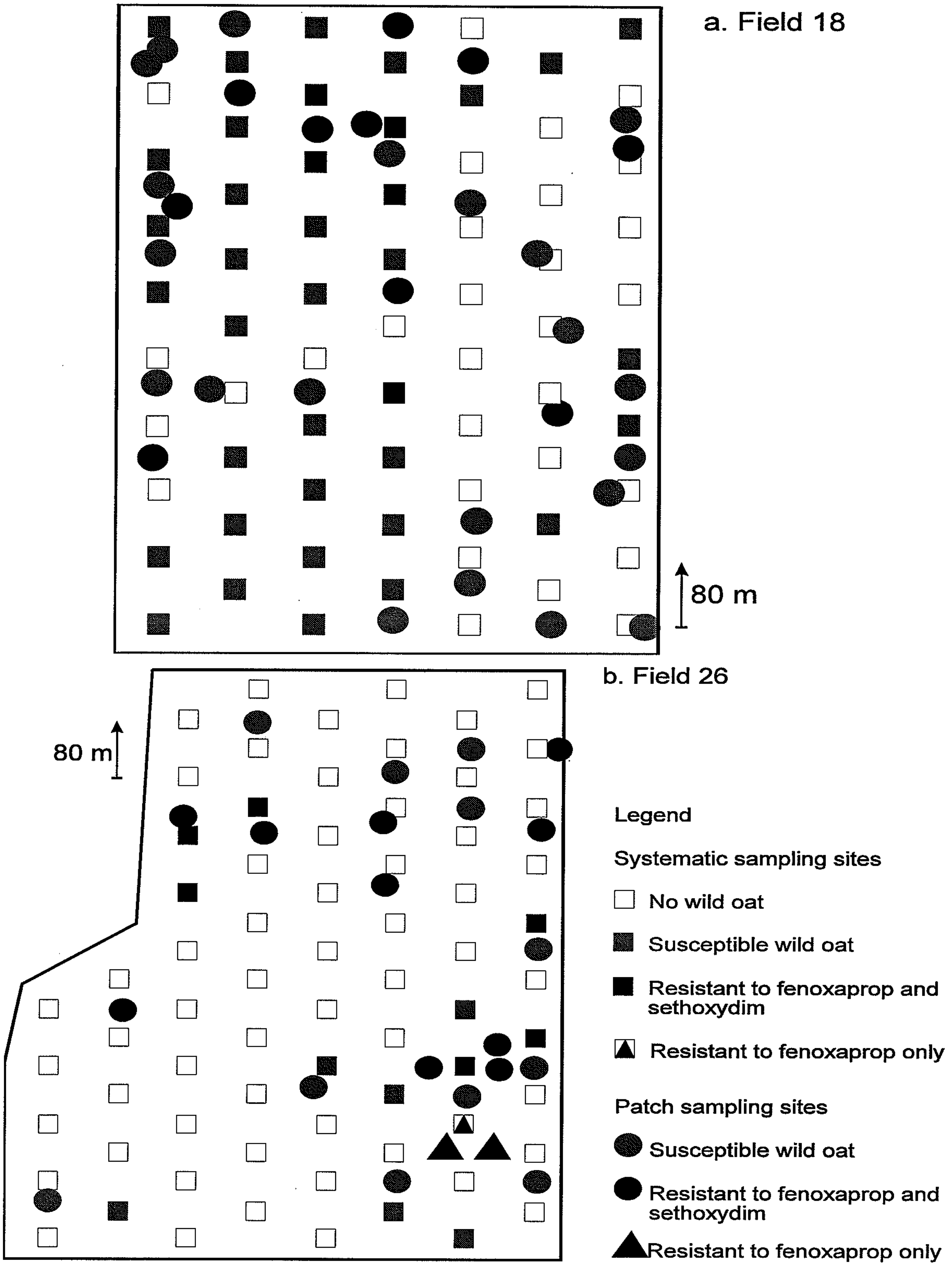

Figure 4-3. Map of resistant and susceptible wild oat a) in field 18, and b) in field 26. Note: The size of the symbols on the map are much larger than the actual size of the sampling sites.

Generally, wild oat tends to grow in dense patches in low-lying areas of fields (Sharma and Vanden Born 1978). Natural spread of wild oat patches is limited because seeds tend to shed out on the ground at maturity. Therefore, the evolution of new wild oat biotypes in a population, such as Group 1 resistant biotypes, is likely to be patchy because of little seed dispersal from the parent plants. This was observed in the surveyed fields since the density of resistant wild oat in sampling sites was generally higher than that of susceptible wild oat (Table 4-1). For example, in field 18 the density in sampling sites with resistant wild oat was 34 plants m^{-2} compared to 13 plant m^{-2} in the sampling sites with susceptible wild oat. Despite the apparent patchiness of resistant wild oat in fields, these plants have the potential to spread throughout the field. Recent research in Manitoba demonstrated that wild oat seed can be carried over 100 m distances by combine harvesters (S. Shirtliffe and M. Entz, personal communication).

As a rule, resistance is identified when 30% of the plants in the population are resistant (Gressel and Segel 1978). In the present study, over 30% of the samples in fields 7, 8, 12, 26, and 30 were resistant to Group 1 herbicides without producers being aware of the problem. Therefore, field scouting should be strongly encouraged in order to detect the first patches of resistant wild oat. Producers should keep a close watch on areas of fields with high wild oat densities. They should also be particularly attentive to the effectiveness of herbicides on wild oat patches. In case of poor control after herbicide treatment, patches could then be destroyed to reduce both seed return and seed movement to clean areas of the fields. Patch monitoring and destruction of areas of high weed density can effectively reduce the spread of resistance by reducing seed production from plants. The prevention of seed spread is clearly one of the most effective practices in combating resistance (Maxwell 1992; Thill et al. 1994; Morrison and Bourgeois 1995).

Whenever resistance is first evolving, the presence of resistant weeds is highlighted by herbicides which eliminate the majority of the susceptible weeds leaving only conspicuous patches of resistant plants. In the 30 fields surveyed here, resistant wild oat was detected in 81% of those sprayed with Group 1 herbicides in 1993. In contrast, resistant wild oat occurred in only 50% of the ones sprayed with a non-Group 1 herbicide.

The probability of finding resistant wild oat was greater in fields with a history of high Group 1 use (Table 4-2). Resistant wild oat was detected in fewer than half the fields in which Group 1 herbicides were used 6 times or less in the previous 11 years. However, resistant wild oat occurred in all fields where Group 1 products were used 9 or more times in the previous 11 years. These results demonstrate that herbicide rotation and/or a reduced reliance on one mode of action can effectively delay the evolution of resistant wild oat populations.

Seedling responses to herbicides were generally homogeneous within samples, and less than 10 samples contained both resistant and susceptible plants (data not shown). However, within certain fields, resistant patches occurred within a few paces of samples consisting only of susceptible seeds (Figures 4-3a and b). On the one hand, the absence of mixed population can be explained by the elimination of the susceptible plants by Group 1 herbicides applied in 1993. On the other hand, the absence of a mixed population may indicate that the resistant wild oat identified in the survey were in place for some time and displaced susceptible ones.

Table 4-2. Relationship between the number of fields with at least one resistant wild oat and Group 1 herbicide use from 1983 to 1993.

Years of Group 1 use from 1983-93	Number of Fields	
	Resistant	Susceptible
4	1	1
5	3	4
6	1	1
7	6	3
8	2	1
9	3	0
11	1	0

Marked differences in response to sethoxydim were observed among samples in field 26, where some samples were resistant to fenoxaprop and sethoxydim while others were resistant to fenoxaprop only (Figure 4-3b). Murray et al. (1995) reported that differences in cross-resistance patterns among populations were caused by different mutations at a single gene locus. However, prior to this study, there was no evidence in the literature of simultaneous evolution of distinctly different wild oat biotypes within the same field. Samples from field 26 clearly illustrate that herbicide resistance can evolve in a mosaic pattern of resistant sub-populations with different cross-resistance patterns. Upon confirmation of a resistant wild oat patch to some, but not all, Group 1 herbicides in a field repeatedly sprayed with Group 1 herbicides, one could be tempted to use Group 1 herbicides which are still active on that particular wild oat patch. Unfortunately, there is a chance that the field may harbour other patches of resistant wild oat which exhibit different resistance patterns. A frequent and radical change in herbicide mode of action or herbicide Group would provide the best alternative for a long-term weed management strategy and herbicide resistance avoidance.

4.4. Conclusion

Group 1 herbicides were used on more than 60% of the fields in township 0810W every year from 1983 to 1993. Despite the high use of herbicides, wild oat was present in all 30 fields surveyed. Worse still, 20 fields out of 30 had wild oat resistant to Group 1 herbicides, and producers were not aware of it. Although resistance was limited to one or a few isolated patches in most fields, the fact that resistance was present at all is cause for concern.

There is little doubt that resistance was identified at the early stage of development in most fields. The localization of the patches in the field and a continuous monitoring of their development should help producers curtail the spread of resistance. By doing so, producers will keep the option of using a Group 1 herbicide in the future. However, future use of Group 1 herbicides in fields comparable to 18 and 26 reported in this study is in jeopardy because of widespread resistant patches throughout these fields. Early identification of resistant wild oat patches can be a difficult task in light of the scale of farming operations in western Canada, where fields are frequently between 20 and 70 ha in size. In Manitoba, most of the resistant cases reported to date were first recognized only after resistant weeds caused a significant crop yield loss (Morrison and Devine 1994).

Development of resistance from small patches was emphasized in field 26 with the presence of two distinct resistant biotypes with different cross-resistance patterns occurring within 50 meters of each other. The patchy development of resistant wild oat emphasizes the importance of limiting the dispersal of seeds within and between fields.

5. FIELD AND PRODUCER SURVEY OF ACCase INHIBITOR RESISTANT WILD OAT IN MANITOBA

Abstract. In a previous chapter, 729 townships in Manitoba were differentiated as being at low, medium or high risk of selecting wild oat resistance to Group 1 herbicides based on herbicide use histories. In this study, 16 townships, representing the three risk categories, were surveyed in order to identify the number of resistant wild oat patches in each of them. As well, a questionnaire was mailed to farmers in these townships requesting information on their attitudes relating to herbicide resistance. The wild oat survey consisted of sampling seed from conspicuous wild oat patches visible from north-south roads in each township. A total of 533 samples were collected and screened with fenoxaprop-P and sethoxydim using a bioassay. An average of 8 resistant wild oat patches was found in each of the high risk townships. This rate was significantly higher than in low and medium risk townships where an average of less than one resistant wild oat patch per township was detected. The attitude of producers towards herbicide resistance was similar in all risk categories. However, the number of respondents suspecting Group 1 resistance on their farms was related to risk categories with producers in high risk areas suspecting the most cases of resistance.

Résumé. Dans une étude préalable, on avait utilisé les antécédents d'utilisation des herbicides pour déterminer le degré de risque (faible, moyen ou élevé) de résistance de la folle avoine aux herbicides du Groupe 1 pour 729 townships au Manitoba. Dans la présente étude, on a effectué l'inventaire de 16 townships, regroupant les trois catégories de risque, afin de déterminer le nombre de regroupements denses de folle avoine résistante dans chacun d'eux. De plus, on a envoyé un sondage par la poste aux agriculteurs vivant dans ces townships en vue de recueillir des renseignements concernant leur attitude vis-à-vis de la résistance aux herbicides. Pour effectuer l'inventaire, il fallait récolter des graines à partir de regroupements denses de folle avoine visibles depuis les chemins nord-sud de chaque township. Un total de 533 échantillons ont été récoltés et testés pour la résistance au fénoxaprop-p et au séthoxydime au moyen d'essais biologiques. En moyenne, on a découvert 8 regroupements de folle avoine résistante dans les townships à risque élevé de résistance. Ce résultat est considérablement plus élevé que dans les townships à risques faible et moyen où, en moyenne, on a identifié moins d'un regroupement de folle avoine résistante par township. L'attitude des agriculteurs concernant la résistance aux herbicides était la même, peu importe la catégorie de risque. Toutefois, le nombre d'agriculteurs interrogés qui soupçonnaient de la résistance aux herbicides du Groupe 1 était lié aux catégories de risque puisque les agriculteurs demeurant dans les régions à risque élevé soupçonnaient un plus grand nombre de cas de résistance.

5.1. Introduction

Only four years after ACCase inhibitor (Group 1) herbicide resistance was first identified in wild oat in Manitoba (Heap et al. 1993), the number of confirmed resistant populations is estimated to number in the hundreds (Morrison and Bourgeois 1995). Despite the growing number of resistance cases, producers remain reliant on Group 1 herbicides for post-emergence control of grassy weeds. In 1993, Group 1 herbicides were applied on approximately half of the sprayed fields in the Province (Chapter 3).

For wild oat, resistance evolution is closely tied to frequent use of Group 1 herbicides over periods of 7 to 10 years (Heap et al. 1993; Seefeldt et al. 1994). However, except for a study by Stephenson et al. (1990) on triazine resistance, there is almost no information relating regional herbicide use histories or cultural practices to the occurrence of resistant weeds. The primary impediment in conducting such studies is the lack of complete records necessary to calculate herbicide use frequencies over time. However, in Manitoba, the Manitoba Crop Insurance Corporation (MCIC) began compiling a database on management practices of insured fields in 1981. The database now includes herbicide use on over 750 000 fields between 1981 and 1993. Information in this database was used to segregate the Province into high, medium, and low risk areas for Group 1 herbicide resistant based on previous herbicide usage (Chapter 3).

Regional differences in herbicide use patterns may reflect differences in producers' attitudes towards herbicide resistance. In 1991, Manitoba Agriculture initiated a strong extension effort to slow the development of herbicide resistance weeds in the Province. Three years after new resistance management concepts were introduced, including herbicide grouping and the benefits of a "1 in 3 rotation" (Goodwin 1994), the use of Group 1 herbicides did not decline (Chapter 3). With the exception of information obtained from a phone survey (Goodwin 1994), little feedback is available regarding the impact of provincial recommendations to avoid or delay the occurrence and spread of resistant weeds.

In view of the above, this study was initiated with two objectives. The first was to conduct a patch survey of wild oat in representative townships to determine the frequency of Group 1 herbicide resistance in relation to their past Group 1 herbicide use histories. The second was to determine production practices and producers' attitudes relating to the problem of herbicide resistance in these same representative townships.

5.2. Materials and methods

5.2.1. Survey of wild oat patches

The survey of wild oat patches took place in 16 townships. The townships were selected on the basis of past Group 1 herbicide use for wild oat control. Herbicide use histories were drawn from the Manitoba Crop Insurance Corporation (MCIC) database (Chapter 3). The selected townships were categorized as being at a high, medium, or low risk of developing Group 1 herbicide resistance. In high risk townships, on average, Group 1 herbicides were applied to over 50% of the sprayed fields annually between 1981 and 1993. Likewise, in medium and low risk townships Group 1 herbicides were applied to between 30 and 50% and less than 30% of the sprayed fields, respectively.

Since wild oat is not normally a problem in south-western Manitoba, townships from this area were not included in the selection. The remainder of the agricultural land of the Province was divided into five regions, these being Winnipeg-Red River Valley, south-central (Portage and Treherne), Brandon-Neepawa, Dauphin, and Swan River districts. While considered desirable, it was not always possible to locate suitable townships in each risk category within each of the five regions. An additional high risk township (0810W near Treherne), was also sampled since it was the location of a detailed field survey conducted in 1993 (Chapter 4). The 16 selected townships are represented on a provincial map (Figure 5-1).

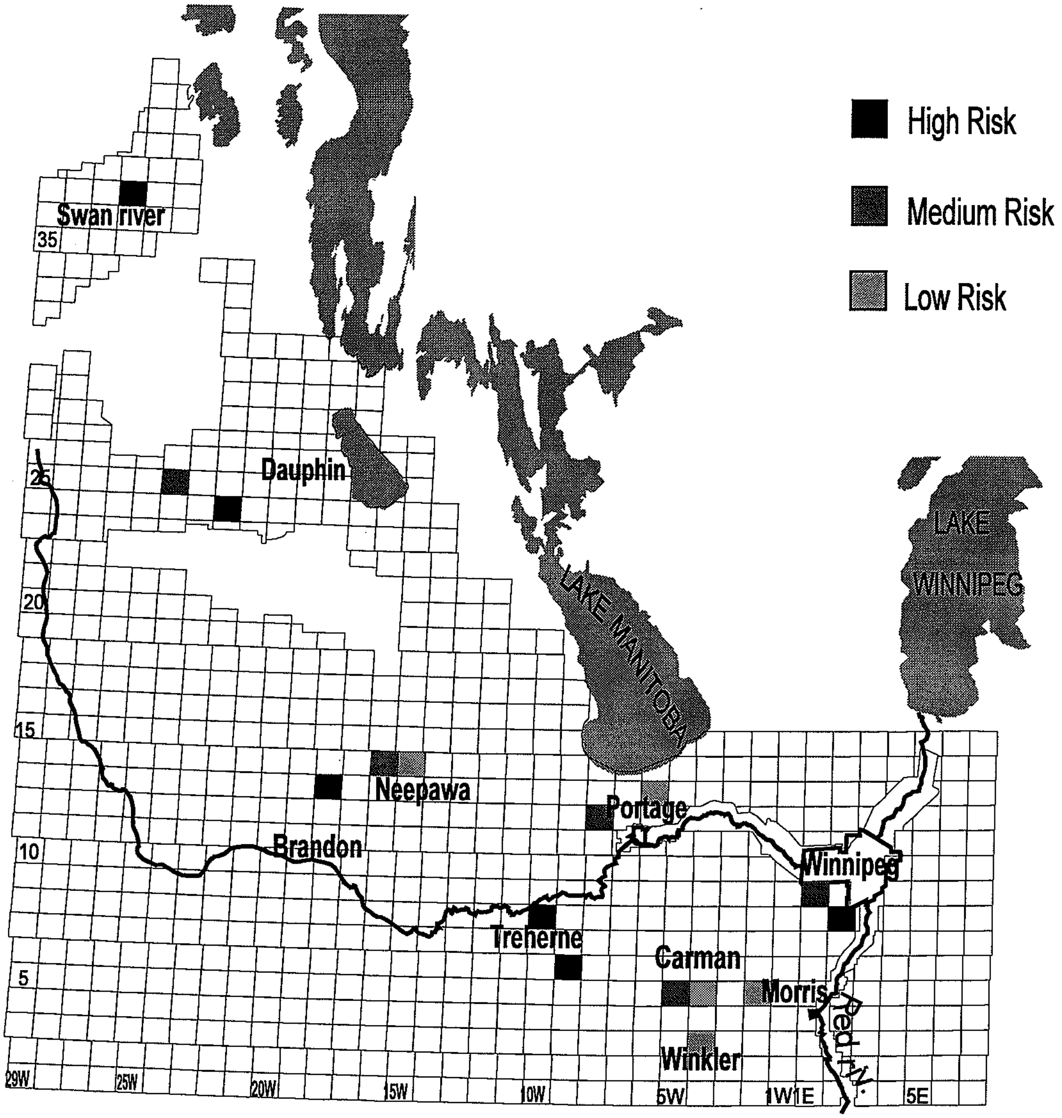

Figure 5-1. Location of the 16 surveyed townships on the provincial map.

Townships in Manitoba are 6 miles by 6 miles in area. A township is divided into 36 square miles referred to as sections. A grid of roads provided access to most of the sections within a township. The sampling procedure consisted of collecting wild oat seed from conspicuous patches spotted in the fields while driving along south-north mile roads. Approximately 200 g of seeds were hand-harvested, and placed in paper bags labeled with the legal land description and a diagram representing the location of the patch in the field. On five occasions, more than one suspicious patch occurred in a particular field. In these cases samples from each patch were kept separate.

Sampling occurred between August 8 and September 5, 1994. Differences in crop maturity from south to north determined the sequence in which townships were surveyed; the more southerly townships were surveyed almost 4 weeks before the most northerly township near Swan River.

Seed samples were stored at room temperature for 6 months in order to reduce seed dormancy. Screening for Group 1 resistance was conducted with fenoxaprop-P and sethoxydim using a bioassay technique (Murray et al. 1996). Agar medium was prepared with either 10μM of fenoxaprop-P or 5μM of sethoxydim and poured into Plexiglas containers. Seeds were dehulled by hand and soaked in chilled (5°C) distilled water for 24 hours. Prior to placing the seeds on the agar, the surface of the agar was slit with a scalpel. Seeds were placed crease down directly over the slits to improve seedling root development within the agar. For each herbicide, one row of 10 seeds per sample was placed on top of each slit in the agar. Each container included six rows of 10 seeds each.

After 5 days in the dark at 25°C, samples which showed only dying seedlings when exposed to both herbicides were rated as susceptible. When one or more seedling(s) survived one or both herbicides, resistance was confirmed by transplanting living seedlings from the agar into wooden flats and spraying them at the two leaf stage. Before and after spraying, the flats were placed in a growth room at a temperature of 21/15°C, a 16/8 h day/night regime and an irradiance of 480 μE m^{-2} s^{-1}. Seedlings that survived the fenoxaprop-P bioassay were sprayed with 150 g ha^{-1} of fenoxaprop-P whereas those that survived the sethoxydim bioassay were sprayed with 100 g ha^{-1} of sethoxydim.

Three weeks after spraying, samples with at least one surviving one or both herbicides were assessed as resistant.

Analysis of variance was conducted for the total number of wild oat patches in the three risk categories using a completely randomized design with unequal replication (Gomez and Gomez 1984). Least significant difference was used to determine differences between the means at $p<0.05$. The relationship between the average Group 1 herbicide use from 1981 to 1993 and the number of resistant patches in the townships was best described by an exponential function. The model fitted was

Equation 5-1. $$y = a \times e^{(b \times x)}$$

where y is the number of patches per township, x is the Group 1 herbicide use frequency between 1981 and 1993 in percent of sprayed fields, e is the base of the natural logarithm, a is the intercept of the regression on the y axis, and b is the slope of the inflection curve. Regression analysis was performed with the procedure Nonlin in SAS[1].

In order to determine a statistical difference in the number of resistant patches between risk categories, the data were linearized using a logarithmic transformation (Gomez and Gomez 1984). Analysis of variance and a least significant difference test were conducted on the transformed data. Finally, a correlation analysis was conducted between the transformed number of resistant patches in each township and the average Group 1 herbicide frequency calculated over periods of 1 to 13 years. The correlation coefficients r were used

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[1] SAS Version 6. 1985. SAS Inst. Inc., Box 8000, Cary, NC 27511-8000

as an indicator of the optimum number of years to be used in calculating Group 1 herbicide use frequency as an indicator of resistance selection.

5.2.2. Producers' view on resistance

A questionnaire was sent by Manitoba Agriculture to farmers in each of the surveyed townships in order to assess practices and attitudes related to herbicide resistance. The questionnaire consisted of 17 questions, an abridged summary of which is included in Table 5-1. The first three questions identified the location, size, and nature of the farming operations. Questions 5 through 13 assessed the severity of resistance from the producer's perspective. These questions also evaluated the producers' knowledge on concepts such as herbicide grouping and the "1 in 3 rotation" of herbicides. Herbicide grouping consists of a classification of herbicides according to their mechanism of action (Goodwin 1994). The "1 in 3 rotation" of herbicides promotes the use of herbicides from the same Group no more than once in three years (Morrison and Bourgeois 1995). The last four questions dealt with opinions and concerns about different cultural practices that might be used as alternatives to herbicide rotation as a means of combating resistance evolution.

Out of 720 surveys, 181 were returned, for a 25% response rate. Among the returned surveys, 17 were not analyzed because of unclear responses. Results were drawn from 59, 53, and 52 surveys returned from high, medium, and low risk townships, respectively. Responses are presented as the percentage of producers who responded to the survey grouped by risk category.

Table 5-1. Abridged list of the questions included in the producers' survey.

Question 2	What crops do you grow, and what is their approximate acreage?
Question 4	Which of the following herbicide rotations would qualify as a 1 in 3 rotation of herbicide groups? Hoe Grass[z] - Achieve[z] - Poast[z]; Hoe Grass[z] - Avenge[z] -Puma[z]; Hoe Grass[z] - Edge[z] - Assert[z]
Question 5.1	Do you suspect that you have herbicide resistance on your farm?
Question 5.2	If yes, has it been confirmed?
Question 5.3	To what weed and herbicide Group do you suspect (or know) that you have a resistance problem?
Question 6	Assuming that resistance is present on your farm, how serious of a problem is it to your farming operation?
Question 10	On how many fields are you practicing a 1 in 3 grassy herbicide Group rotation?
Question 12	If you don't already have the problem, do you believe that herbicide resistance could occur on your farm?
Question 14	Rate the following cultural practices in a weed management program. Crop rotation; Tillage; Competitive varieties; Early seeding; Good seedbed preparation; Cleaning equipment; Mowing weed patches and ditches or headlands.

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[z] Hoegrass (diclofop-methyl) trademark of Hoechst Schering AgrEvo; Achieve (tralkoxydim) trademark of Zeneca Corp; Poast (sethoxydim) trademark of BASF; Avenge (difenzoquat) trademark of American Cyanamid; Puma (fenoxaprop-P ethyl) trademark of Hoechst Schering AgrEvo; Edge (ethalfluralin) trademark of Dow Elanco.

5.3. Results and discussion

5.3.1. Survey of wild oat patches

A total of 533 wild oat seed samples were collected from the 16 townships. The number of samples per township varied from 12 to 61 (Table 5-2). The average number of samples per township in each risk category was 38, 32, and 29 for the high, medium and low risk categories, respectively. These values were not significantly different ($p=0.05$). Hence, past herbicide use histories had no influence on the number of wild oat patches found in any of the townships.

While the number of wild oat patches was unaffected by herbicide use history, the number of resistant patches was significantly different between risk categories ($p<0.05$). In the high risk townships an average of almost 8 Group 1 resistant wild oat patches were identified per township (Figure 5-2), ranging from 3 to 18 resistant patches (Table 5-2). In high risk townships 0609W and 0810W more than one-third of the patches were resistant (Table 5-2). The number of resistant wild oat patches in the medium and low risk townships were not significantly different (Figure 5-2). In low and medium risk townships, there was an average of less than 1 resistant wild oat patch per township with a minimum of 0 and a maximum of 2 (Table 5-2). There were 2 and 3 townships with no resistant wild oat patches in low and medium risk categories, respectively. Therefore, medium risk townships do not differ from low risk townships in terms of resistant wild oat frequency. However, over time the incidence of resistance is more likely to increase in medium risk townships than low risk ones. This is because Group 1 herbicide use has increased drastically in the medium risk townships since 1990 (Chapter 3).

Table 5-2. Results of the 1994 survey of wild oat patches in 16 townships of Manitoba.

Risk categories	Township	Patch numbers		
		Resistant	Susceptible	Total
High	3726W	6	26	32
	0810W	13	34	47
	0609W	18	39	57
	1318W	4	24	28
	0802E	3	41	44
	2422W	3	19	22
	Total	**47**	**183**	**230**
Medium	0901E	0	34	34
	1208W	2	23	25
	0505W	0	61	61
	1416W	1	20	21
	2524W	0	17	17
	Total	**3**	**155**	**158**
Low	1415W	1	18	19
	1306W	2	10	12
	0502W	0	46	46
	0504W	0	45	45
	0304W	1	24	25
	All Low	**4**	**143**	**147**

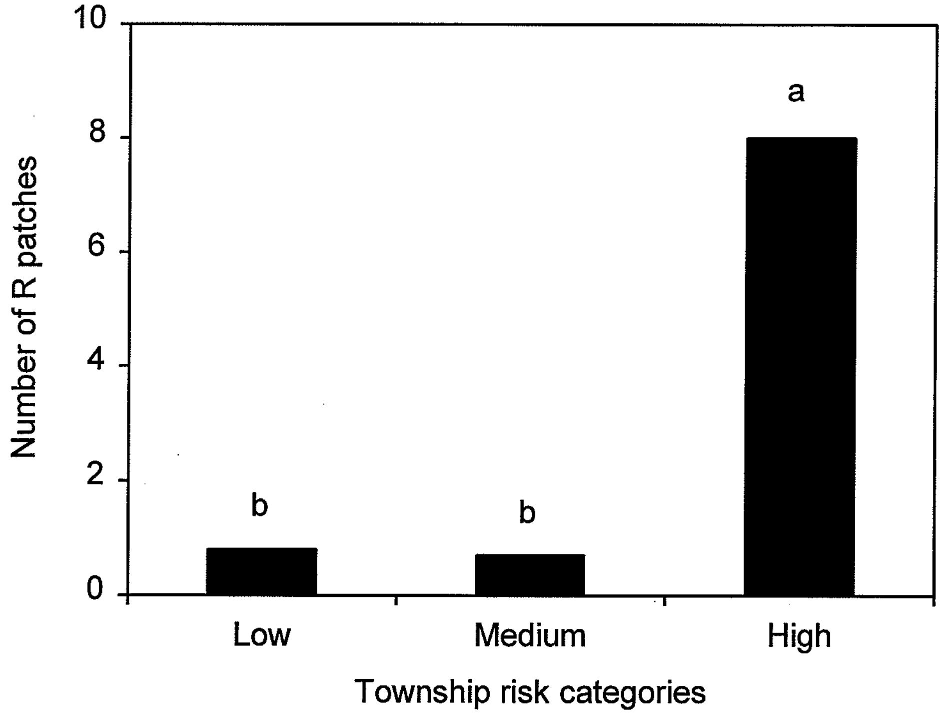

Figure 5-2. Mean number of resistant patches in townships grouped by risk categories.

The relationship between the number of resistant patches and Group 1 herbicide use frequency between 1981 and 1993 was described by an exponential function (Equation 5-1 and Figure 5-3). This function described the high number of resistant patches in townships with Group 1 use frequencies averaging more than 50% compared to the townships with less than 50% Group 1 use from 1981 to 1993. This relationship can be explained by a more intense selection pressure in the high risk townships compared to the low and medium risk townships. In high risk townships, there is a greater chance of a field being sprayed continuously with a Group 1 herbicide than in medium and low risk townships. Continuous use of a similar type of herbicide year after year is the leading cause of herbicide resistance evolution (Jaseniuk et al. 1996).

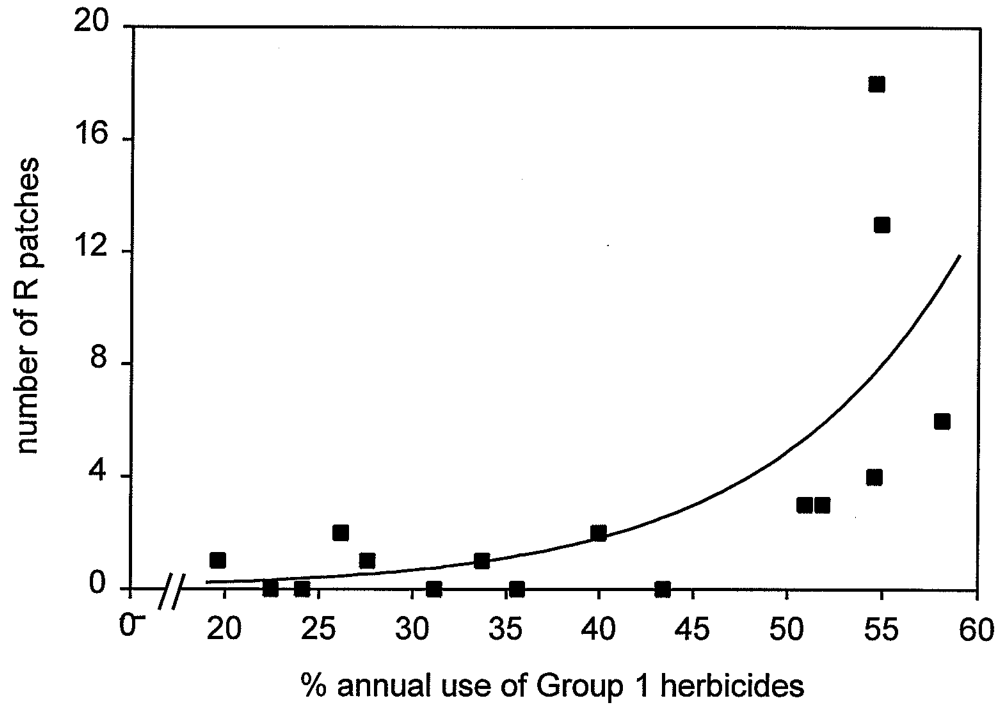

Figure 5-3. Regression analysis between the number of resistant patches and the annual frequency of Group 1 herbicide use from 1981 to 1993 in 16 townships. Parameter estimates: a=0.035 and b=0.099.

Despite exponential relationship between the number of resistant patches and Group 1 herbicide use frequency between 1981 and 1993, a large amount of variation was observed in the number of resistant patches in high risk townships (Table 5-2). Some of this variation may be related to the variation in frequency of Group 1 herbicide use in fields within each township. Other variation in the number of resistant wild oat patches may be related to herbicide use in the spring of 1994. In a previous study, herbicide use in the year of a wild oat survey was found to influence the chances of finding resistant wild oat (Chapter 4).

Correlation coefficients between Group 1 herbicide use histories over periods of 1 to 13 years and the number of resistant patches were variable (Table 5-3). Group 1 herbicide use frequencies calculated over periods of 8 to 13 years accounted for more than 64% (r=0.8) of the variation observed in the number of resistant patches. The percentage variation was reduced to less than 48% (r=0.68) with Group 1 use frequencies calculated for periods of less than 3 years. In other words, an increase in the number of years increases the accuracy of the establishment of risk of selection of Group 1 herbicide resistant wild oat. A minimum of 4 years of herbicide use history is necessary to establish accurate risk areas (Table 5-3).

Table 5-3. Correlation coefficients between the number of resistant patches and Group 1 herbicide use histories over periods of 1 to 13 years.

	Period used to calculate Group 1 herbicide use frequency (years)												
	13	12	11	10	9	8	7	6	5	4	3	2	1
r	0.81	0.81	0.82	0.82	0.82	0.82	0.79	0.79	0.78	0.78	0.68	0.64	0.61

r: correlation coefficient

Table 5-3 emphasizes the paramount importance of keeping records of herbicide use in order to access risk of selecting for resistant weeds. Moreover, the data validate the risk area established in a previous study (Chapter 3), where the definition of risk areas was based on herbicide use histories between 5 and 13 years. Group 1 herbicide resistant weeds were assumed to be more frequent in an area with a high Group 1 herbicide use frequency compared to an area with a low use of these herbicides. Evolutionary models supported this assumption (Gressel and Segel 1978, Jasieniuk et al 1996). These models were also used in implementing herbicide rotations strategies to delay the onset of resistance. Therefore, the survey data validate resistant evolution models and clearly indicate that the high use frequency of Group 1 herbicides increase the probability of resistant weed selection.

The frequency of resistant patches in high risk townships was 21% (Table 5-2). This result is slightly higher than the 1993 field survey conducted in 0810W where 15% of the conspicuous patches turned out to be resistant (Chapter 4). Similar to that survey, some samples collected in this survey were resistant to fenoxaprop-P but not to sethoxydim. As previously reported by Heap et al. (1993) the level of resistance to both herbicides was variable from sample to sample (data not shown) and is consistent with the evidence that different alleles induce Group 1 herbicide resistance in wild oat (Murray et al. 1995).

5.3.2. Producers' view on resistance

For the most part, cropping practices were similar between the three risk categories. The number of crops per farm averaged between three and five. Wheat, canola, flax, and barley were the most common crops. In townships in the low risk category farmers produced more "special" crops such as peas, corn, and sunflowers than those in medium and high risk townships. This can be explained by the high heat unit requirement of corn and sunflowers which limit these crops to the Carman-Morris-Winkler area where 3 out of 5 low risk townships were selected. The diversity of the farming operations within each risk category made it difficult to identify specific management practices related to the presence of herbicide resistant wild oat except for herbicide use history.

Concepts of herbicide grouping and the "1 in 3 rotation" were generally well understood by all producers (Table 5-4). Overall, 76% (96 out of 126) of the respondents that provided an answer were able to point out the correct "1 in 3 rotation" out of three scenarios. The majority of the producers likely answered the question without reference material indicating a good understanding of the herbicide Group concept. Nevertheless extension effort is still necessary for the producers that provided a wrong answer and for those providing no answer at all.

Table 5-4. Producers' answers on determining the correct "1 in 3 rotation" of herbicide among three choices.

	Risk categories High	 Medium	 Low	 All
	.... number of respondents			....
Hoe Grass-Achieve-Poast	5	7	5	17
Hoe-Grass-Avenge-Puma	6	6	1	13
Hoe Grass-Edge-Assert[z]	**37**	**27**	**32**	**96**
Total answers	48	40	38	126
No response	11	13	14	38
Total respondents	59	53	52	164

[z] Correct answer.

On average, respondents claimed to practice a "1 in 3 rotation" of grass weed herbicides on 67% of their fields (Table 5-5). The proportion of fields claimed to be in a "1 in 3 rotation" was higher in the high and medium risk townships (72% and 70%, respectively) than in the low risk townships (59%). These statements are not supported by regional use figures compiled by MCIC which showed that Group 1 herbicides were used on 50% of sprayed fields in the

1990's (Chapter 3). However, this discrepancy may relate to the fact that many producers have only started to practice a "1 in 3 rotation" in the past three or four years. Overall, the number practicing a "1 in 3 rotation" of herbicides was significantly higher than reported by Goodwin (1994), indicating a increase in adoption of this practice from 1993 to 1994.

In all three risk categories, crop rotation was the principal impediment in the adoption of a "1 in 3 rotation" of herbicides (Table 5-5). In high risk townships, five respondents did not practice herbicide rotation because they already had resistance, resulting in a lack of suitable rotation options. In medium and low risk townships, the convenience, as well as habit, of using particular herbicides and poor autumn weather interfering with application of pre-plant incorporated herbicides discouraged 15 producers from rotating herbicides.

A total of 13 respondents neglected to practice herbicide rotation because "they did not have herbicide resistance on their farm" (Table 5-5). This result indicates that the concept of "1 in 3 rotation" of herbicides is viewed by these farmers as a curative, rather than as a preventive, method.

Table 5-5. Average number of fields on which a "1 in 3 rotation" of herbicides was practiced, and reasons for not adopting this practice.

	Risk categories			
	High	Medium	Low	All
	.... %			
Fields in a "1 in 3 rotation" of herbicides	72	70	59	67
Reasons for not rotating	.... number of respondents			
• Crop rotations	9	7	12	28
• Not enough options because of resistance in the field	5	0	0	5
• Not enough options because of zero-tillage	1	0	0	1
• Not necessary because no resistance	5	3	5	13
• Fall weather interfering with soil applied herbicides	0	5	4	9
• Convenience and habit	0	5	1	6

Altogether, 42% of the respondents suspected resistance on their farm. In high risk townships, 49% of the producers suspected resistance. In medium and low risk townships, the proportion dropped to 38%. The replies were based on any weed / herbicide resistance combination, so it was not possible to directly relate Group 1 herbicide use to the number of farmers who suspected resistance on their farm. However, a breakdown of herbicide resistance by weed and herbicide Group clearly indicated a strong relationship between risk categories and Group 1 resistant wild oat (Figure 5-4). Group 8 (triallate and difenzoquat) resistance in wild oat was suspected in both medium and low risk townships which may indicate that some producers in these townships relied more heavily on Group 8 herbicides rather than on Group 1 herbicides for wild oat control. Although Group 3 (trifluralin and ethalfluralin) resistant green foxtail was suspected in all townships regardless of risk category, a higher proportion was suspected to occur in low risk townships compared to the medium and high risk townships. High Group 1 herbicide use decreases the need for Group 3 or Group 8 herbicides since Group 1 herbicides control both wild oat and green foxtail, and provide a wide choice of tank mix options to control a broad spectrum of broadleaf weeds. Surprisingly, respondents acknowledged that only 16 out of 68 suspected resistant cases had been confirmed by provincial extension staff or industry representatives. Producers seemed to rely mostly on their own judgment to assess herbicide resistance.

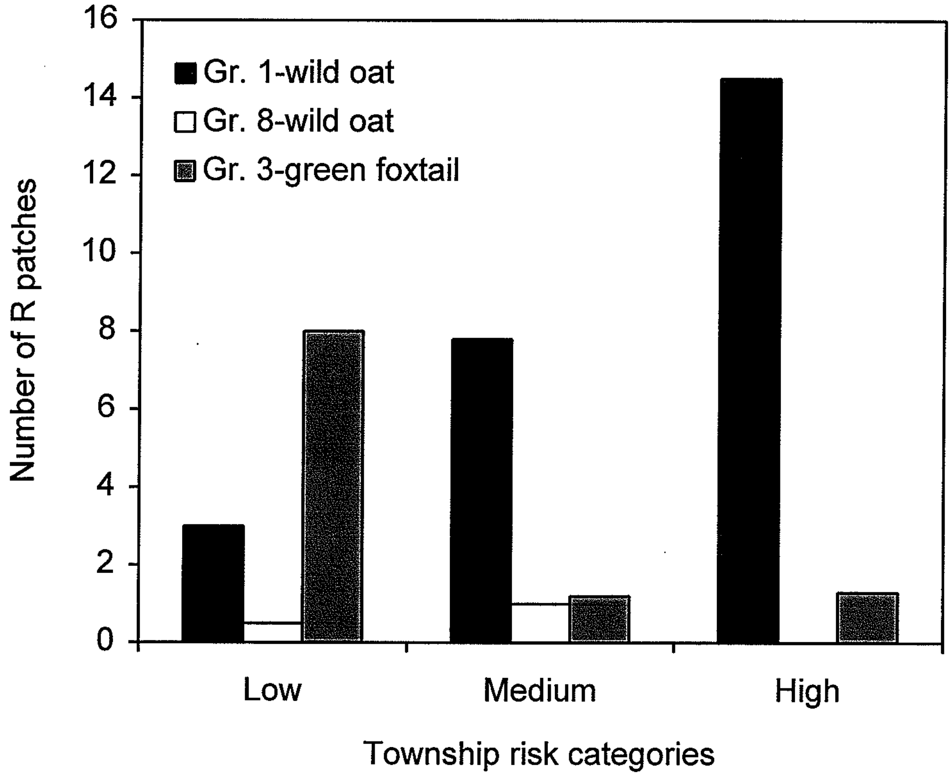

Figure 5-4. Percent of respondents suspecting resistance on at least one of their fields, grouped by risk categories.

Although 42% of the producers suspected resistance on their farm, only 15% of them reported resistance as being a very serious problem. The majority of the respondents (74%) felt that herbicide resistant weeds were of some concern, but 12% reported that they were of little or no concern. Nevertheless, nobody doubted the fact that resistance could happen on their farm. This attitude was significantly different than that portrayed by 9 regional weed specialists out of 31 that were interviewed by Goodwin (1994). According to these 9 specialists, the majority of producers believed that herbicide resistance could not happen in their region (Goodwin 1994). This radical change in attitude may have changed with the rapid development of resistance cases throughout the Province and heavy media coverage on the problem.

In order to reduce the selection pressure imposed by herbicides on the weed population, cultural techniques are promoted by scientists and provincial extension specialists to control weeds. Out of a choice of 7 techniques, respondents classified crop rotation as the most important cultural practice in a weed management program. The ranking was unanimous in all three risk categories. Seedbed preparation and tillage were ranked second and third overall. Cleaning equipment and mowing weed patches, ditches, and headlands were ranked in fourth and fifth position. These practices reduce weed seed production and dispersal. Finally, early seeding and the use of competitive varieties were ranked in sixth and seventh positions. These last two practices improve the competitive ability of crops against weeds. Clearly, next to herbicides producers rely mostly on traditional cultural practices such as crop rotations, and cultivation (including soil seedbed preparation) to control weeds. Surprisingly, producers put little stock in the ability of the crop itself to compete with weeds despite clear evidence to the contrary (Kirkland and Hunter 1991).

Reducing weed seed production and seed dispersal by mowing weed patches and cleaning equipment may have ranked low because they are time consuming. This fact was summed up by one respondent who stated that "with my farm growing in size there is less time available for things like spot spraying".

In general, producers' attitudes toward herbicide resistance was not related to past herbicide use history. The only significant relationship was observed between Group 1 herbicide use frequency and the number of suspected Group 1 resistant wild oat sites (Figure 5-4). However, producers in medium risk townships clearly overestimated the number of Group 1 resistant wild oat cases compared to the actual number of sites found in the road side survey. Producers in medium risk townships may be aware that Group 1 herbicide resistance in wild oat is inevitable because of a significant increase of Group 1 herbicide use in these townships since 1990 (Chapter 3).

5.4. Conclusion

The number of resistant wild oat patches in townships was related to the frequency of Group 1 herbicide used in the past 13 years. The relationship was best described with an exponential function since the number of resistant patches was significantly greater where Group 1 herbicides were used annually on more than 50% of the fields since 1981. No difference in the number of resistant patches was established between low and medium risk townships. However, medium risk townships probably have the highest potential for Group 1 herbicide resistance because of recent increased use of these herbicides.

Producers' attitudes toward herbicide resistance was similar in the three risk categories. The "1 in 3 rotation" of herbicides was accepted as a management practice on over half of fields of the survey respondents. However, 8% of respondents did not include herbicide rotation in their weed management strategy because they claimed not to have resistance. Producers also rely mostly on traditional cultural practices to help them delay the evolution of herbicide resistant weeds. Improvement of crop competition against weeds by using competitive varieties and early seeding were considered the least important cultural techniques in weed management strategies.

Although the majority of the producers have adopted a "1 in 3 rotation" of herbicides in recent years, the rotation options become limited because of the presence of resistant wild oat. This now is the case for approximately one field out of five in high risk townships in Manitoba.

6. CHARACTERIZATION OF CROSS-RESISTANCE PATTERNS IN ACCase INHIBITOR RESISTANT WILD OAT

Abstract. The purpose of this study was to determine cross-resistance patterns among wild oat lines resistant to acetyl-coenzyme A carboxylase (ACCase) inhibitors, and to determine if any cross-resistant type was more common than another. Discriminatory concentrations of two aryloxyphenoxy-propionates (APP) and three cyclohexanediones (CHD) were determined using a petri-dish bioassay. These concentrations were then applied to 82 resistant wild oat (*Avena fatua* L.) lines identified in previous Manitoba studies. In addition, two resistant standards (UM1 and UM33), and a susceptible standard (UM5) were included in the experiments. Coleoptile lengths, expressed as percentages of untreated controls, were used to assess the level of resistance to each herbicide. Large variations were observed among wild oat lines and herbicides. However, cluster analysis summarized the relationship between the five herbicides (variables) and the wild oat lines into three main cross-resistance types. Type A included wild oat lines with high resistance to APP herbicides and no, or low, resistance to CHD herbicides. Types B and C included those with low to moderate resistance and high levels of resistance to all five herbicides, respectively. Type C was the most common cross-resistance type. Relationships among herbicides were determined using pairwise correlation and principal component analysis (PCA). All correlations were high between APP herbicides and between CHD herbicides but not between APP and CHD herbicides. The first two axes of the PCA accounted for 88.35 % of the total variance with the first axis correlated to the CHD herbicides and the second axis correlated to the APP herbicides. In the PCA, wild oat lines were segregated into the three types identified in the cluster analysis. Although CHD and APP herbicides bind at the same locus on the ACCase, resistant wild oat lines respond differently to them.

Résumé. Le but de la présente étude était de déterminer les types de résistance croisée qui existent entre 82 lignées de folle avoine résistante aux inhibiteurs de l'acétyl coenzyme-A carboxylase (ACCase) et de determiner si l'un des types est plus commun que les autres. On a pu établir des concentrations discriminatoires de deux aryloxyphénoxy-propionates (APP) et de trois cyclohexanediones (CHD) au moyen d'essais biologiques conduits en boîtes de Pétri. Ces concentrations ont ensuite été utilisées pour traiter les 82 lignées de folle avoine résistante découvertes au Manitoba dans des études préalables. Deux témoins résistants (UM1 et UM33) ainsi qu'un témoin susceptible (UM5) ont également été testés. La longueur des coléoptiles, exprimée comme pourcentage par rapport aux témoins non traités, a permis d'évaluer le niveau de résistance à chacun des herbicides. De grandes variations ont été observées entre les lignées de folle avoine et les herbicides. Cependant, une analyse typologique a établi que la relation entre les cinq herbicides (variables) et les lignées de folle avoine correspondait à trois types principaux de résistance croisée. Parmi le type A figuraient les lignées de folle avoine hautement résistantes aux herbicides APP et ayant aucune résistance ou une résistance faible aux herbicides CHD. Le type B comprenait les lignées peu ou moyennement résistantes aux cinq herbicides, tandis que le type C incluait les lignées hautement résistantes aux cinq herbicides. Le type C constituait le type de résistance croisée le plus courant. L'analyse corrélative et l'analyse en composantes principales (PCA) ont permis d'établir les relations entre les herbicides. Les corrélations étaient élevées parmi les herbicides APP, d'une part, et parmi les herbicides CHD, d'autre part, mais elles étaient plutôt faibles

entre les herbicides APP et CHD. Les deux premiers axes de l'analyse PCA correspondaient à 88,35 % de la variance totale, le premier axe étant corrélé aux herbicides CHD et le deuxième, aux herbicides APP. L'analyse PCA a séparé les lignées de folle avoine selon les trois mêmes types établis par l'analyse typologique. Malgré le fait que les herbicides CHD et APP se fixent au même locus de l'ACCase, les lignés de folle avoine résistante se comportent différemment entre eux.

6.1. Introduction

Aryloxyphenoxypropionate (APP) and cyclohexanedione (CHD) herbicides inhibit acetyl coenzyme A carboxylase (ACCase), an enzyme essential to fatty acids biosynthesis (Devine and Shimabukuro 1994). Diclofop methyl (an APP herbicide) was the first commercially available ACCase inhibitor to be registered as a post-emergent herbicide in western Canada, and has been used frequently in Manitoba since 1980. With the registration of additional APP and CHD herbicides over half the sprayed acreage in Manitoba was treated with these herbicides by the early 1990's (Chapter 3).

The first reported case of wild oat resistance to ACCase inhibitors occurred in a field that had been repeatedly sprayed with APP's and CHD's over the previous ten years (Heap et al. 1993). Resistance to ACCase inhibitors has also been found in a number of other grassy weeds including Italian ryegrass, *Lolium multiflorum* (Stanger and Appleby 1989); blackgrass, *Alopecurus myosuroides* (Moss 1990); goosegrass, *Eleusine indica* (Marshall et al. 1994); large crabgrass, *Digitaria sanguinalis* (Wiederholt and Stoltenberg 1995), and giant foxtail, *Setaria faberi* (Stoltenberg and Wiederholt 1995). In Manitoba, resistance has also been reported in green foxtail (Heap and Morrison 1996).

Physiological evidence indicates that APP's and CHD's both bind to the same region of the target enzyme (Rendina et al. 1989). However, different cross-resistance patterns characterize ACCase inhibitor resistance in weeds (Stanger and Appleby 1989; Moss 1990; Heap et al. 1993). In wild oat populations, the levels of resistance vary from no resistance to 300-fold resistance to specific ACCase inhibitors (Heap et al. 1993). Murray et al. (1995) demonstrated that the difference in cross-resistance between two resistant wild oat populations (UM1 and UM33) was genetically controlled by semi-dominant point mutations at a single nuclear gene locus. An altered ACCase enzyme confers herbicide resistance in these two wild oat populations (Devine, personal communication). However, the actual site and type of mutation have not been identified on the gene encoding for ACCase.

A total of 150 resistant wild oat lines collected in two separate field surveys have been identified in previous studies (Chapters 4 and 5). Resistant wild oat lines occurred primarily in areas where APP and CHD herbicides were used repeatedly. Only a few of these have been characterized based on cross-resistance patterns (Heap et al. 1993). The objectives of this study were to: a) determine the discriminatory rates of the herbicides clodinafop, clethodim and tralkoxydim on the wild oat line UM5, and b) determine cross-resistant patterns, and quantify differences in herbicide resistance patterns, among 82 Manitoba wild oat lines collected in recent field surveys. The main purpose of the study was to describe the range in response types among lines and to determine which type, if any, was most common.

6.2. Materials and methods

6.2.1. Determination of Discriminatory Rates.

A discriminatory concentration for a specific herbicide is the minimum herbicide concentration required to distinguish susceptible from resistant lines. Murray et al. (1996) developed seed bioassays for rapid identification of resistance to fenoxaprop-P (an APP herbicide) and sethoxydim (a CHD herbicide). Presence or absence of resistance in wild oat was based on coleoptile and radicle length of seedlings placed on an agar medium[1] containing a discriminatory concentration of herbicide. In this study, additional bioassays were developed for the APP herbicide clodinafop, and for the CHD herbicides clethodim and tralkoxydim. The dose response curves were developed using the susceptible wild oat population UM5, using procedures comparable to those described by Murray et al. (1996). The origin of UM5 is described in Heap et al. (1993).

The bioassays were conducted on agar media with concentrations of 0, 0.05, 0.1, 0.15, 0.25, 0.5, 0.75, 1, 1.5, 3, and 5 µmol for clodinafop and clethodim, and 0, 1, 2, 3, 4, 5, 7.5, 10, 20, 30 µmol for tralkoxydim. Plexiglas boxes, 13.5 x 12.5 x 3.5 cm deep, contained 3 rows of five seeds each of UM5 wild oat on a 1 cm-thick agar medium containing the herbicide. Plates were left in the dark for 5 days at 21°C. After the five days, the length of the coleoptiles of 10 seedlings were measured. These 10 seedlings were picked at random from among those that germinated from the 15 plated seeds. The same process was replicated three times with new batches of agar each time. A mean coleoptile length was calculated for each dose and herbicide. Dose response curves were

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

fitted to the means using a sigmoidal model with the NONLIN procedure in SAS[2]. The model fitted was:

Equation 6.1. $$y = k/(1+e^{bg}x^{b})+d$$

where y is the dependent variable (mean coleoptile length in mm), x is the herbicide concentration (mol), e is the base of the natural logarithm, *k* is the difference between the upper and lower asymptotes, *d* is the lower asymptote, and *b* and *g* determine the shape of the curve. Parameter estimates were considered to be statistically significant at p=0.05 where the standard error was less than half the numerical value of the estimate (Koutsoyiannis 1977). A large standard error of a parameter is indicative of a poor estimation or that the equation is not representative of the dataset.

6.2.2. Screening of Herbicide Resistant Lines.

Eighty-two lines were selected from among resistant wild oat populations collected in two field surveys (Chapters 4 and 5). Forty-three of these lines were collected in a field survey conducted in 1993 in a single township (0810W) near Treherne, Manitoba (Chapter 4). The remaining 39 lines were from among those collected in a more extensive survey conducted in 1994 (Chapter 5). These lines were from nine townships located throughout the southern part of the province. In addition, three populations (UM1, UM5, and UM33) were included as "standards". UM1 and UM33 are both resistant to ACCase inhibitors, but have different cross-resistance patterns (Heap et al. 1993). UM5 was included as a susceptible standard. The origins of UM1, UM5, and UM33 were described by Heap et al. (1993). In total, the 85 wild oat lines were screened at the

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

discriminatory rates of two APP herbicides (fenoxaprop-P and clodinafop) and three CHD herbicides (sethoxydim, clethodim and tralkoxydim).

Fifteen hand-peeled seeds were placed on media without herbicide, and at the discriminatory concentration of each herbicide. A similar procedure to the one used for the determination of the discriminatory concentration was used for each of the wild oat lines. Coleoptile lengths were measured to differentiate responses of the 85 lines to each of the five herbicides. The mean coleoptile length was calculated for each wild oat line - herbicide combination. Values were expressed as a percentage of the coleoptile length of untreated controls, since coleoptile lengths of untreated wild oat varied considerably from one population to another (Murray et al. 1996).

6.2.3. Statistical Analysis.

The wild oat lines were classified into cross-resistance types using a multivariate cluster analysis procedure. Cluster analysis produces a hierarchical dendrogram summarizing the relationships between objects based on the measured variables. In our analysis, the objects are the 85 wild oat lines and the variables are the 5 herbicides. Variable values are herbicide resistance expressed as a percentage of the coleoptile length of untreated control. The clustering algorithm minimized the increase in error sum of squares at each fusion (Ward's method, Podani 1994) based on the 'similarity ratio' resemblance measure. Cluster analysis was performed using the SYNTAX multivariate package (Podani 1994).

The herbicide cross-resistant types delineated by cluster analysis were tabulated by township, and were mapped onto township and field maps for the wild oat lines originating from 0810W. These maps were used to discuss possible relationships among resistant wild oat populations.

Pairwise Pearson product-moment correlations (r) were computed between the five herbicides to examine trends in cross-resistance patterns among the lines. This correlation matrix was also input into principal component analysis (PCA; Podani 1994). PCA is a linear multivariate ordination method that produces a parsimonious, low-dimensional representation of the variation present in the original five-dimensional variable (herbicide-resistance) space. If the original variables are correlated, PCA takes advantage of these correlations to obtain new, derived variables (principal components, or PCA axes) that offer a more efficient summarization of the major trends present in the data. A PCA 'biplot' consists of the coordinate position of each individual (wild oat line), together with vectors indicating the direction of variation of each variable (herbicide resistance). PCA was performed using the SYNTAX package (Podani 1994).

6.3. Results and discussion

6.3.1. Determination of discriminatory rates.

As reported for fenoxaprop-P and sethoxydim (Murray et al. 1996), increasing dosages of clodinafop, clethodim and tralkoxydim resulted in a reduction of coleoptile length in the susceptible UM5 wild oat line (Figure 6-1). Parameter estimates of the dose response curves are provided in Table 6-1. The discriminatory concentration represented the lowest dosage that resulted in at least 80% inhibition of coleoptile elongation compared to the untreated control. Using this criterion, the discriminatory concentrations were 1.5 µmol for clethodim, 3 µmol for clodinafop and 5 µmol for tralkoxydim. Discriminatory concentrations of 10 µmol for fenoxaprop-P and 5 µmol for sethoxydim were determined using comparable procedures (Murray et al. 1996).

Table 6-1. Parameter estimates (std. errors in parentheses) describing the coleoptile length of UM5 seedlings on agar medium treated with 5 ACCase inhibitor herbicides.

	g	*b*	*d*	*k*
Clodinafop	0.8(0.1)	1.8(0.3)	10.4(2.7)	55.5(3.9)
Fenoxaprop-P^{z}	1.8(0.2)	1.6(0.4)	6.8(1.8)	48.2(4.2)
Clethodim	1.0(0.1)	2.4(0.3)	8.3(1.3)	54.0(2.0)
Sethoxydim^{z}	0.3(0.1)	2.0(0.3)	7.8(0.8)	30.8(1.2)
Tralkoxydim	-0.9(0.1)	2.7(0.3)	9.7(1.6)	55.9(2.1)

^{z} from Murray et al. (1996)

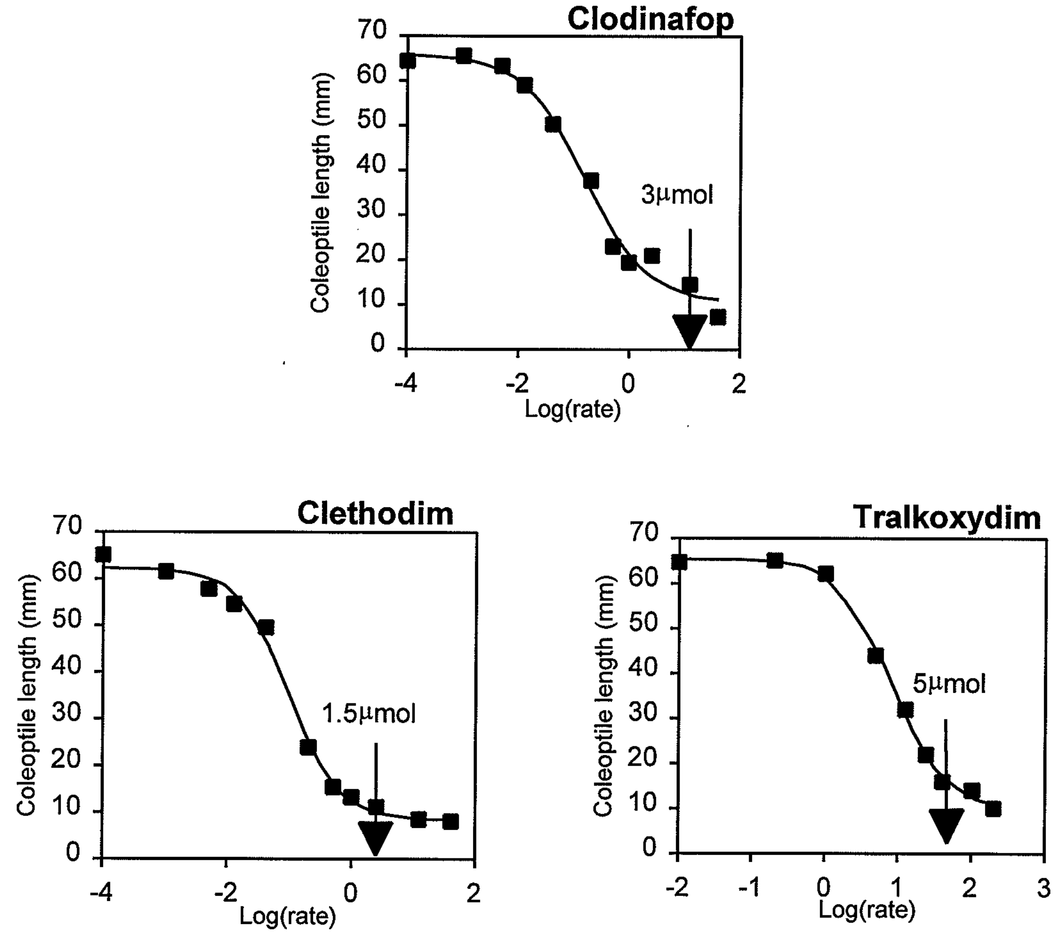

Figure 6-1. Coleoptile growth of UM5 wild oat as influenced by clodinafop, clethodim and tralkoxydim. Refer to Table 6-1 for parameter estimates.

6.3.2. Screening of herbicide resistant lines.

Three main herbicide cross-resistant types (denoted A, B and C) were separated by cluster analysis (Figure 6-2). Type C, which includes 44 wild oat lines, is separated from types A and B in the first dendrogram dichotomy. A second dichotomy separates types A and B, which include 23 and 17 wild oat lines, respectively. UM33 and UM1 are placed in types A and C, respectively. Each branch of the dendogram further divides into smaller clusters indicating greater ressemblance in cross-resistance patterns among some wild oat lines. Indeed, wild oat lines originating from a similar field often had similar cross-resistance patterns (data not shown).

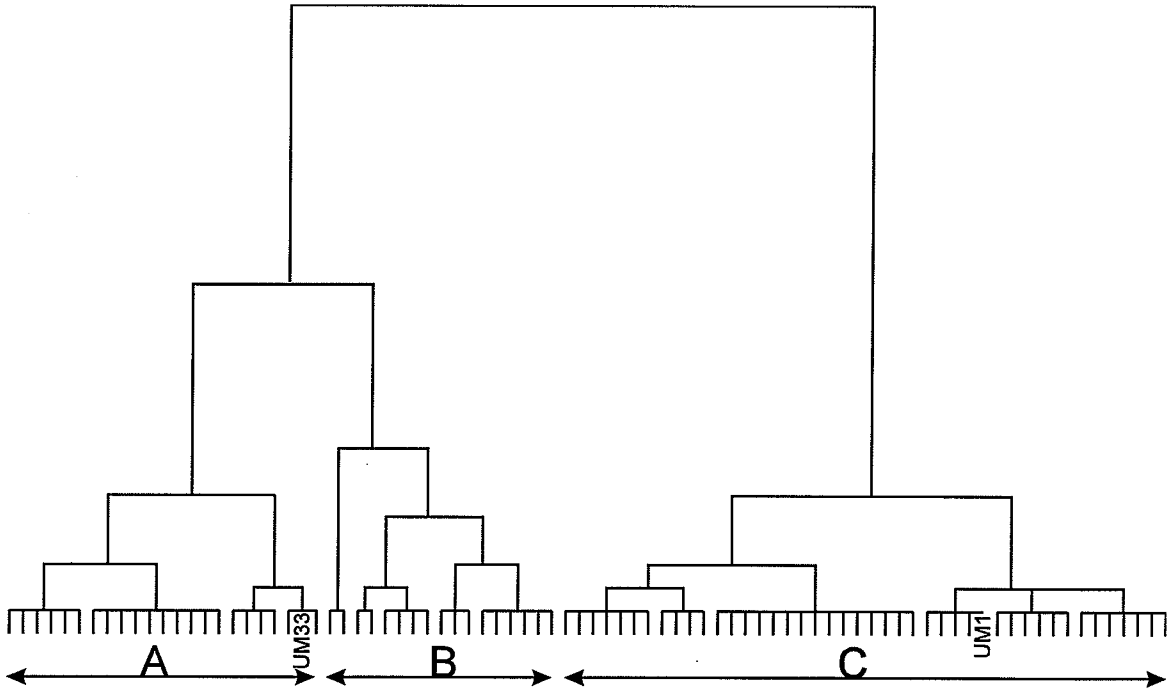

Figure 6-2. Clustering by a dendrogram of 84 wild oat lines according to similarity in cross-resistance to 5 ACCase inhibitor herbicides.

Mean herbicide cross-resistance for the three types (A, B and C), the susceptible standard (UM5), and the two known ACCase resistant lines (UM1 and UM33), are summarized in Table 6-2. Cross-resistant type C was most common, accounting for 44 of the 84 resistant wild oat lines. This type, which is characterized by high levels of resistance for both APP and CHD herbicide groups, is more resistant to a broad selection of ACCase inhibitors than the other types. Cross-resistance type A (which includes line UM33) is highly resistant to the APP herbicides, but shows little or no resistance to the CHD herbicides. Cross-resistant type B shows low to moderate resistance to both herbicide groups.

Table 6-2. Mean coleoptile lengths (as a percentage of control) of wild oat lines assayed with five ACCase inhibitor herbicides in cross-resistance types.

Types	Number	APP		CHD		
or Lines	of lines	fenoxaprop-P	clodinafop	sethoxydim	clethodim	tralkoxydim
A	23	70%	81%	41%	28%	14%
B	17	43%	54%	45%	33%	25%
C	44	59%	73%	76%	65%	56%
UM1 (R)		67%	83%	102%	56%	54%
UM33 (R)		71%	71%	31%	14%	22%
UM5 (S)		14%	17%	15%	13%	20%

(R) resistant line
(S) susceptible line

In Manitoba, the three wild oat cross-resistance types were often found to develop within the same locality (Table 6-3). In township 0810W, all three cross-resistance types occurred together with no discernible spatial pattern

(Figure 6-3). Indeed, different types grew within 100 meters of each other in fields 18 and 26 (Figure 6-4). However, the frequency of use of APP and CHD herbicides may have affected the establishment of type A and B. Type A wild oat occurred in a small patch in field 26 while type C flourished on the north part of the field. On the other hand, wild oat of all three types were scattered across field 18. Past herbicide use histories may explain these differences in distribution. Both fields 18 and 26 were sprayed 6 times with APP herbicides but field 18 was never sprayed with a CHD herbicide while field 26 was sprayed twice with sethoxydim (Bourgeois, unpublished). Sethoxydim may have impeded the development of type A wild oat in field 26 while type C wild oat were not affected by this CHD herbicide. The low level of resistance to both CHD and APP herbicides may explain a slower development of type B wild oat compared to type A and C. Injury resulting from the application of the herbicides may reduce seed set in type B wild oat compared to the other types.

Table 6-3. Classification of the resistant wild oat lines by townships of origin and the three cross-resistance types (A,B,C).

	Cross-resistance types		
Township	A	B	C
0802E	0	0	1
0609W	1	2	6
0810W	17	13	31
1208W	1	0	0
1318W	0	1	3
1415W	1	0	0
1416W	1	0	0
2422W	1	0	2
3726W	0	1	0
Total	22	17	43

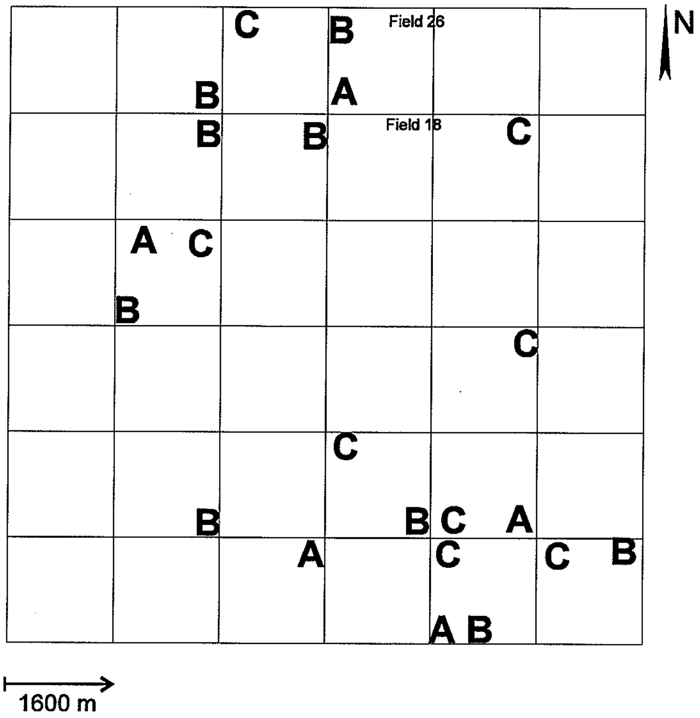

Figure 6-3. Sampling locations of resistant wild oat lines in the township 0810W with reference to their cross-resistance types A, B, and C.

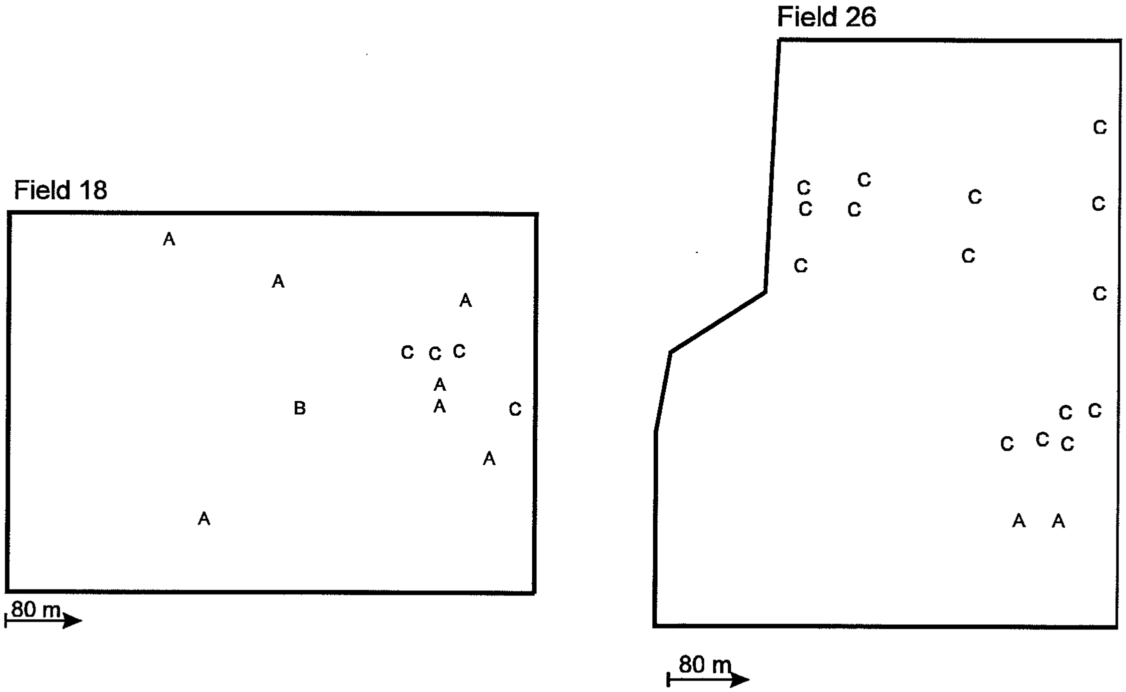

Figure 6-4. Sampling locations of resistant wild oat lines in two fields with reference to their cross-resistance types A, B, and C.

Pairwise product-moment correlations of herbicide resistance are presented in Table 6-4. All correlations are positive. Patterns of resistance indicate a high correlation between the two APP herbicides (fenoxaprop-P and clodinafop), and high correlations among the three CHD herbicides (sethoxydim, clethodim and tralkoxydim). However, all cross-correlations between the APP and CHD herbicides are low. Therefore, wild oat that are resistant to fenoxaprop are generally resistant to clodinafop but not always to the CHD herbicides. These correlations are in agreement with results of the cluster analysis where cross-resistance types were defined according to level of resistance to either APP or CHD herbicides.

Table 6-4. Pairwise Pearson product-moment correlations coefficients between herbicide responses of 85 wild oat lines.

	APP		CHD		
	fenoxaprop-P	clodinafop	sethoxydim	clethodim	tralkoxydim
fenoxaprop-P	1.0				
clodinafop	0.7	1.0			
sethoxydim	0.2	0.3	1.0		
clethodim	0.2	0.3	0.9	1.0	
tralkoxydim	0.0	0.2	0.8	0.8	1.0

In the PCA analysis, the first PCA axis is strongly correlated with CHD herbicide resistance, and the second axis with APP herbicide resistance (Figure 6-5). The first two axes account for 88.35% of the total variance observed (69.50% and 18.85% on the first and second axis, respectively). The importance of the first axis indicates that cross-resistance patterns in the 85 lines are based primarily on levels of resistance to CHD herbicides. The three cross-resistance types A, B and C delineated by cluster analysis are well separated in the two-dimensional ordination space. Type A lines are positively weighted on the second ordination axis, indicating that they have the highest resistance to APP herbicides. Type C lines are weighted positively on the first axis, indicating high resistance to the CHD herbicides compared to types A and B. The type B lines (and the susceptible line UM5) are negatively weighted on both ordination axes, indicating that they have comparatively low levels of resistance (or no resistance) to all five herbicides. The scattering of the wild oat lines in the two dimensional space highlights the variation in cross-resistance even within the defined type A, B and C. These variations are not caused by vigour differences among lines since resistance was established as a percentage of coleoptile length compared to untreated control. Therefore, the variations within cross-resistance types may indicate that each type includes several mutations confering somewhat comparable cross-resistance patterns.

The apparent untrended distribution of wild oat patches with different cross-resistant patterns indicates that resistance in wild oat develops from independent sources, at least during the early stages of development. As previously reported (Chapters 4 and 5), wild oat lines were collected from clearly defined patches within fields and with few exceptions did not constitute a serious problem in terms of major crop losses. The frequent application of ACCase inhibitors in all fields in 0810W (Chapter 3) would appear to have provided sufficient selection pressure to support the development of several types of ACCase mutants at random locations in the township.

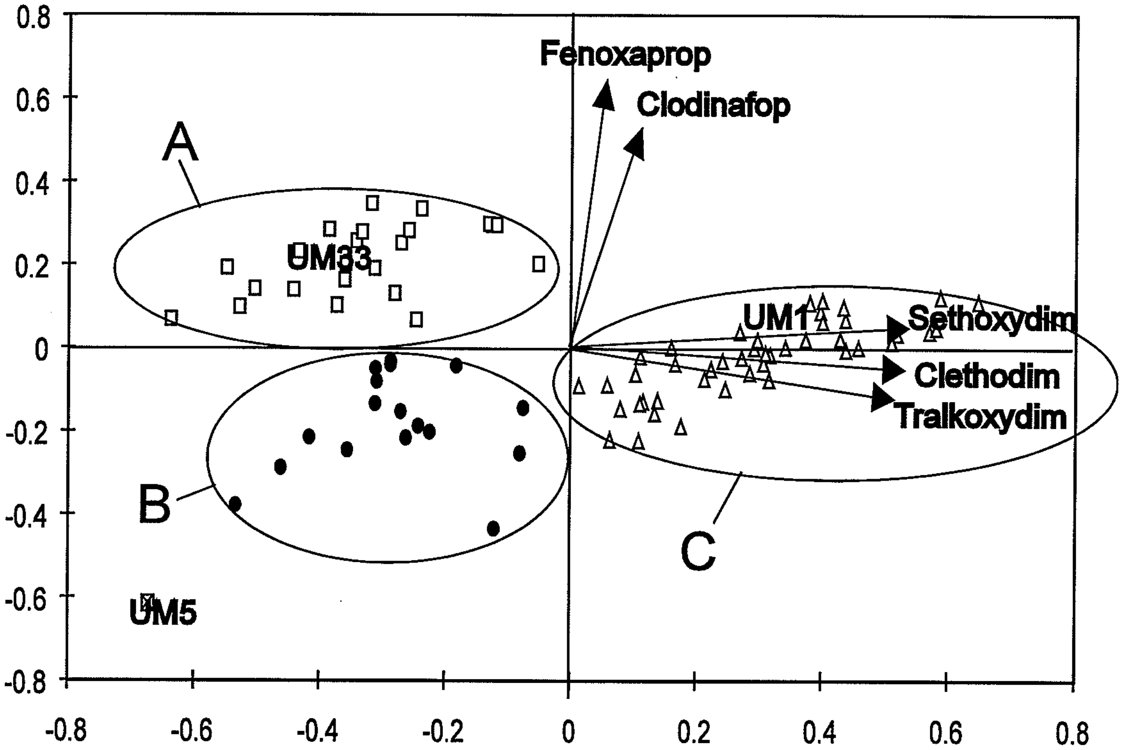

Figure 6-5. PCA biplot for the five ACCase inhibitor herbicides and the 85 wild oat lines.

The incidence of several cross-resistant patterns in a single field reduces the chances of finding one ACCase inhibitor that will be active on all wild oat present in the field. In field 18, most wild oat were type A, which had no, or only low, resistance to CHD herbicides (Figure 6-4). Although the use of a CHD herbicide would kill type A wild oat, type B and C would not be controlled and would expand in the field. Clearly, fields with high ACCase inhibitor use histories should not be sprayed with these herbicides more than once every three years. The use of alternative mode of actions is recommended on a rotational basis in addition to non-chemical weed control methods such as mowing or cultivating dense wild oat patches.

The development of different cross-resistance types within a field increases the complexity of weed resistance management because each case of resistance can be different. Moreover, weed populations benefit from selection of several cross-resistance types by maintaining genetic variability through mutations in the population. A high genetic variability within a population increases the probability of some individuals being resistant to new stresses after the occurrence of selection. Wild oat is known for its ability to maintain genetic diversity under strong selection pressure (Jana and Thai 1987). The ability of wild oat to maintain genetic variability is probably a key factor in weediness of the species.

Grassy weeds with ACCase inhibitor resistance have a variety of cross-resistance patterns. Some of these cross-resistance patterns resemble the ones described in this study. For example, populations of Italian ryegrass (Gronwald et al. 1992), and oat species (Maneechote et al. 1994; Mansooji et al. 1992) have high resistance to APP herbicides and almost no resistance to CHD herbicides. Cross-resistance in green foxtail (Heap and Morrison 1996), large crabgrass (Wiederholt and Stoltenberg 1995), giant foxtail (Stoltenberg and Wiederholt 1995), and goosegrass (Leach et al. 1995) resemble the type of patterns characterizing types B and C.

Physiological and biochemical evidence suggests that the development of ACCase resistance in grassy weeds resulted from point mutation altering the ACCase binding sites (Betts et al. 1992; Marshall et al. 1992; Marles et al. 1993, Leach et al. 1995). Furthermore, alteration in binding sites on the ACCase enzyme was described as the mechanism of resistance in UM1 wild oat (Devine personal communication). Based on genetic studies, the difference in cross-resistance patterns between UM1 and UM33 resulted from two different alleles occurring at the same gene locus (Murray et al. 1995). Marshall et al. (1992) reported that at least three different alleles conferred resistance to ACCase inhibitors in maize. Therefore, the basis of the difference between cross-resistance types may be related to specific point mutations that alter the ACCase binding sites in different ways.

Variation in cross-resistance patterns induced by point mutations was documented for imidazolinone and sulfonylurea resistance. Resistance to imidazolinone and sulfonylurea herbicides is confered by point mutation on the gene encoding for the acetohydroxyacid syntase (ALS) (Mazur and Falco 1989; Newhouse et al. 1991). In yeast, Mazur and Falco (1989) identified 24 different amino acid substitution on the ALS enzyme responsible for herbicide resistance to the sulfonylurea. Three mutations of the ALS enzyme in corn resulted in distinct cross-resistance patterns to imidazolinone and sulfonylurea herbicides (Newhouse et al. 1991). The situation respecting ACCase resistance in wild oat may be comparable to the ALS resistance situation.

6.4. Conclusion

This study clearly indicated similar responses of ACCase resistant wild oat among APP herbicides and among CHD herbicides, but not between CHD and APP herbicides. Three major types of cross-resistance were identified; type A were resistant to APP and susceptible to CHD herbicides, type B had low levels of resistance to all herbicides, and type C had comparatively high levels of resistance to all herbicides. However, no wild oat line exhibited high CHD herbicide resistance and low APP herbicide resistance. Since APP's and CHD's bind in the same region of the ACCase enzyme (Rendina et al. 1989), the clear differentiation between type A and C indicates that binding of APP herbicides may be more sensitive to changes in the ACCase than binding of CHD herbicides. Although unproven, it may well be that some mutations or conformational changes in the vicinity of the ACCase inhibitor binding site may prevent APP herbicides from binding but not CHD herbicides.

Further work is necessary in order to understand the mechanisms of action of the ACCase inhibitors as well as the mechanisms of resistance. However, the difference in cross-resistance found in wild oat at the phenotypic level should provide interesting leads in the study of these mechanisms at the molecular level.

7. SUMMARY AND CONCLUSIONS

Weed resistance to herbicides was primarily limited to the triazine family of herbicides until the late 1980's (Holt and Lebaron 1990). At that time resistance was believed to occur only with soil-applied herbicides with long residual activity. Therefore, there was little surprise when a population of green foxtail resistant to dinitroanilines herbicides was identified in Manitoba in 1987. Like triazines, dinitroanilines are soil-applied herbicides with season-long residual activity. In 1990, however, the occurrence of ACCase inhibitor resistance in wild oat came as a surprise. ACCase inhibitors, or Group 1 herbicides, are post-emergence herbicides with no-residual activity. Moreover, resistance was not confined to one field, but occurred in many fields throughout the province. The results of this thesis confirm the widespread occurrence of Group 1 resistant wild oat in Manitoba.

The common factor in all new cases of Group 1 resistant wild oat was high Group 1 herbicide use frequency in spring sown crops. Heavy dependency on chemical weed control technology coupled with a lack of herbicide rotation were the primary reasons for the selection of resistant weeds. The real or perceived lack of equally effective alternatives for the control of wild oat and green foxtail throughout the 1980's discouraged producers from rotating to other herbicides. Even though most farmers are currently aware of the resistance problem, many are still reluctant to decrease their reliance on Group 1 herbicides and adopt more holistic weed control practices. The challenge for researchers and extension workers together is to provide practical, economical alternatives to farmers to curb the rapid increase in new resistant populations.

In this final chapter, the status of resistant wild oat is discussed and advice is given regarding different ways in which to impede the selection of

Group 1 resistant wild oat in Manitoba. The discussion is organized to address the three following questions:

1. Can we predict occurrences of Group 1 resistant wild oat?
2. How do resistant wild oat spread?
3. What direction should future research take?

7.1. Can we predict occurrences of Group 1 resistant wild oat?

Literature on the evolution of resistance weeds has been descriptive rather than predictive. Although general models have been proposed, no one has verified the reliability of the models to predict the evolution of resistance to new herbicides. Authors of models are the first to acknowledge the difficulties in predicting resistance in a particular species. Some difficulties are related to a lack of information on weed biology such as seed viability, pollen dispersal, and mating systems. However, from a practical standpoint, one of the most significant limitations is the inadequacy of detailed field records relating to herbicide usage.

Strictly from a weed management stand-point, the most important factor in delaying evolution of resistance through major gene mutation is to lower the selection pressure. One way of doing so is to alternate herbicides with different modes of action. All models and evidence relating to new weed resistance cases illustrate that repeated applications of herbicides with a similar mode of action will select resistant individuals in a population. Therefore, where a particular herbicide, or a group of herbicides, have been repeatedly used over many years the risk of selecting resistant weeds should be greater than in other areas where herbicides were used less frequently The novelty of this study was to actually create herbicide resistance risk maps for Manitoba based on the principle that intensive Group 1 herbicide use will increase the risk of resistance evolution in wild oat and green foxtail populations. Although herbicide use histories were averaged over entire townships from 1981 to 1993, high risk areas corresponded to those localities where Group 1 resistant wild oat and green foxtail were first detected. Moreover, subsequent surveys indicated a higher frequency of Group 1 resistant wild oat in townships at high risk than in the other townships. This, then constitutes one of the first studies where the occurrence of resistance has been directly linked with herbicide usage on a regional basis.

The study of herbicide use histories indicated a steady increase of Group 1 herbicide use in Manitoba. As a result, the number of townships at high risk of developing Group 1 herbicide resistance has risen significantly. If this trend continues the future use of Group 1 herbicides is certainly in jeopardy. The loss of Group 1 herbicides may reduce cropping options, since alternatives for grassy weed control in crops such as flax are limited. In the southwestern part of the province, producers are now relying almost exclusively on Group 1 herbicides for green foxtail control because of widespread resistance to dinitroaniline (Group 3) herbicides. Unless new products with different modes of action become available to producers, occurrences of Group 1 and Group 3 resistant green foxtail will force producers out of broadleaf crops with the exception of herbicide resistant canola where 'non-selective' herbicides such as glyphosate or glufosinate can be applied.

The presence of resistant wild oat in 20 out of 30 surveyed fields in a high risk township was above expectations since none of the producers suspected resistance in these fields. However, these findings substantiated estimates from evolutionary models on weed resistance enrichment in populations. For example, Jasieniuk et al (1996) concluded that there was a 100% chance of resistant wild oat occurring in fields of 30 ha with an initial frequency of resistant individuals of 1 in 10^{-6} and wild oat densities of 30 plants m^{-2}.

The survey of the 30 fields in a high risk township also illustrated large variations in the frequency of Group 1 herbicide application and the frequency of resistance in a field. While resistance occurred in 12 fields where Group 1 herbicides were used more than 7 years out of 11, no resistance was detected in four others. Clearly, the initial frequency of resistant wild oat is not the same from one field to another. Variation in resistant wild oat occurrences also appeared in the wild oat patch survey conducted in six high risk townships where resistant wild oat frequency varied from 6 to 30%. These differences may result, in part, from variation in mutation frequencies from one population to another as reported for *Lolium* (Matthews and Powles 1992). These differences may also result from different densities of wild oat occurring in different fields or they may be related to differences in management practices such as crop rotation, tillage systems, and time of seeding.

Rotation of herbicides with different modes of action should become a common weed management practice in order to slow the development of resistance. Although this would provide a good start, it is probable that the rotation of herbicides will only delay the evolution of resistance. Non-chemical weed control methods should also be included in a weed management strategy in order to further reduce the chances of resistant weed selection. Non-chemical weed management techniques include late-seeding, crop rotation, seed and equipment sanitation, and field mowing. Rotating between spring sown and more competitive winter sown cereals will also impede evolution of resistance.

The weed problem encountered in mono-crop production are well documented (Barrett 1983). Highly specialized weeds are selected from the population to thrive under the conditions prevailing in the field. Group 1 resistant wild oat is an example of a specialized weed selected by high use of Group 1 herbicides in spring sown crops. Breaking cycles of annual crops with short-term perennial forages (3-4 years) reduces the number of annual grassy weeds in subsequent crops. For example, Ominski et al. (1994) observed less wild oat in wheat fields following alfalfa than in wheat fields following wheat. A reduction in wild oat density within a field will reduce the probability of a resistant mutant being present in the field. Moreover, if wild oat densities are low it may be possible for a producer to refrain from using a herbicide that year, which in turn, will reduce the selection pressure on the population.

7.2. How do resistant wild oat spread?

In the previous section, high use of herbicides with the same mode of action was shown to be the key factor for the selection of resistance. However, for resistance to evolve in a population, the presence of at least one resistant individual is necessary. In order to spread across the field, the resistant gene requires transfer through seed or pollen.

The first resistant individual in a field can have two origins. An individual resistant mutant can occur within the population. Alternatively, a resistant individual may be imported into a susceptible population through seed or pollen movement. In both cases, resistance will evolve from single plants. Since wild oat seed tends to fall on the ground at maturity, resistant wild oat will develop in patches around the first resistant parent plant. The patchy distribution of resistant wild oat is a key component of detecting resistance in a field (Heap and Morrison 1991).

The presence of resistant wild oat in patches was confirmed in the surveys conducted in fields and townships of Manitoba. Sampling conspicuous patches of wild oat proved to be more effective in identifying resistance than systematic sampling. Moreover, most patches in fields sprayed with a Group 1 herbicide in the same year were resistant. These findings should encourage producers to map weed patches by scouting fields annually either soon after herbicide application or after harvest. This would alert them to early development of suspicious wild oat patches and facilitate close monitoring in the future.

The development of geographic positioning system (GPS) devices and geographic information system (GIS) software may improve the speed and accuracy of constructing field maps. These techniques should enhance the detection of resistant wild oat at an early stage. Early detection of resistant wild oat provides opportunities to use alternative control options such as mowing or cultivating. Destruction of new patches of wild oat, whether or not they are resistant, will improve the chances of confining the weed to a small area of the field.

As previously stated, the spread of resistance within a field can be through either pollen flow or seed-flow. Wild oat is mostly selfing which reduces the chance of exchanging resistant gene through pollen-flow (Murray 1996). Moreover, in fields sprayed with Group 1 herbicides, few susceptible plants remain to cross with resistant ones. The surveys have shown that no, or only a few, susceptible plants grow in resistant patches. For these reasons, pollen flow is probably not a major factor contributing to the spread of resistance within a field.

Spread of resistance through seed is often downplayed with wild oat because seeds tend to fall on the ground prior to harvesting the crop (Sharma and Vanden Born 1978). However, spread of wild oat seed by a combine harvester occurred over distances of 100 meters (Shirtliffe and Entz pers. communication), and may also occur through contaminated crop seeds and tillage. Although movement may be limited to a few seeds, these could become a new epicenter of resistance development.

7.3. What direction should future research take?

In this study, resistance was determined by seed bioassay in order to process over 1300 samples in limited time and space. Cross-resistance patterns were characterized on the basis of coleoptile length at a discriminatory dosage relative to an untreated control. The discriminatory dosage was determined as the minimum dosage required to kill a susceptible population (UM5). Unfortunately, this dosage does not correspond to a commercial herbicide field rate. Therefore, the level of resistance observed in the bioassay may not translate to the actual level of resistance observed in the field. Additional pot and field experiments would be required to correlate the bioassay results to whole plant responses.

Resistant wild oat to Group 1 herbicides had large variations in cross-resistance patterns. These variations certainly indicate that several mutations can confer resistance in wild oat. Similar variations were observed in wild oat populations from Oregon (Seefeldt et al 1994). However, most of the variation observed among the 84 resistant wild oat lines characterized in this study was summarized into three main cross-resistant types. The principal differences between the three cross-resistance types were related to the levels of resistance to herbicides from the cyclohexanedione (CHD) chemical family which varied from no resistance to high resistance. Levels of resistance to aryloxyphenoxypropionate (APP) herbicides varied from moderate to high. Interestingly, none of the wild oat lines were highly resistant to CHDs and only low or moderately resistant to APPs. Overall, the correlation between herbicides from a similar chemical family were high while correlation between herbicides from different chemical families were low.

The occurrences of three cross-resistance types may indicate three major types of mutations resulting in different levels of resistance between CHD and

APP herbicides. Since both APP and CHD herbicides bind in a similar region of the ACCase enzyme (Rendina et al. 1989), the physiological implications are that some mutations may alter the ACCase enzyme binding site enough to prevent CHD's from binding but not APP's, while other mutations prevent both CHD and APP herbicides from binding.

Wild oat lines with high levels of resistance to all Group 1 herbicides were the most common. These lines have a selective advantage compared to the others since most fields are sprayed with APP as well as CHD herbicides. Therefore, the mutation frequency for each cross-resistant type may be equal. The difference in the frequency of wild oat of each cross-resistant type may depend on historical CHD and APP herbicide use patterns.

In terms of herbicide resistance research, the finding of three major types of cross-resistance patterns generates several questions and concerns. Firstly, what is the mechanism of resistance to Group 1 herbicides? Secondly, what mutation(s) confer resistance to APP's but not CHD's? Thirdly, how different is this mutation compared to mutations that confer resistance to all Group 1 herbicides? Answers to some of these questions will require detailed sequencing of the gene encoding the ACCase enzyme to identify nucleotide substitutions comparable to those reported for ALS resistant mutants (Mazur and Falco 1989).

The resistant wild oat lines gathered from the surveys will provide a basis for the study of relationship among these lines using molecular biology techniques. Absence of differences in DNA among lines growing in adjacent fields may indicate movement of seed from one field to another. The study of the genetic relationship among these lines should provide clues about weed movement both within fields and between fields in the same region.

The situation of resistant wild oat to Group 1 herbicides is critical in Manitoba. The number of fields with resistant wild oat continues to increase and producers still rely heavily on Group 1 herbicides. *Ceteris paribus* (if the situation remains the same), Group 1 herbicides may become useless in the next 5 to 10 years.

In order to impede further selection of Group 1 resistant wild oat in Manitoba, producers will have to rotate herbicides in an integrated weed management program. Producers should also be encouraged to enact a long term weed control strategy. Consequently, education remains essential for producers to change their current practice of overusing Group 1 herbicides. Future identification of herbicides according to Groups on product labels and promotional material may encourage producers to practice herbicide rotation.

Evidently, the lack of substitutes to Group 1 herbicides remains a key complaint of numerous producers. Chemical companies need to fill the gap by offering quality weed control with herbicides using different modes of action. The registration of triallate as a surface application for minimum tillage as well as the development of canola varieties resistant to glyphosate and glufosinate are some examples of new options available to producers in place of Group 1 herbicides. The registration of quinclorac, a new mode of action for green foxtail control, is also promising for improving rotational choices where they are most limited.

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Appendix 1. Analysis of the MCIC database using SYBASE. Text in italics represents SYBASE program in SQL language.

SQL stands for Structured Query Language. It is an English like language used to retrieve and manipulate data in database. The MCIC database used in this study was structured into two tables. Table MCIC includes data fields id, ldesc, qtr, sec, tow,mun, qcrop, qvar, cropy, qacres, yield, and herbap (see Table 1 for data field description). Table HERB includes data fields id and herb.

Table 1. Description of the data fields in tables MCIC and HERB.

Data Field	Description
id	Identification number
ldesc	Legal land description
qtr	Quarter
sec	Section
tow	Range and township
mun	Municipality
qcrop	Crop
qvar	Variety
cropy	Year
qacres	Acres planted
yield	Yield in bu acre^{-1}
herbap	Number of herbicide applied.
id	Identification number
herb	Commercial name of herbicide(s) applied

The id field description enable to correspond herbicides applied to each field.

Example 1 of query: To count the fields sprayed with at least one herbicide in 1981 for each township.

```
select tow, count(herbap)
from MCIC
where cropy ='81'          Note: cropy is in alphanumeric format.
group by tow
```

Appendix 1. (continued)

Example 2 of query: To count the number of Group 1 herbicides applied in 1981 on fields, grouped by township.

```
select mcic.tow, count(herb.herb)
from mcic, herb
where mcic.id=herb.id
    and mcic.cropy = '81'
    and herb.herb in (5, 45, 795, 370, 381, 383, 793,400, 405, 401, 428, 526,
    791,607, 722)          Note: these codes correspond to Group 1 herbicides
group by tow
```

Appendix 2. Field histories of the surveyed fields

Year	Field 1 (NE230810W)		Field 2 (NE240810W)		Field 3 (SE170810W)	
	Crop	Herbicide	Crop	Herbicide	Crop	Herbicide
1983	Wheat	Fortress	Wheat	Fortress	Wheat	Avenge
1984	Flax	Poast	Peas	Treflan	Potatoes	Eptam
1985	Barley	Hoegrass II	Wheat	Hoegrass II	Wheat	Avenge
1986	Peas	Poast	Flax	Poast	Wheat	Hoegrass
1987	Wheat	Avenge Stampede	Wheat	Avenge	Potatoes	Eptam
1988	Sunflower	-	Canola	-	Wheat	Hoegrass II
1989	Wheat	Laser	Wheat	Laser	Wheat	Hoegrass
1990	Wheat	Hoegrass	Wheat	Hoegrass	Flax	Poast
1991	Wheat	-	Wheat	-	Flax	Poast
1992	Potatoes	-	Canola	Edge	Canola	Treflan
1993	Wheat	-	Wheat	-	Wheat	-

Year	Field 4 (SW110810W)		Field 5 (NW330810W)		Field 6 (NE250810W)	
	Crop	Herbicide	Crop	Herbicide	Crop	Herbicide
1983			Wheat	Hoegrass II	Wheat	Stampede
1984			Flax	Poast	Wheat	Stampede
1985			Wheat	Hoegrass II	Flax	Poast
1986			Wheat	Hoegrass II	Wheat	Stampede
1987			Flax	Poast	Wheat	Hoegrass
1988			Wheat	Hoegrass II	Oat	-
1989			Flax	Poast	Flax	Poast
1990	Wheat	Hoegrass II	Wheat	Hoegrass II	Fall rye	-
1991	Wheat	Triumph Achieve	Wheat	Laser	Canola	Treflan
1992	Wheat	Triumph Laser	Canola	Poast	Wheat	Laser
1993	Wheat	Achieve	Wheat	Hoegrass	Wheat	-

Year	Field 7 (SW200810W)		Field 8 (NE080810W)		Field 9 (SE080810W)	
	Crop	Herbicide	Crop	Herbicide	Crop	Herbicide
1983	Wheat	Hoegrass II	Barley	Avenge	Flax	Treflan
1984	Flax	Poast	Wheat	Hoegrass	Barley	Avenge
1985	Wheat	Hoegrass II	Flax	Poast	Wheat	Hoegrass
1986	Wheat	Hoegrass	Wheat	Hoegrass II	Flax	Poast
1987	Flax	Poast	Flax	Poast	Barley	Avenge
1988	Wheat	Hoegrass II	Wheat	Hoegrass II	Oat	-
1989	Wheat	Hoegrass II	Canola	Poast	Wheat	Hoegrass
1990	Flax	Poast	?	?	Flax	Poast
1991	Wheat	-	?	?	Wheat	Laser
1992	Lentils	Edge	?	?	Canola	Treflan
1993	Wheat	Triumph	Wheat	Puma Laser	Wheat	Puma

Appendix 2. (continued)

Year	Field 10 (NW150810W)		Field 11 (NW210810W)		Field 12 (NE020810W)	
	Crop	Herbicide	Crop	Herbicide	Crop	Herbicide
1983	Flax	Poast	Wheat	Hoegrass II		
1984	Wheat	Hoegrass	Barley	Hoegrass		
1985	Canola TR	Bladex	Flax	Poast		
1986	Wheat	-	Wheat	Hoegrass		
1987	Wheat	Hoegrass	Canola	Treflan		
1988	Canola TR	Bladex Edge	Wheat	Hoegrass II		
1989	Wheat	Hoegrass	Flax	Poast	Wheat	Hoegrass II
1990	Wheat	-	Wheat	Triumph	Flax	Treflan
1991	Wheat	-	Barley	Stampede	Canola	Poast
1992	Canola	Edge	Canola	Treflan	Wheat	Triumph
1993	Wheat	Puma	Wheat	-	Wheat	Fortress

Year	Field 13 (NE130810W)		Field 14 (NW350810W)		Field 15 (NE350810W)	
	Crop	Herbicide	Crop	Herbicide	Crop	Herbicide
1983	Wheat	Fortress	Peas	Treflan	Wheat	Hoegrass
1984	Wheat	Hoegrass II	Wheat	Hoegrass II	Canola	Treflan
1985	Wheat	Hoegrass II	Wheat	Hoegrass II	Wheat	Hoegrass
1986	Wheat	-	?	?	Canola	Treflan
1987	Wheat	Hoegrass	Peas	Treflan	Wheat	Hoegrass
1988	Wheat	Hoegrass	Flax	Poast	Wheat	Hoegrass
1989	Flax	Treflan	?	?	Canola	Treflan
1990	Wheat	-	Wheat	Triumph	Wheat	Triumph
1991	Canola	Edge	Flax	Poast	Wheat	Triumph
1992	Wheat	-	Canola	Edge	Flax	Poast
1993	Wheat	Hoegrass	Wheat	Laser	Wheat	Mataven

Year	Field 16 (NE060810W)		Field 17 (NE240810W)		Field 18 (NE270810W)	
	Crop	Herbicide	Crop	Herbicide	Crop	Herbicide
1983	Flax	Treflan			Wheat	Avenge
1984	Wheat	Avenge			Wheat	Stampede
1985	Flax	Poast			Canola	Poast
1986	Wheat	Hoegrass			Wheat	Hoegrass
1987	Flax	Poast			Wheat	Avenge
1988	Wheat	Hoegrass	Wheat	Hoegrass II	Wheat	Hoegrass II
1989	Wheat	Hoegrass	Flax	Poast	Wheat	Hoegrass II
1990	Flax	Poast	Wheat	Hoegrass II	Canola	Treflan
1991	Wheat	Triumph	Canola	Treflan	Wheat	Laser
1992	Canola	Edge	Wheat	-	Wheat	Triumph
1993	Wheat	Triumph	Wheat	Triumph	Wheat	Triumph

Appendix 2 (continued)

	Field 19 (SW020810W)		Field 20 (SW290810W)		Field 21 (NW340810W)	
Year	Crop	Herbicide	Crop	Herbicide	Crop	Herbicide
1983	Wheat	Hoegrass	Canola	Treflan	Wheat	Fortress
1984	Wheat	Hoegrass II	Wheat	Hoegrass	FLax	Poast
1985	Wheat	Hoegrass II	Flax	Poast	Wheat	Hoegrass II
1986	Alfalfa	-	Wheat	-	Flax	Poast
1987	Alfalfa	-	Canola	Rival	Wheat	Hoegrass II
1988	Alfalfa	-	Wheat	-	Wheat	Hoegrass II
1989	Alfalfa	-	Flax	Poast	Wheat	Hoegrass II
1990	Barley	Hoegrass	Wheat	Laser	Flax	Poast
1991	Canola	Poast	Wheat	Laser	Wheat	Triumph
1992	Wheat	Achieve	Canola	Edge	Canola	Edge
1993	Wheat	-	Wheat	Assert	Wheat	Fortress

	Field 22 (NW090810W)		Field 23 (NE260810W)		Field 24 (NW200810W)	
Year	Crop	Herbicide	Crop	Herbicide	Crop	Herbicide
1983	Wheat	Fortress	Wheat	Fortress	Canola	Treflan
1984	Canola	Treflan	Corn	-	Wheat	Hoegrass
1985	Wheat	Hoegrass II	Canola	Poast	Wheat	Hoegrass
1986	Barley	Hoegrass	Wheat	-	Wheat	-
1987	Wheat	Hoegrass	Wheat	Hoegrass	?	?
1988	Oat	TCA	Canola	Poast	Wheat	-
1989	Wheat	Hoegrass	Flax	Poast	Flax	Poast
1990	Wheat	Triumph	Wheat	Hoegrass	Wheat	Laser
1991	Wheat	Laser	Flax	Poast	Wheat	Laser
1992	Oat	-	Wheat	Laser	Canola	Edge
1993	Barley	Achieve	Wheat	-	Wheat	-

	Field 25 (NE030810W)		Field 26 (NE340810W)		Field 27 (SE110810W)	
Year	Crop	Herbicide	Crop	Herbicide	Crop	Herbicide
1983	Wheat	Hoegrass	Barley	Hoegrass	Wheat	Treflan
1984	Wheat	Hoegrass II	Wheat	Hoegrass II	Wheat	-
1985	Wheat	Hoegrass	Mustard	Treflan	Wheat	-
1986	Oat	-	Wheat	Hoegrass II	Wheat	Hoegrass
1987	Wheat	Hoegrass	Wheat	Hoegrass II	Canola	Treflan
1988	Wheat	Hoegrass	Wheat	Hoegrass II	Wheat	Hoegrass
1989	Barley	Hoegrass	Fallow	Treflan	Flax	Treflan
1990	Barley	Hoegrass	Flax	Poast	Wheat	Hoegrass
1991	Canola	Poast	Wheat	Triumph Laser	Canola	Edge
1992	Wheat	-	Flax	Poast	Wheat	Hoegrass
1993	Wheat	Achieve	Wheat	Puma Laser	Wheat	Triumph

Appendix 2. (continued)

	Field 28 (NE040810W)		Field 29 (NW280810W)		Field 30 (NE140810W)	
Year	Crop	Herbicide	Crop	Herbicide	Crop	Herbicide
1983	Canola	Treflan	Wheat	Fortress	Wheat	Hoegrass
1984	Wheat	Hoegrass II	Flax	Poast	Wheat	Hoegrass
1985	Wheat	Hoegrass II	Wheat	Hoegrass II	Wheat	Hoegrass
1986	Wheat	Hoegrass	Canola	Poast	Flax	Poast
1987	Canola	Poast	Wheat	Hoegrass II	Wheat	-
1988	Wheat	-	Wheat	Hoegrass II	Flax	Poast
1989	Corn	Rival	Flax	Poast	Wheat	Hoegrass
1990	Wheat	Hoegrass II	Wheat	Triumph	Canola	Edge
1991	Wheat	Hoegrass II	Canola	Edge	Wheat	-
1992	Canola	Assure	Wheat	Fortress	Flax	Poast
1993	Wheat	-	Wheat	Fortress	Wheat	Puma

Appendix 3. Field map of the surveyed fields with locations of sampling sites

Maps of the 30 surveyed fields are presented in the next 13 pages.

Legend:

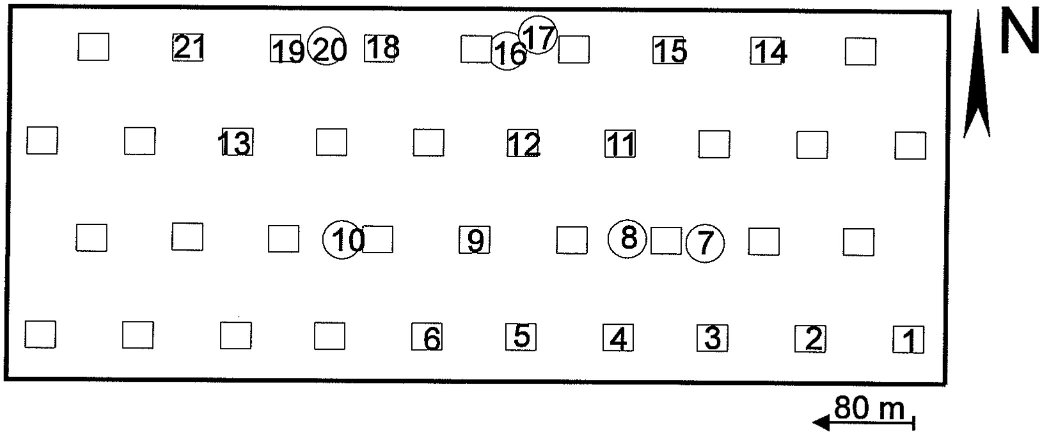
☐ systematic sampling sites

○ patch sampling sites

Shading represents the presence of resistant wild oat.

The numbering system identifies samples using a four digit system. The first two numbers indicate the field number, and the last two the sample number indicated on the maps. For example, sample 1206 was collected on field 12. The exact location of the sample in the field correspond to the number 6 in field 12.

Field 1 NE230810W



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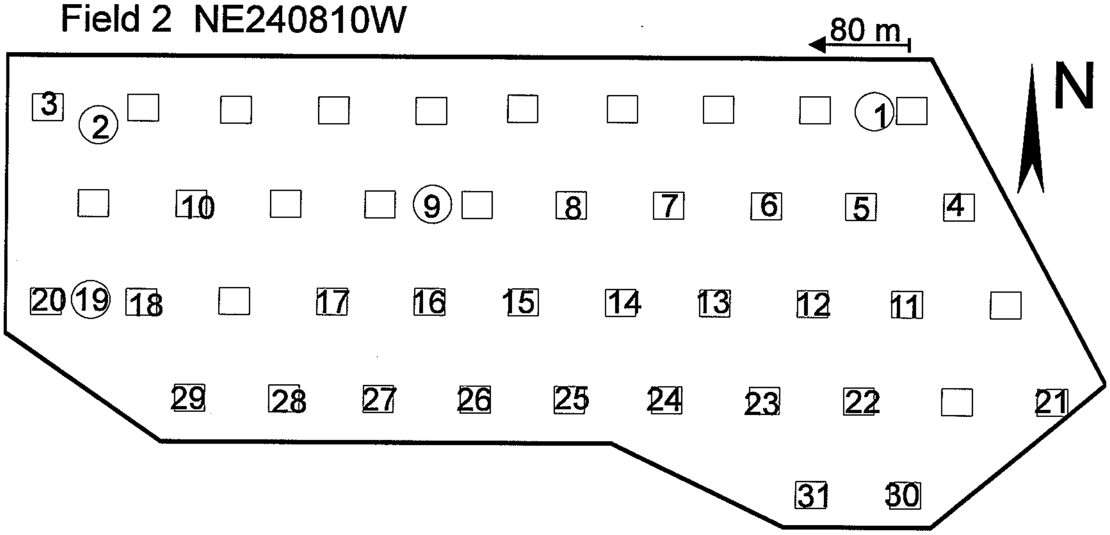
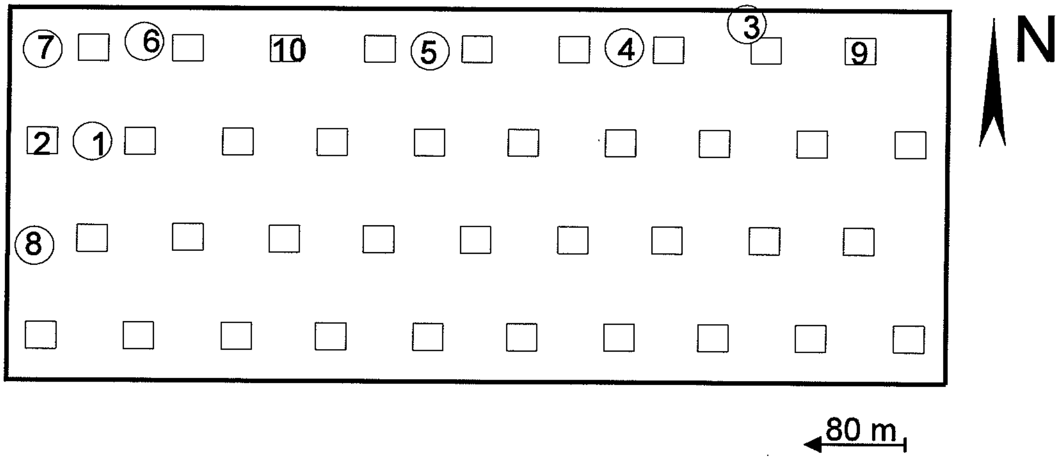


Field 3 SE170810W



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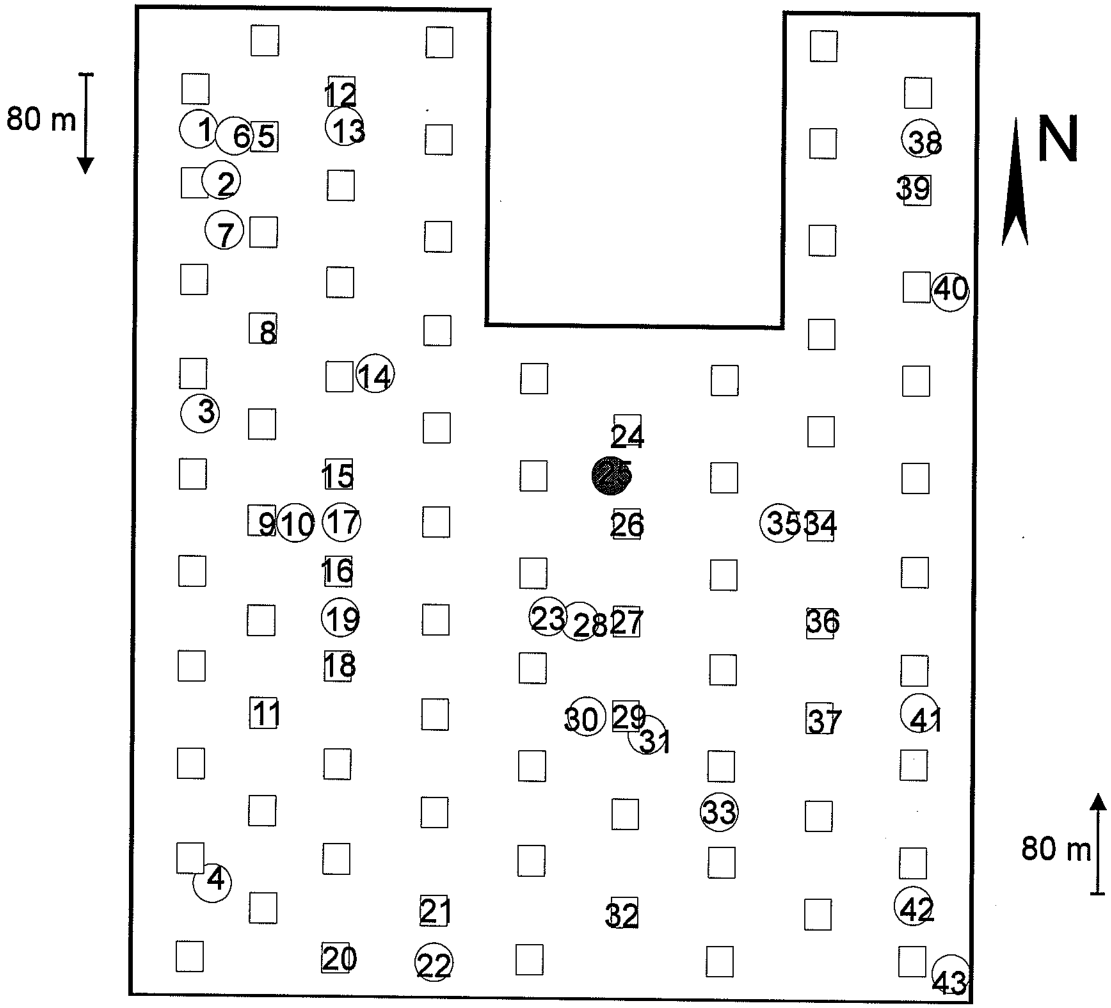
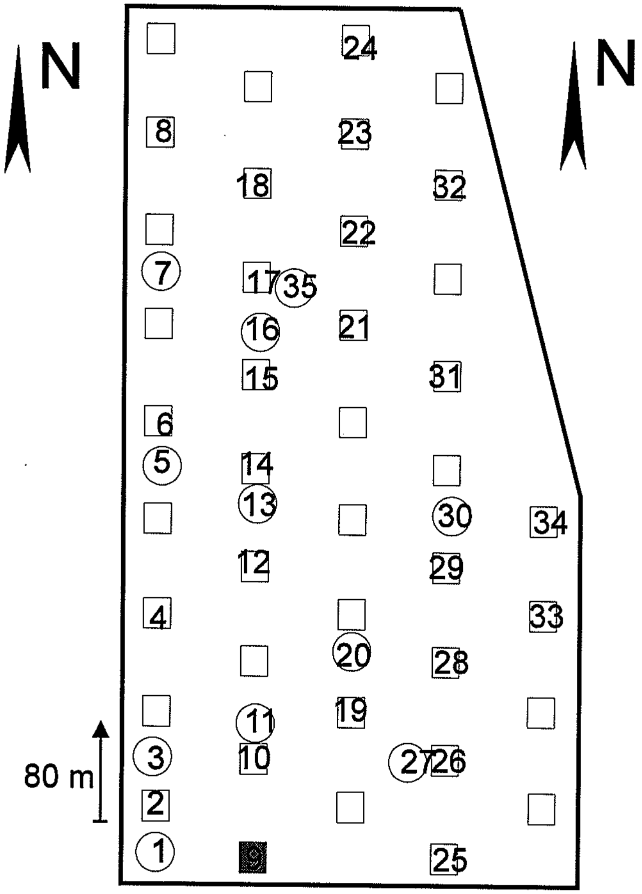


Field 04 SW110810W



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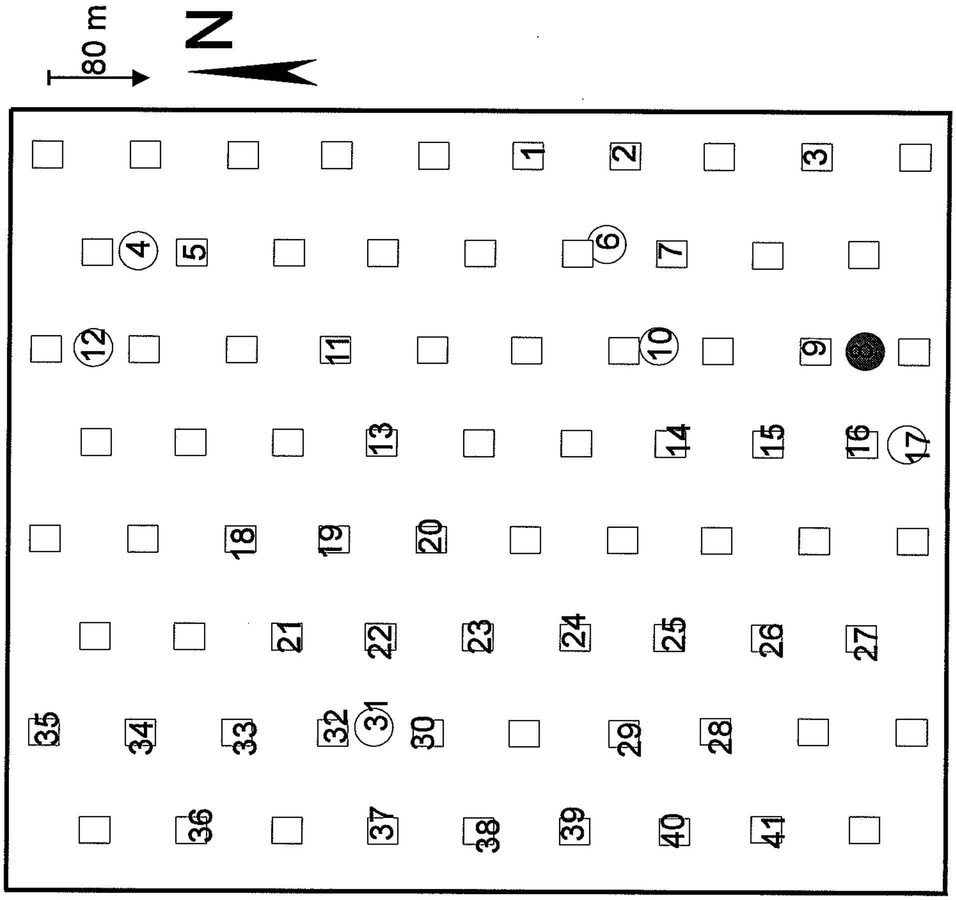

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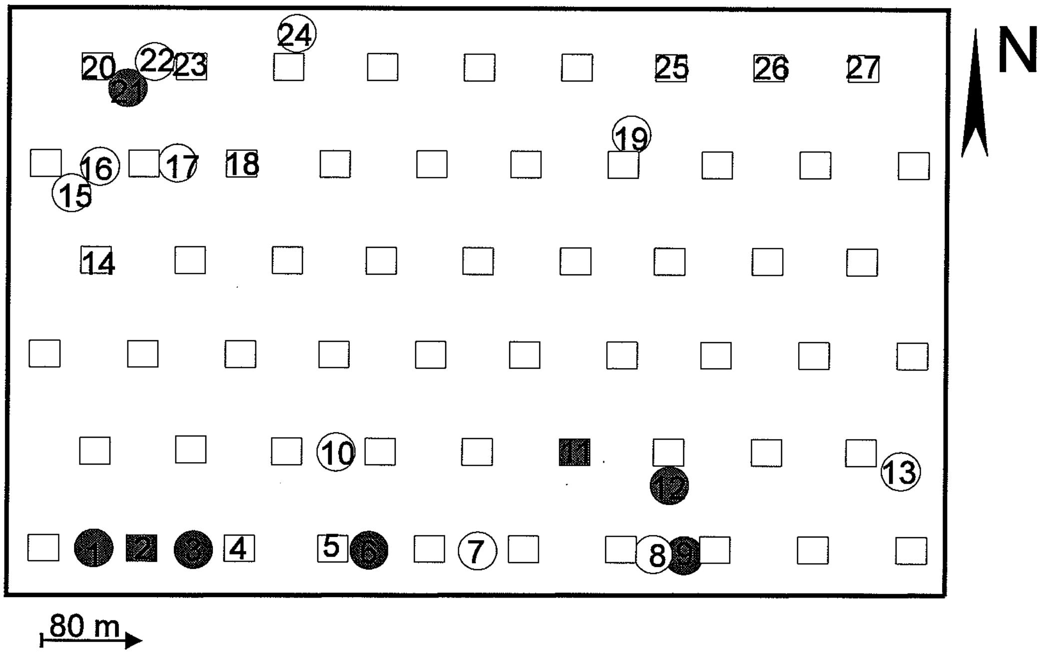
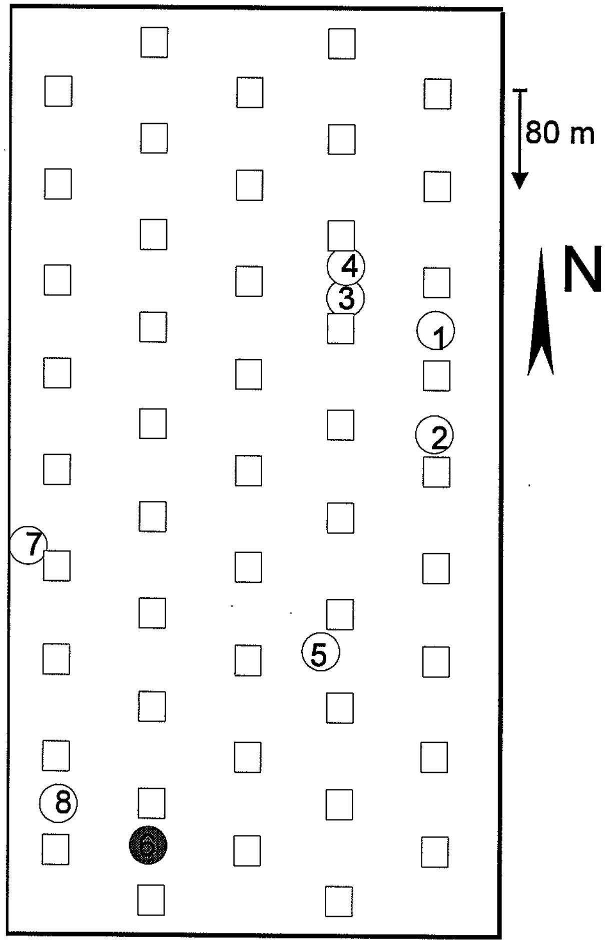


Field 09 SE080810W



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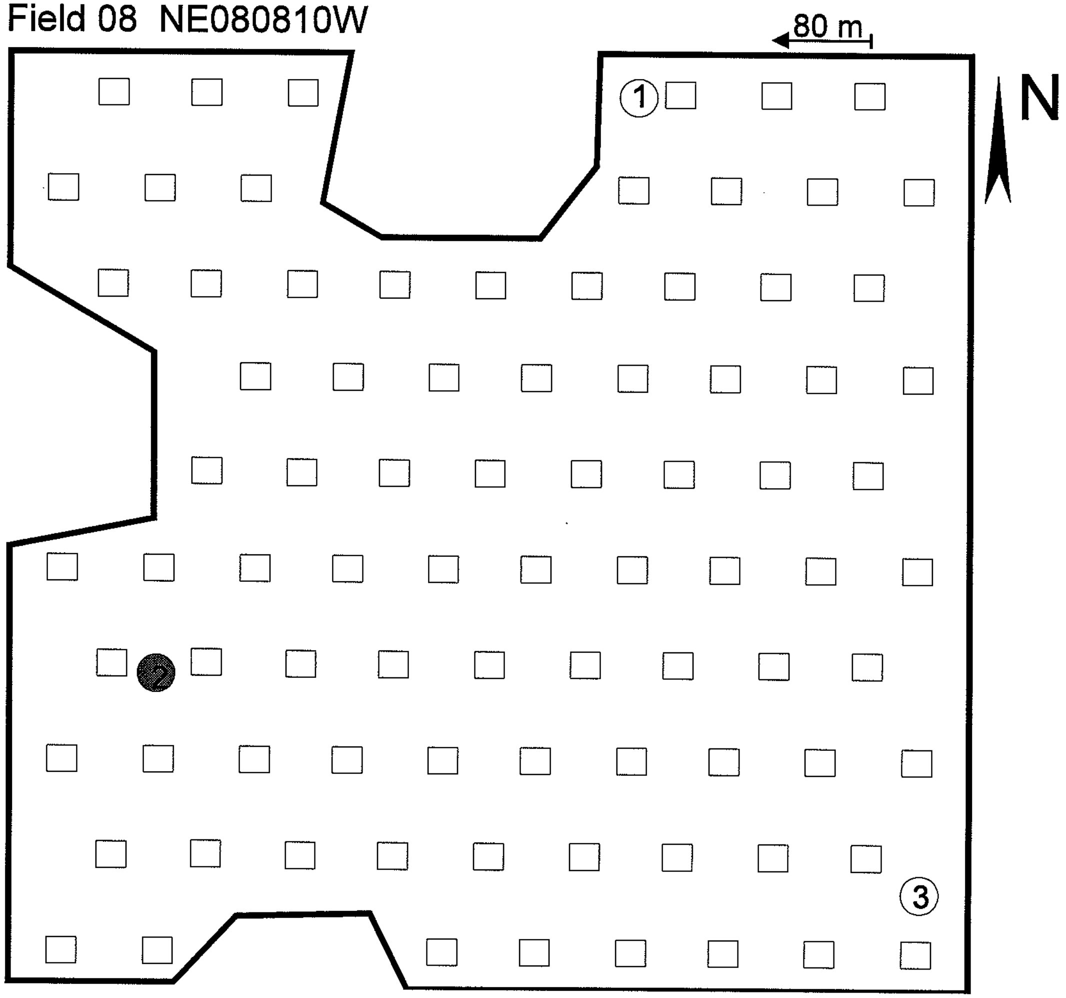

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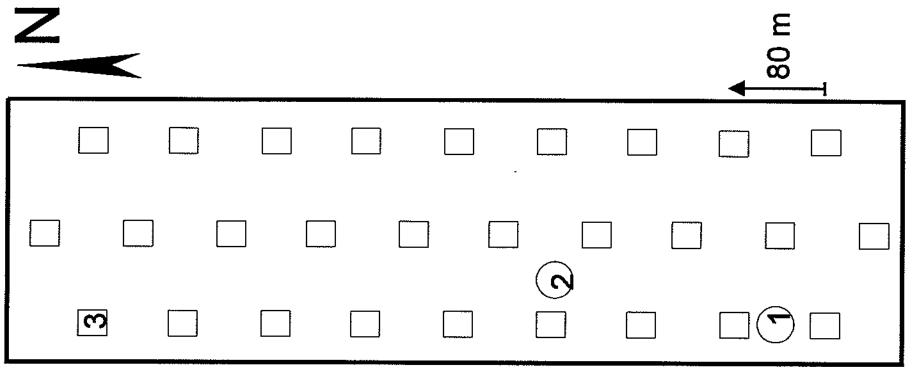

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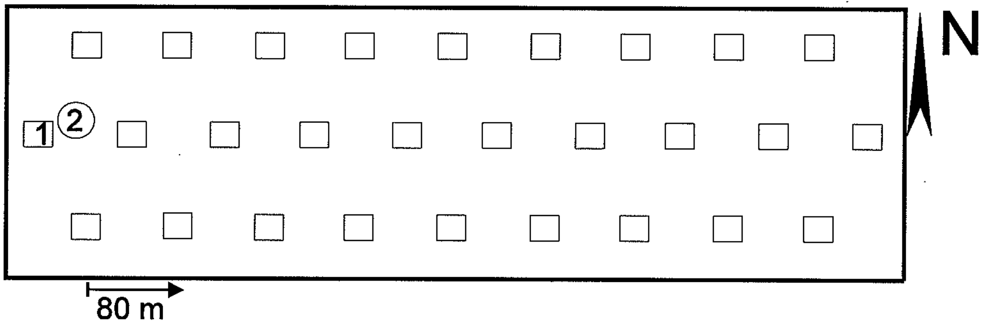

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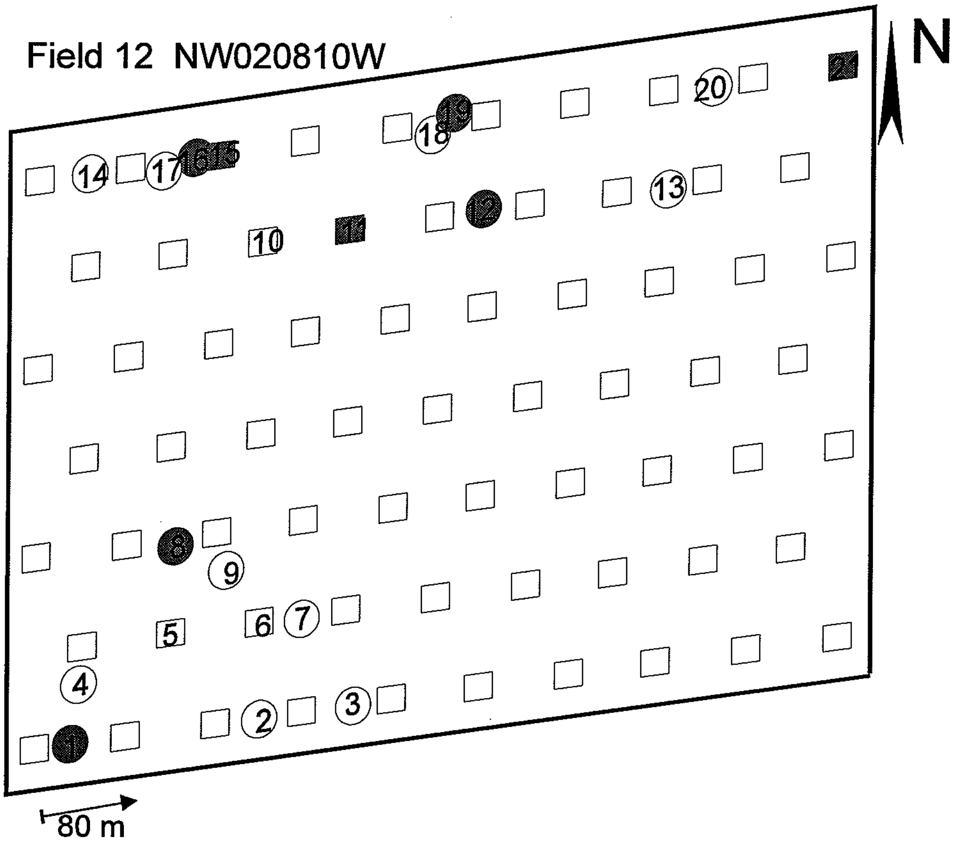

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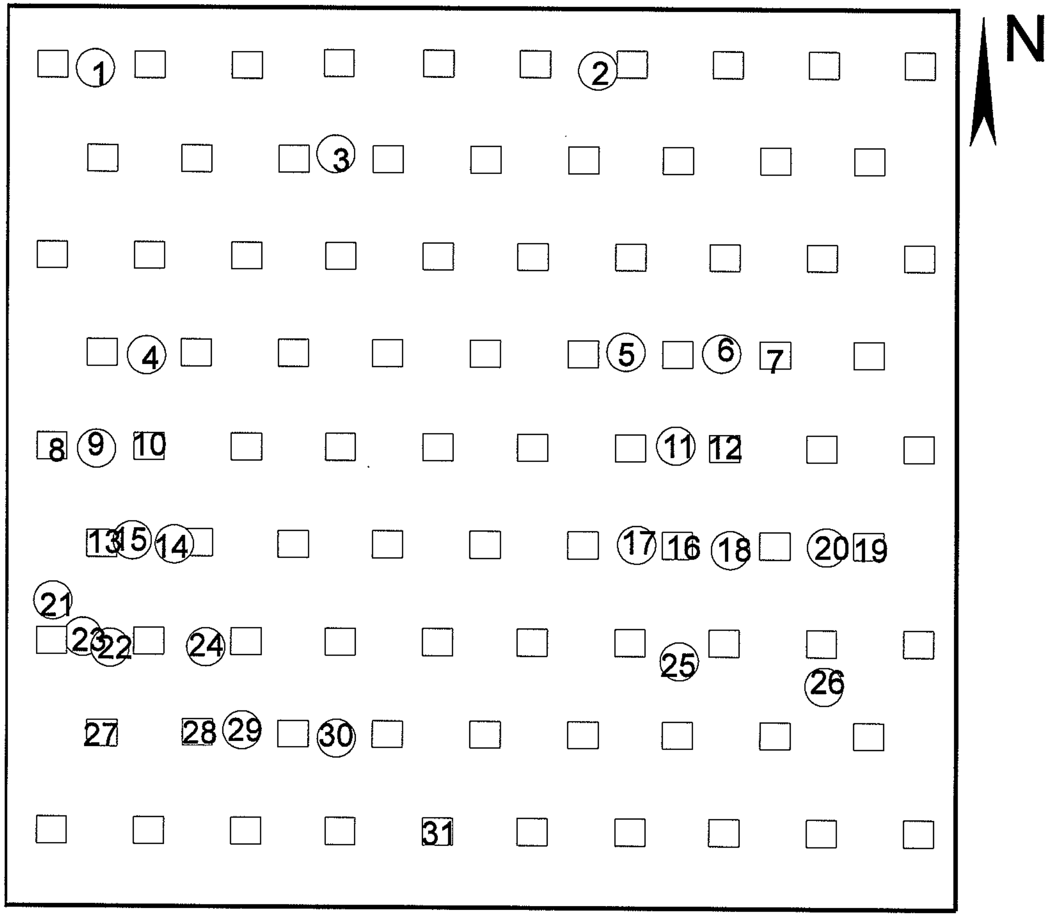

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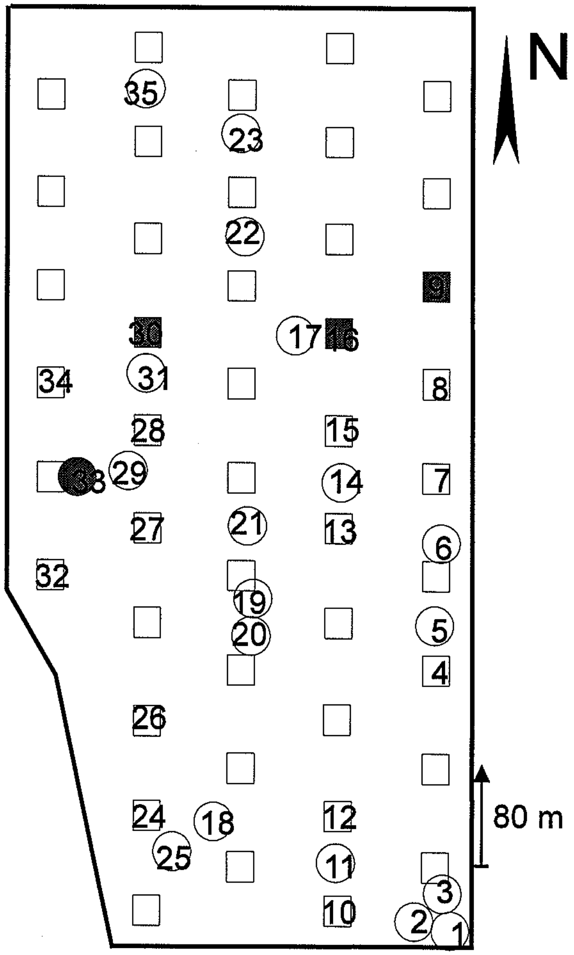

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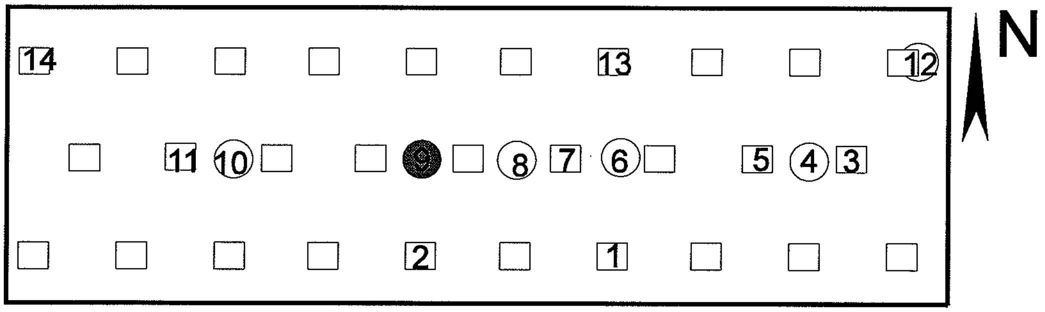

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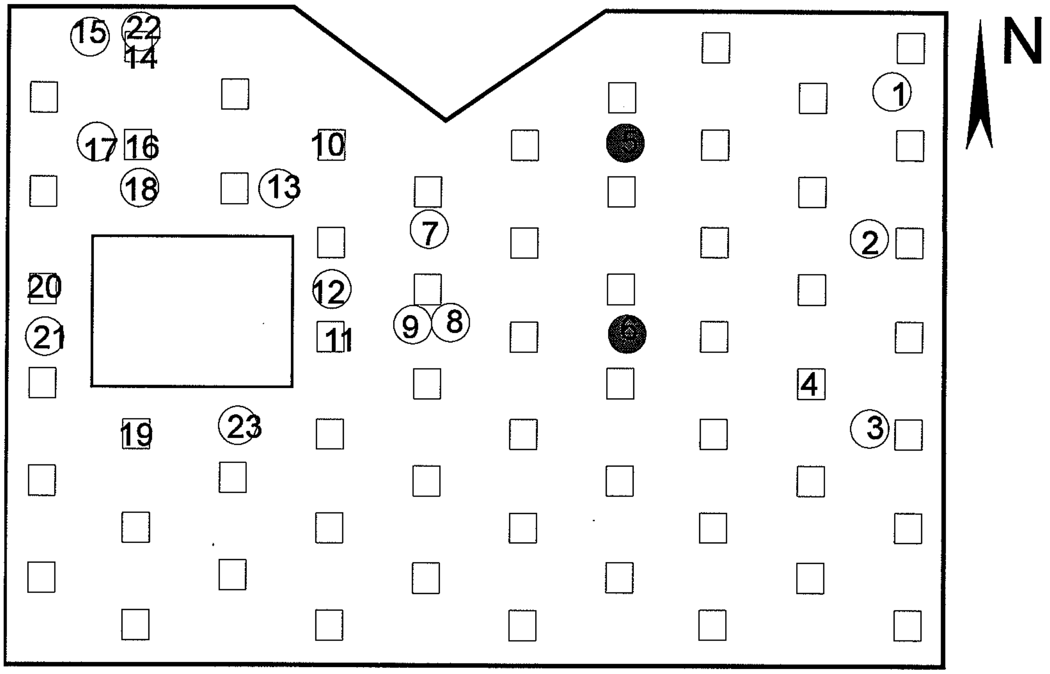

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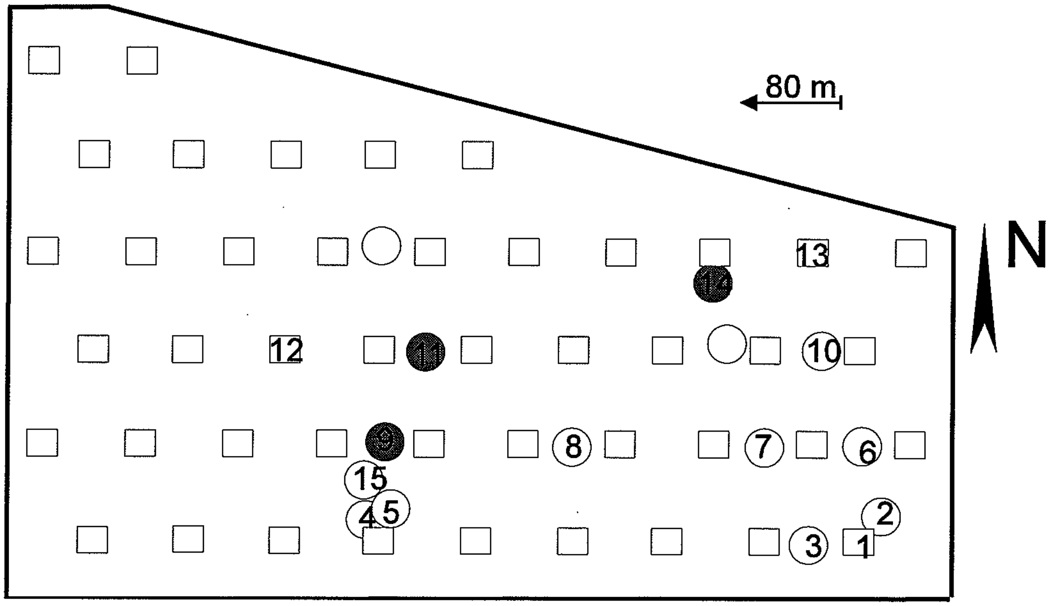

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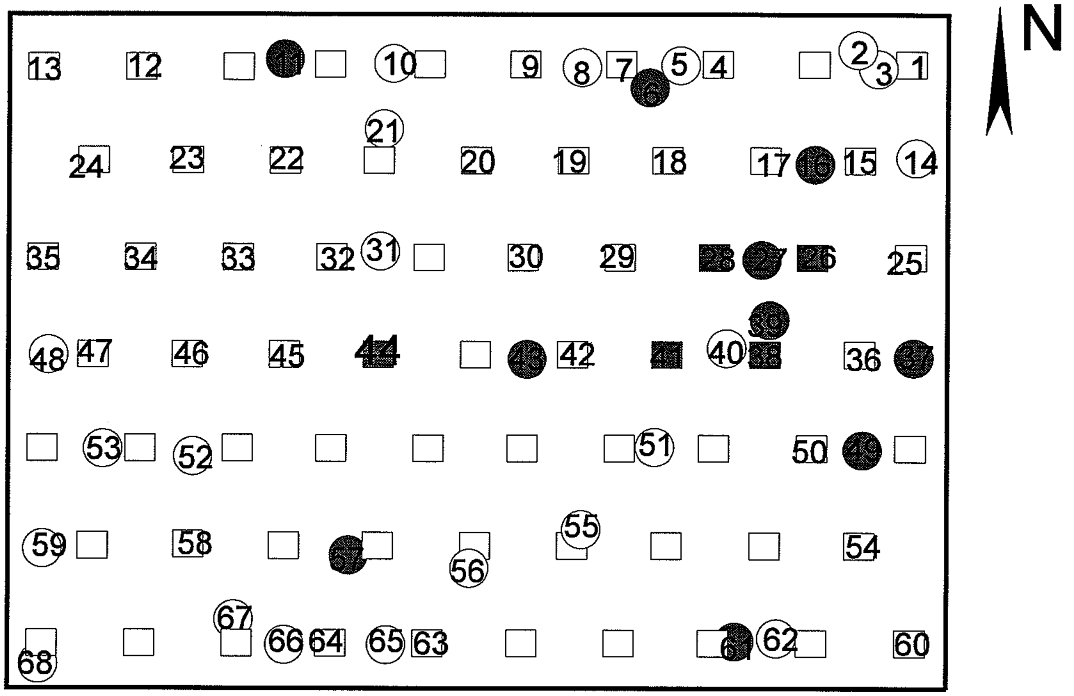
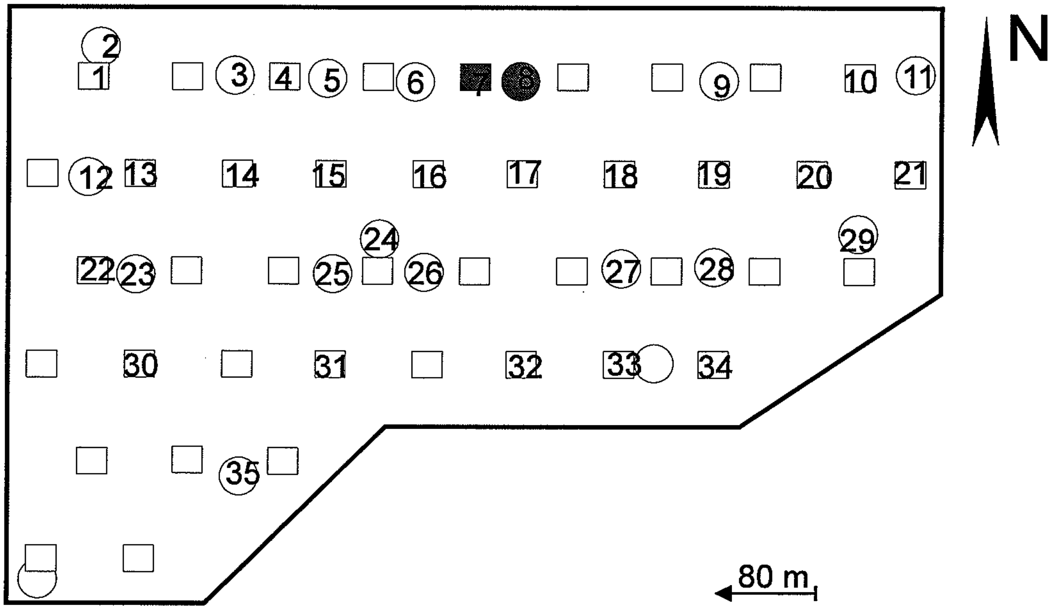


Field 19 SW020810W



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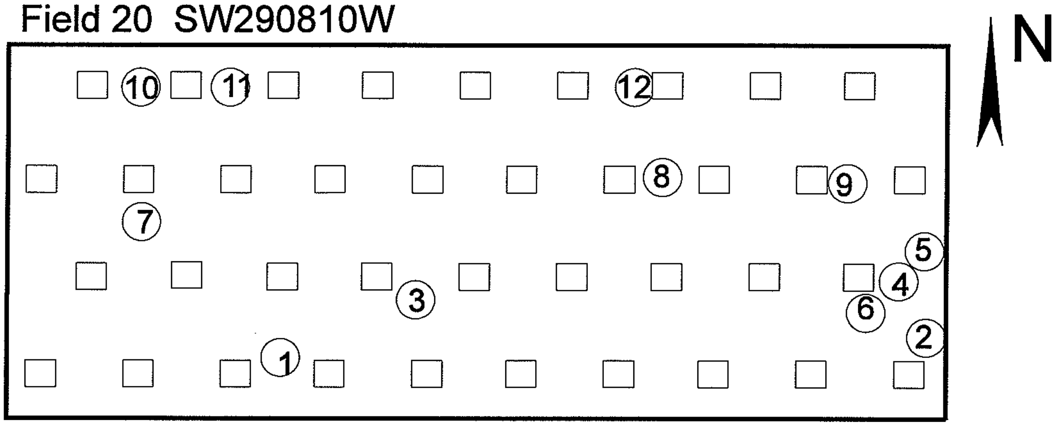

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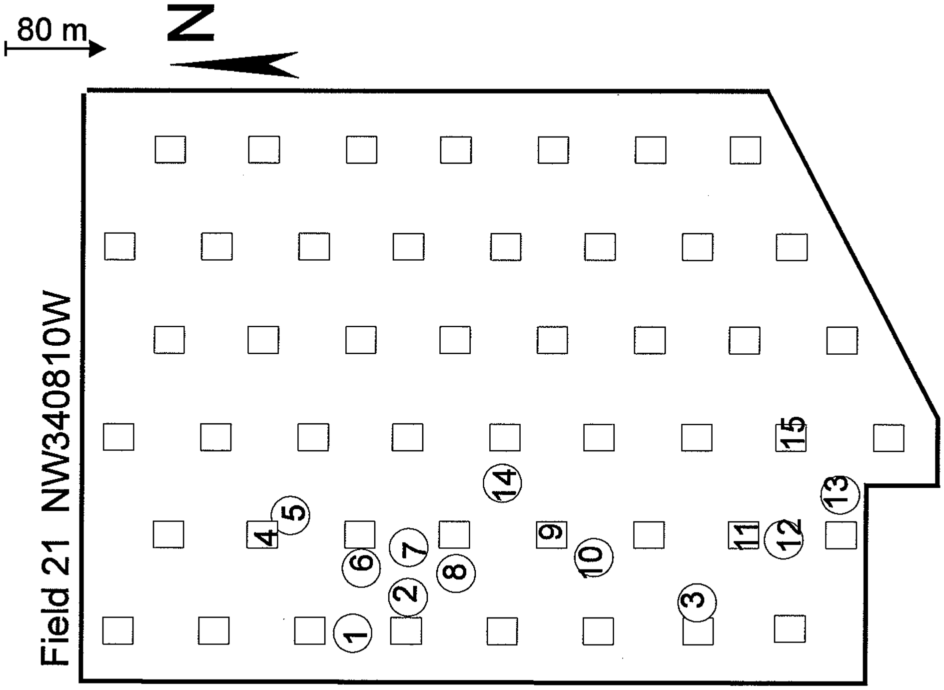

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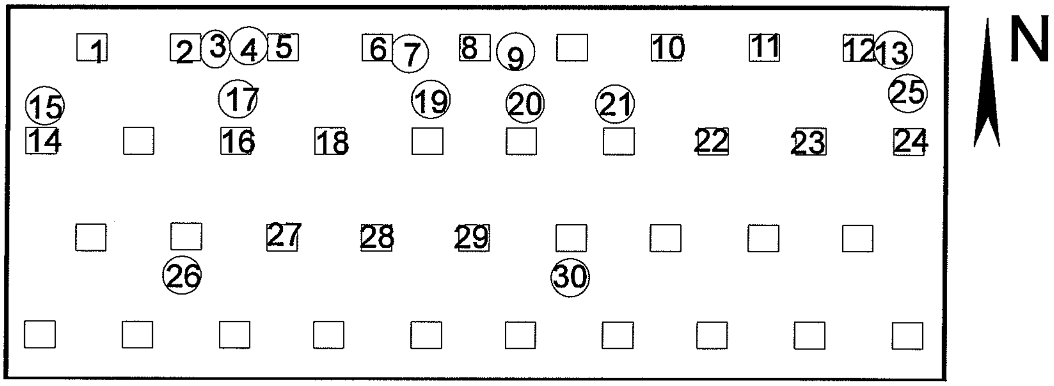

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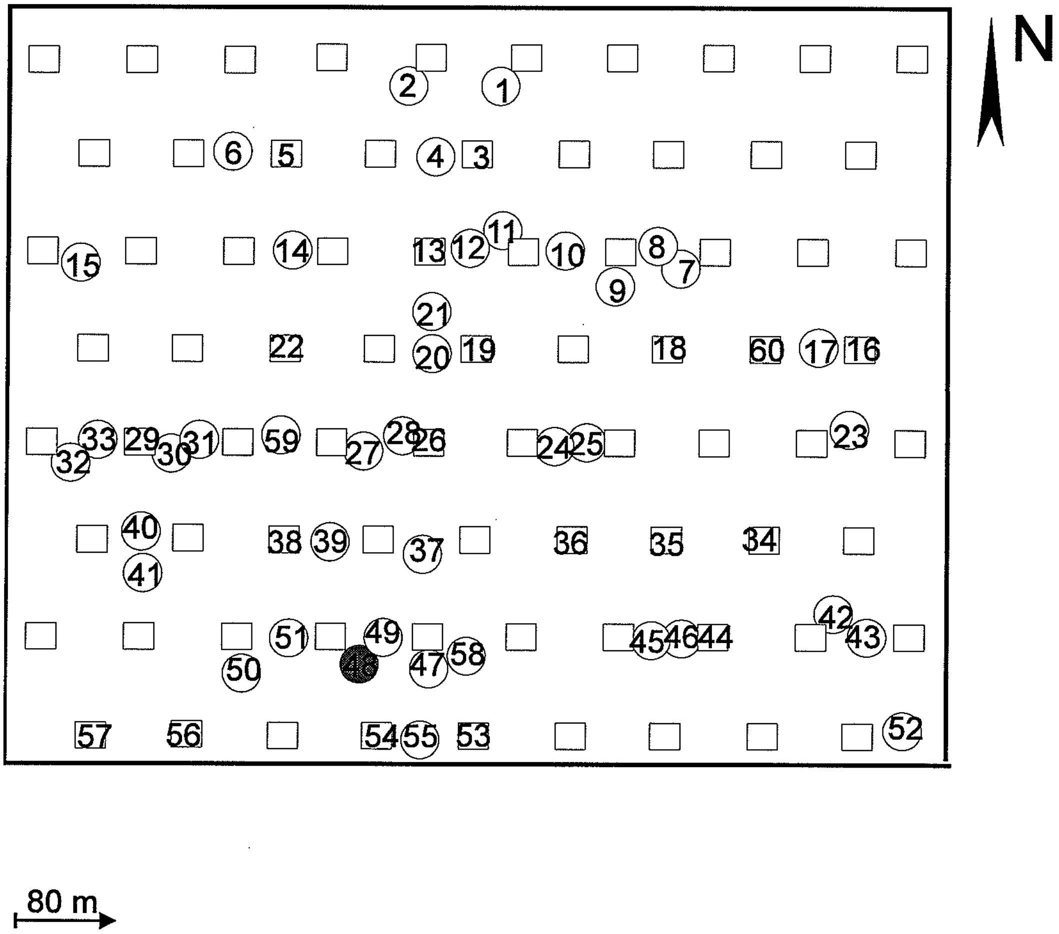
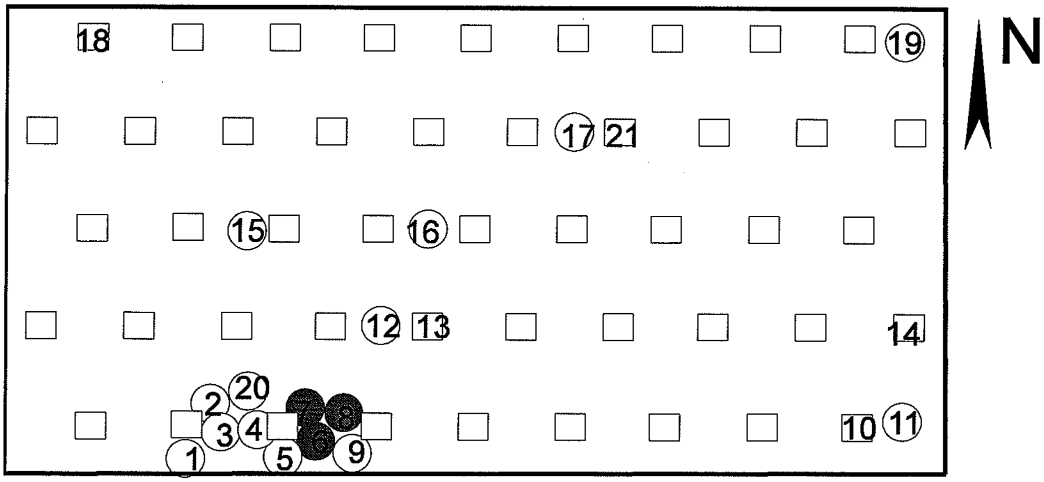


Field 24 NW200810W



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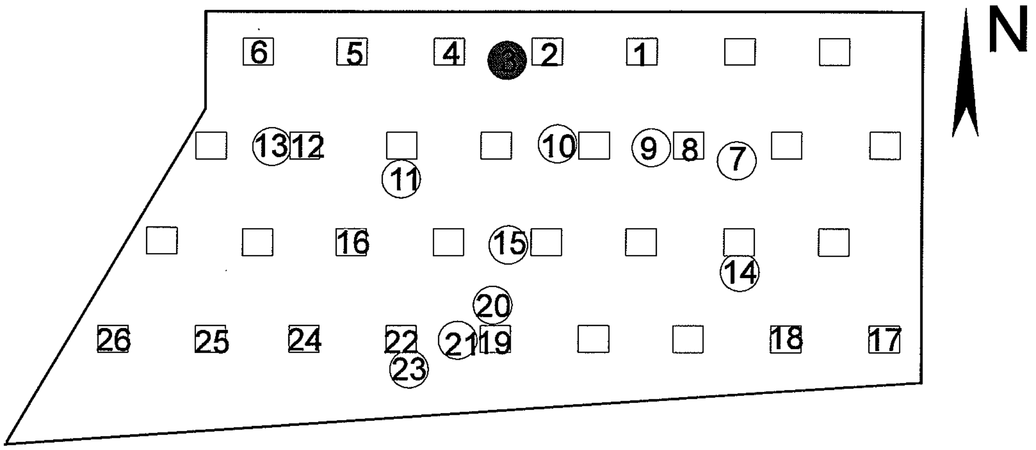

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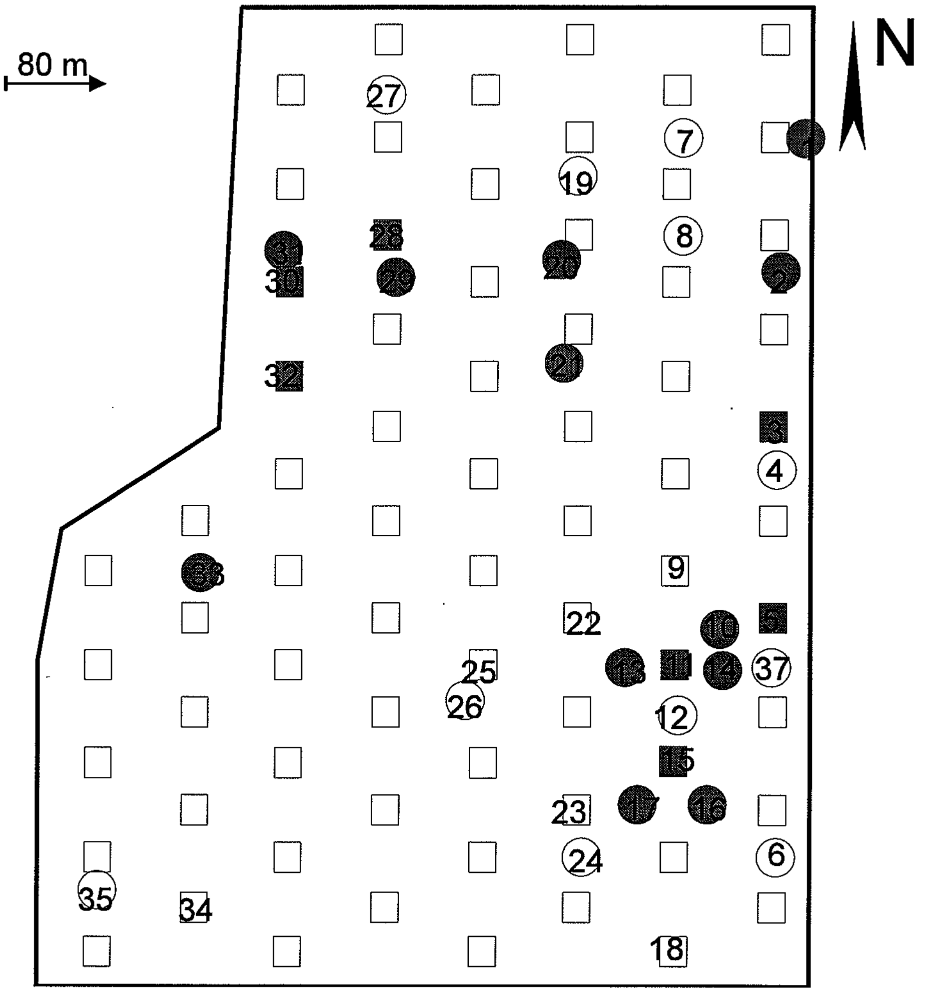

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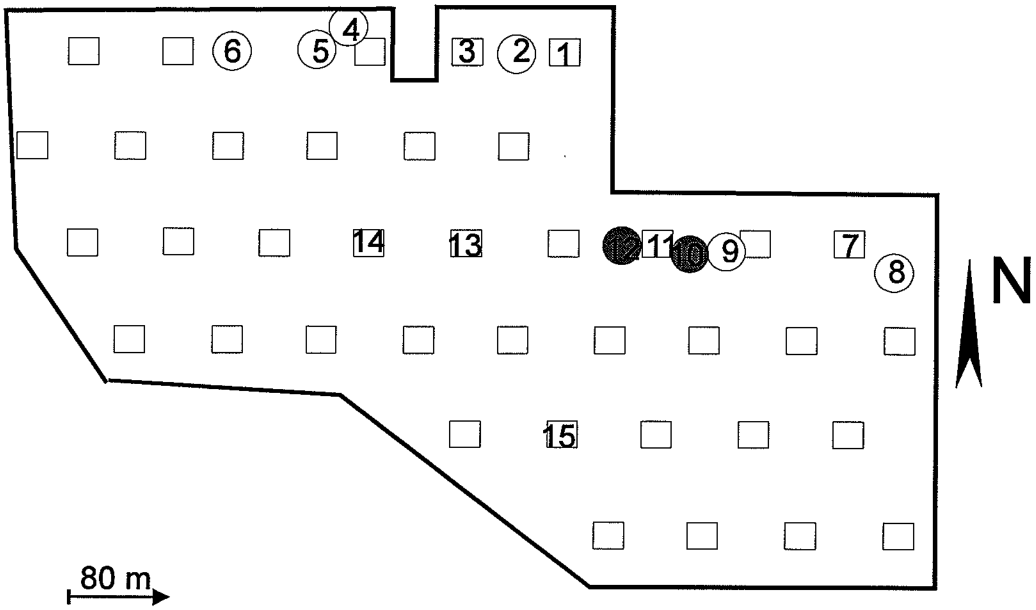

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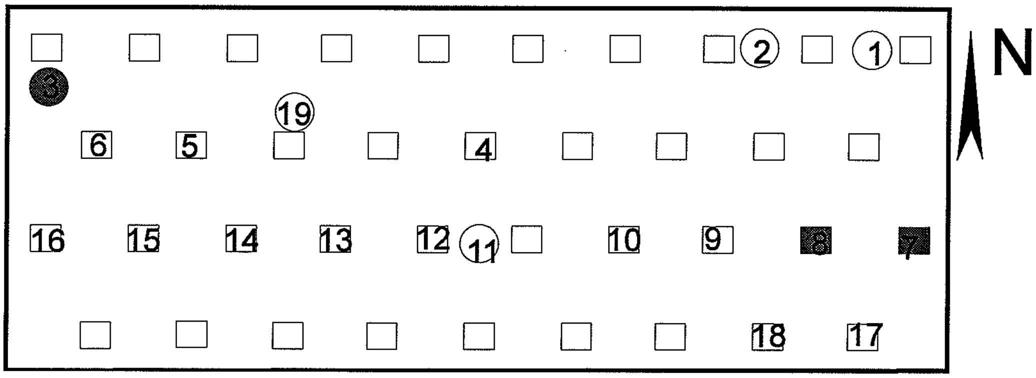

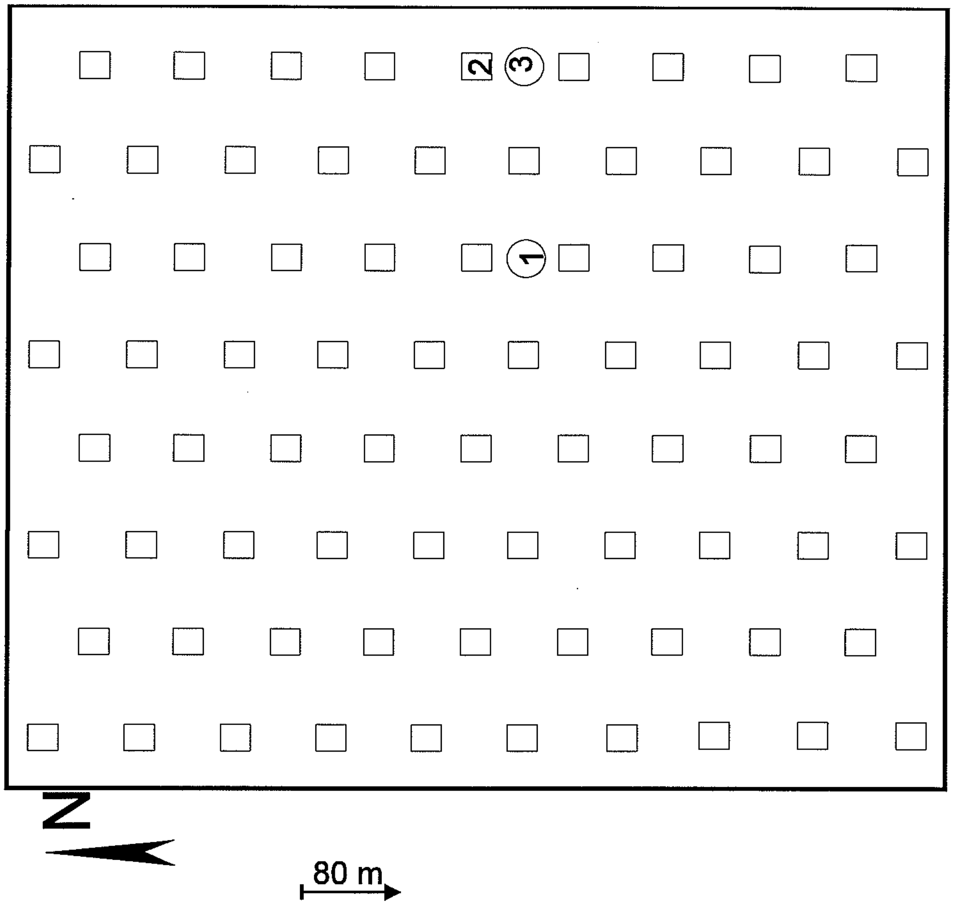

Field 29 NW280810W

Field 30 NE140810W