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HERBICIDE RESISTANCE IN WILD OATS, *AVENA* SPP.

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Abstract

This thesis reports the first instance of an *Avena* population resistant to very high levels of aryloxyphenoxypropionate (AOPP) herbicides. An *Avena sterilis* ssp. *ludoviciana* population from South Australia (SAS1) had been exposed to six aryloxyphenoxypropionate herbicides during the last 8 years. The population is highly resistant to the aryloxyphenoxypropionate herbicides diclofop, fluazifop, fenoxaprop, quizalofop, haloxyfop, paraquizafop and quinfurop. Although cyclohexanedione herbicides controlled SAS1 at the recommended rates, at lower rates SAS1 exhibited less mortality than a susceptible population. The reason for this difference in response to cyclohexanedione herbicides is yet to be determined.

Breeding experiments established that herbicide resistance in SAS1 is controlled by a single, partially dominant, nuclear encoded gene and can be transferred from resistant wild oat to cultivated oats.

Pot and field competition experiments reveal no significant differences between SAS1 and a susceptible biotype for time to first flowering, stem number, aboveground dry weight or seed yield. There is no evidence for a competitive disadvantage associated with herbicide resistance in population SAS1.

Various cultural practices had profoundly different effects on the persistence of resistant wild oat in the seedbank. Three glyphosate treatments or three cultivations per season dramatically reduced the seedbank over a period of three years. Glyphosate reduced the soil seedbank at a similar rate to multiple cultivations but is faster, cheaper and involves less soil structure damage.

Fifty nine *Avena* populations from wheat belt regions of Australia were tested in addition to SAS1. Thirty seven of these populations were resistant to at least one aryloxyphenoxypropionate or cyclohexanedione herbicides. Diclofop resistance usually appears after 3-5 years of the herbicide application either in rotation or in sequential seasons for *A. sterilis* and *A. fatua* . The number of instances of herbicide resistance in wild oats in Australia can be expected to increase in coming years.

DECLARATION

This thesis contains no material which has been accepted for award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, it contains no material previously published or written by another person, except where due reference has been made in the text.

I give my consent to the thesis being made available for photocopying or loan from the university library.

Ali Mohammad MANSOOJI

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Chapter 1

General Introduction

Pesticides are a major technical tool used successfully throughout the world. However, an adverse consequence of persistent application has been the appearance of resistant pest populations. Resistance to pesticides is a global phenomenon and now exists for fungicides, bactericides, rodenticides, nematocides and herbicides. Even in the world of medicine some organisms have been shown to develop resistance. Weeds that are resistant to herbicides are rapidly becoming very important factors in crop production and agricultural technology (LeBaron and MacFarland, 1990).

Naturally existing, genetic variation exists within large populations of a single biotype or between different biotypes of any weed species and this results in differential response to herbicide. Through these intraspecific differences in response to herbicide and the presence of herbicide selection pressure, resistant individuals survive herbicides and are able to reproduce free of competition from the susceptible biotypes (LeBaron and Gressel, 1982).

Resistant plants need to be studied both because of their usefulness for basic research and to solve the practical problems of weed control which they present. Farmers must consider the problem according to precise population dynamics if they are to achieve long-term weed control using

herbicides. If not, they will become involved in accumulating difficulties. These problems may be unsuccessful chemical control, multiple and/ or cross resistance, loss of yield and disappointment. Using different herbicides from the same chemical group or from different groups but with the same mode of action can only intensify their problem.

In Chapter 2 a review is presented of pertinent biology of wild oats (*Avena spp.*), the weed studied in this thesis. The development of herbicide resistance in wild oats is reviewed, followed by an introduction to the different wild oat populations reported in this thesis. An introduction to how resistant biotypes were found in Australia and relevant paddock histories are included. The main herbicides used for evaluation of resistance in wild oats are also discussed. Some general aspects of materials and methods are described in Chapter 3.

Clear evidence for resistance can be obtained with plants grown and treated with herbicides under field conditions. Herbicide dose responses for wild oat biotypes are shown in Chapter 4. The dose response curves were used to determine LD_{50} (fifty percent mortality dose) for the resistant biotypes.

When a new biotype is found to be resistant it is important to study some agro-ecological features. One of these features is the fitness of the resistant biotype in its natural habitat, compared with the susceptible biotype, for production of total dry matter, tillers and seed yield. In Chapter 5, the results of a competition experiments are used to indicate the potential of the resistant biotype to compete with the susceptible

biotype in the absence of herbicide. The aboveground biomass production in the absence of competition is also discussed.

In Chapter 6, the results of seedbank studies demonstrate what happens to the gene pool preserved in the seedbank and how long the seeds will survive in the soil. Such studies can help to organise a proper management program to combat the problem of resistance.

The genetic work in Chapter 7 demonstrates the number of genes responsible in controlling resistance in biotype SAS1. Studies of this character may help to identify whether there is any possibility of transferring the trait through pollen to susceptible biotypes.

The results in Chapters 4 to 7 concern a single resistant wild oat biotype, SAS1. Many wild oat biotypes from different parts of Australia were studied. These biotypes include suspected resistant populations as well as putative susceptible populations. Pertinent results of this survey are presented in Chapter 8. Some conclusions and recommendations in Chapter 9 finalise this thesis.

Chapter 2

Review of literature

2.1. Introduction

The impact of herbicides on modern agricultural technology has been remarkable. It is almost impossible to quantify the importance of herbicides in helping overcome famine, pestilence, poverty and crop losses due to weeds. Many of the weeds prevalent throughout the history of mankind are now easily controlled by herbicides introduced since 1950. Herbicides have been essential, not only for the direct effects on weed control improving crop yield but have also enabled more efficient use of water and fertiliser and for the optimum development of improved crop varieties and hybrids. They have also been important tools in the recent development of conservation tillage methods, which have reduced topsoil erosion and moisture losses in many areas (LeBaron and MacFarland, 1990).

Although the discovery and development of herbicides came after the discovery and development of organic insecticides and fungicides, herbicide use has far surpassed other pesticides in the marketplace (Table 2.1). Herbicides represent approximately two thirds of the volume of all agricultural chemicals sold in the USA (Table 2.1). They also represent 85% of the total pesticide use on major crops.

In response to the massive use of herbicides in most western nations, a number of plant species (weeds) have developed herbicide resistance following selection with herbicides. Most cases of herbicide resistance have occurred in situations where the same herbicide (or herbicides with the same mode of action) have been used repeatedly, usually associated with intensive agricultural or horticultural systems involving crop monoculture and minimum tillage, in which herbicides have been relied upon to achieve a high level of weed control (Moss and Rubin, 1993).

Table 2.1. U.S.A. pesticide sales in 1986*

	Agriculture		Total	
	($ million)	%	($ million)	%
Herbicides	2775	63	3625	56
Insecticides	1050	24	1980	30
Fungicides	310	7	515	8
Others	290	6	370	6
Total	4425	100	6490	100

World-wide total sales of all pesticides in 1986 is estimated to be $16,150,000,000

*From Economic Analysis Branch, Office of Pesticide Programs, EPA (LeBaron and MacFarland, 1990).

Biological flexibility and ecological adaptability have been recognised as laws of nature for a long time. The ability of living organisms to compensate for, or adapt to, adverse or changing environmental conditions

is remarkable. As stated by LeBaron and Gressel (1982) "regardless of how and when the various living species began, natural selection has occurred and still continues".

Many cases of herbicide resistance have appeared in the northern hemisphere and involve resistance to triazine herbicides in areas where these herbicides have been extensively used. Resistance has also developed to a diverse range of other herbicides. The importance of herbicide resistance, relative to resistance in other organisms, has increased over the past decade. In 1982 of 428 species resistant to one or more insecticides about 60% were agricultural pests and many affect human health (LeBaron and Gressel,1982). There were 81 plant pathogens resistant to benzimidazole fungicides and 8 cases resistant to the bactericide streptomycine. Thirty weed species (23 dicots and 8 monocots) contained biotypes resistant to triazine herbicides and a few to phenoxys, trifluralin, paraquat and ureas. Seven years later, in 1991, LeBaron reported that there were biotypes of 102 herbicide resistant weed species which had evolved in various locations around the world. This included biotypes of 57 species (40 broadleaf and 17 grasses) resistant to triazine herbicides and 45 biotypes (27 broadleaf and 18 grasses) resistant to other herbicides. Resistant weeds were reported in all but 11 of the 50 States of the U.S.A., all but 4 of the 10 provinces of Canada, all except 9 of 29 countries of Europe and 9 other countries, including Australia (LeBaron, 1991). LeBaron, (1991) and Holt, (1992) reported at least 50 species (33 dicots and 17 monocots) with biotypes resistant to one or more of 14 other herbicides or herbicide classes.

The situation in Australia has followed a similar trend with herbicide resistance confirmed in six species to 1989. All these are important and widespread weeds of crops and pastures throughout Southern Australia (Powles and Howat, 1990). Of greatest practical and specific concern to Australian farmers is the appearance of biotypes of the grass weed, annual ryegrass, with cross resistance to a range of chemically dissimilar herbicides. In 1992 more than 9 weed species in Australia had developed resistance. In 1990 one biotype of wild oat was formally reported as resistant in Australia (Piper, 1990; Mansooji *et al.*, 1992). The results of this thesis reveal that at least 29 resistant biotypes of wild oats had developed resistance by 1993 (Chapter 8).

2.2. Biology and agronomic importance of wild oat

2.2.1. Introduction

The genus *Avena* contains both crop and weed species. In this section the botanical classification of *Avena* species is described, followed by a discussion of the biological features of the weedy species.

2.2.2. Botany and classification

The status of different species of wild oat (*Avena* spp.) can be appreciated only by placing them in the context of the genus as a whole.

According to plant systematic methods wild oats can be classified as:

Kingdom	Plantae (plants)
Division	Vascular plants
Class	Angiosperms
Sub-class	Monocotyledons
Order	Graminales
Family	Gramineae (Poaceae), (grasses)
Tribe	*Avena*
Genus	*Avena*

Cultivated members of this genus are agronomically categorised as cereals or small seeded graminacious plants. Some common names of some species are as follows:

barbata: slender oat, bearded oat, the barbed oat

fatua: common wild oat, spring wild oat

sterilis: sterile oat, animated oat, red oat, fly oat, Barren oat

sterilis ssp. *ludoviciana*: winter wild oat

strigosa: black oat, small oat, lop-sided oat, bristle oat

(Findlay, 1956; Cooper 1979, CIBA-GEIGY, 1981)

In naked oats (a cultivated form) the caryopsis is loosely enclosed by the lemma and palea, but in all other species of *Avena* they tightly enclose the kernel and form the husk of the mature grain.

The most useful characters in the identification and classification of *Avena* species relate to the morphology of the spikelet (Thomas and Jones, 1976). The spikelet of the oat species is made up of the glumes and florets, the

latter forming the seeds at maturity. Morphological characters such as number of flowers in spikelets, disarticulation, length of floral parts, number of nerves on the glume and lemma, shape of awn are used to differentiate species. A key for using such features to identify some species is given in Figure 2.1.

Figure 2.1. Key for identifying some *Avena* species (from CIBA-GEIGY, 1981).

Spikelets 2-5 flowered, disarticulation*, if any, above the glumes and, in some species also, between the florets; glumes about equal, longer than the lower floret, 5-11 nerved, acute, not keeled. lemmas 5-9 nerved, bidentate, bearing a dorsal bent and twisted awn

1-a lemma glabrous (or nearly so); spikelets not breaking up at maturity	*strigosa*
1-2 lemma long-hairy (at least in the lower part); spikelets breaking up at maturity	2
2-a lemma ending in 2 slender 3-7 mm long bristles	
glumes 9-11 nerved	*barbata*
glumes 5-7 nerved	*wiestii*
2-b lemma ending in 2 small teeth (1-2 mm)	3
3-a disarticulation above the glumes and between the florets.	*fatua*
3-b disarticulation above the glumes only; all florets falling as a unit of dispersal:	
spikelet 20-30 mm long, 2-3 flowered	*ludoviciana*
spikelet 30-50 mm long, 2-5 flowered	*sterilis*

*Disarticulation= Separation of spikelet at maturity

The species *Avena sterilis* has been divided into the subspecies, *A. sterilis* ssp. *sterilis* and *A. sterilis* ssp. *ludoviciana*. Many workers have referred to the whole species *A. sterilis* as *A. ludoviciana*, because *A. sterilis* ssp. *sterilis* does not appear to be as common as *A. sterilis* ssp. *ludoviciana* in wheat growing areas of Britain (Thurston,1956) and Europe (Barralis, 1961), and is absent from collections at the national Herbarium of New South Wales in Australia (Whalley and Burfitt, 1972). However, *A. sterilis* is undoubtedly the correct nomenclature at the specific level and is used in this thesis.

2.2.3. Evolution, history and distribution

The numerous species of *Avena* fall into three categories according to their agronomic status (Thomas and Jones, 1976):

1) Cultivated oats include *A. sativa* L. and some varieties of *A. strigosa* Schreb.; also *A. byzantina* C. Kock and *A. nuda* L.

2) Weeds (the wild oats of agriculture) include *A. fatua* L. and certain varieties or subspecies of *A. sterilis*, of which the best known is commonly referred to as *A. sterilis* ssp. *ludoviciana* or *A. ludoviciana* Dur. These are characteristically weeds of cereals and certain other arable crops.

3) Truly wild plants, normal constituents of local vegetation in areas where they are native. These include *A. hirtula* (Lag.) Maltzew, *A. wiestii* Steud., *A. canariensis* Baum, Rajhathy and Sampson, *A. murphy*; Ladizinsky. At best these may provide some grazing (Thomas and Jones,1976).

Although the areas of domestication do not coincide with the primary areas of distribution, or the centres of origin of the weed progenitors, the

genetic relationships between the wild and cultivated species clearly indicate the derivation of the latter from the wild hexaploid species (Thomas and Jones,1976).

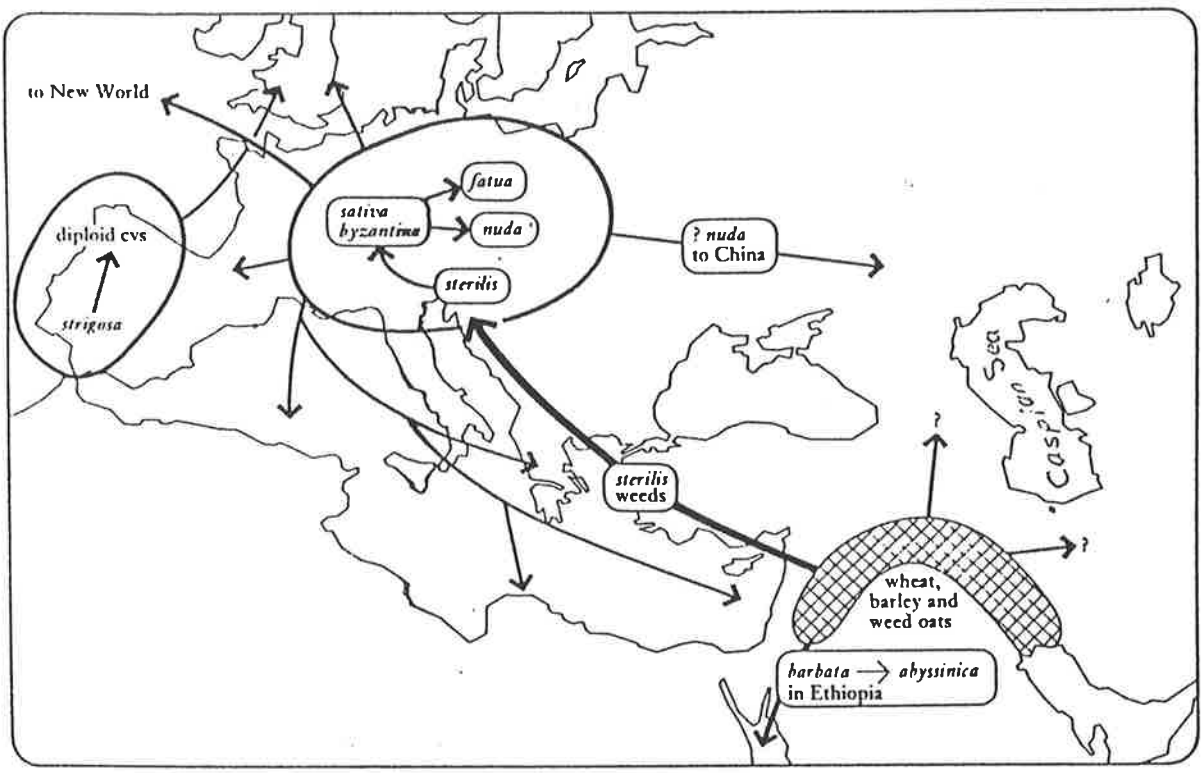
2.2.4. Centre of origin

According to Malzew, (1930) the precise origin of wild oats species is unclear. The most likely place of origin for *Avena fatua* is in south west Asia, particularly in Pamir. For *A. sterilis* including *A. ludoviciana* the most likely place of origin is in Asia Minor. In a later determination (Holden, 1976) states that the genus *Avena* has its origin in the "fertile crescent" area between the Persian Gulf and the Mediterranean.

According to Thurston and Phillipson, (1976) wild oats are thought to have originated as a contaminant in wheat grown in Persia and spread mostly by Neolithic man. More recently this has occurred through the movement of Caucasians.

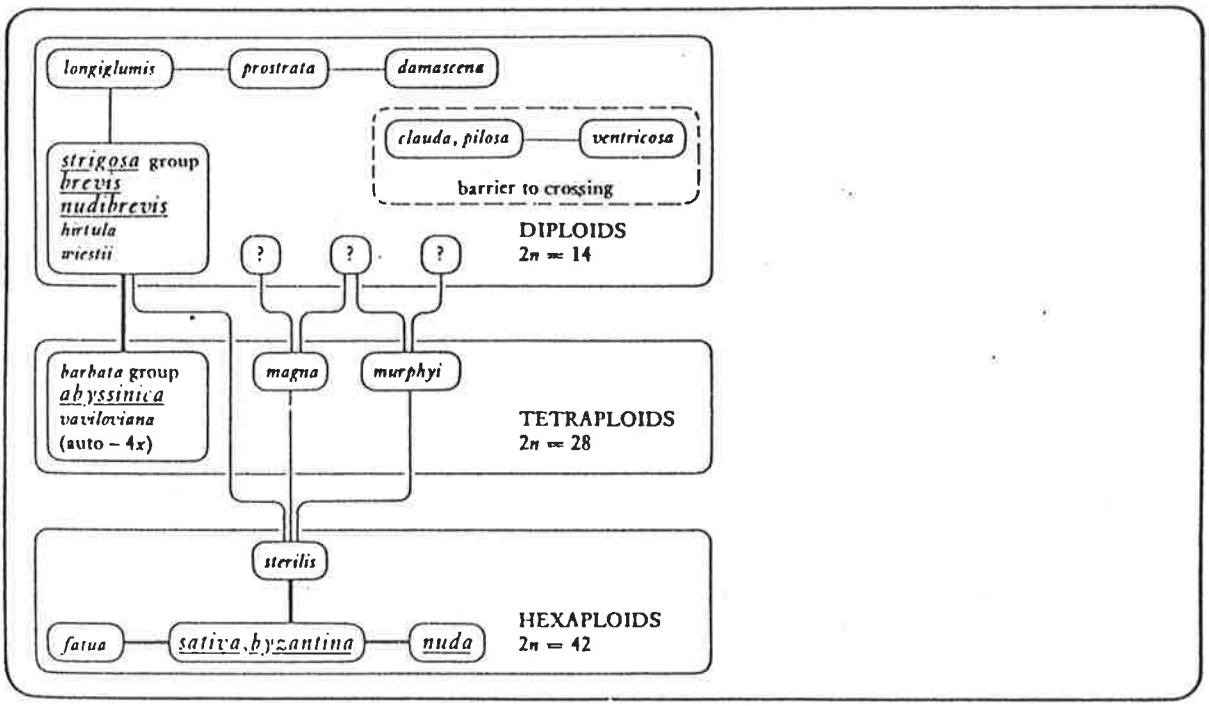
Wild oats are now found from Alaska to Iceland and Iraq to California (USA) in the northern hemisphere and in most non-tropical agricultural areas of the southern hemisphere (Holm *et al.*,1977). Wild *Avena* species such as *A. sterilis* are weeds of wheat and barley crops. As contaminants of wheat and barley grain, *A. sterilis* was carried north west into temperate Europe. The cultivated hexaploids (e.g. *A. sativa*) evolved from *A. sterilis* while the other important weedy hexaploid, *A. fatua*, may have either evolved from *A. sativa* or from *A. sterilis* (Holm *et al.*, 1977).

Figure 2.2 Evolutionary geography of the cultivated oats, *Avena* (From Holden, 1976).



The *Avena* species has three levels of ploidy: diploids (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42). All are regular bivalent formers. Cultivated forms with non-shedding grains occur in each group (Holden, 1976). A summary of present knowledge on species relationships is given in Figure 2.3. This summary is based on genome affinities.

Figure 2.3. Relationships in *Avena*. Cultivated forms in large italics. Thickness of connecting lines indicates degree of relationship, not necessarily evolutionary connection (Holden,1976).



Two main evolutionary mechanisms appear to have promoted speciation

(1) structural divergence of chromosomes

(2) hybridisation followed by chromosome doubling (Barr, 1990).

"The hexaploid oats incorporate 3 genomes designated "A", "C", and "D". "A" is derived from the diploid A genome species, "B" from *A. clauda* - *A. pisola* type while the origin of the "D" genome is unknown. In many ways, the *Aveneae* are similar to the *Triticeae* in the method of speciation and the genetic structure of the cultivated hexaploids (Barr, 1990).

A. fatua has been reported as a serious weed of cropping since 700 B.C. Today, wild oat is a weed of more than 20 crops in 55 countries including Australia (Holm *et al.*, 1977). The exact date of introduction into Australia is unknown, but it is thought that introduction probably occurred from South Africa in the early 1800s and by 1895 *A. fatua* was recognised as a pest (Grigg 1974, Maiden,1895; Patterson,1976; Thurston and Phillipson, 1976). It is distributed throughout the entire wheat belt (zone of 250-500 mm annual rainfall) in Australia, especially where the break of the season occurs during April-May (Thurston and Phillipson, 1976).

2.2.5. Biology of weedy *Avena* species

Wild oats usually germinate during autumn or winter in the wheat belt of Australia. They grow very tall and vigorously compete for light, as they grow above the crop. Wild oats species found in Australia are predominantly self-pollinating (Jones,1976), with natural intraspecific outcrossing rates typically less than 0.5-1% (Stanton and Coffman, 1924; Coffman and Wiebe,1930; Purseglove, 1972; Jain,1975; Burdon *et al.*, 1992). Derick (1933) reported an interspecific outcrossing of 0.1% in artificial block plantings of *A. fatua* and *A. sativa*. Burdon *et al.*, (1992), reported quantitative estimates of the frequency of outcrossing between *A.*

fatua and *A. sativa* in four naturally occurring, mixed populations to vary from 0 to 0.93% (mean=0.65%).

The inflorescence is a panicle. Seeds rapidly come to maturity, mostly before the crop they are infesting. The first florets ripen early and shatter, enriching the seed bank. Due to the indeterminate growth habit of the panicle, the last seeds are just emerging when the first, ripe ones are shattering. These late seeds, mature with the crop, ensuring contamination of the crop seeds at harvest. Wild oats are fecund species. Up to 7000 seeds can be produced from one plant, in favourable conditions (Barr, 1990).

Three species of wild oats, *Avena fatua* L. ssp. *fatua* (L.) Thell (hexaploid 2n=6x=42), winter wild oat, *Avena sterilis* ssp. *ludoviciana* (Dur.) G.& M. (hexaploid 2n=6x=42) and slender wild oats, *Avena barbata* Pott. (tetraploid 2n=4x=28) commonly infest the cereal growing areas of Australia. Usually the hexaploid *A. fatua* and *A. sterilis* are weeds of crops while the tetraploid *A. barbata* is mainly restricted to roadsides (Whalley and Burfitt, 1972; Patterson, 1976; Barr, 1990; Thurston and Philipson, 1976). *Avena barbata* is typically a plant of wastelands.

2.2.5.1. Seedling establishment

A. sterilis ssp. *ludoviciana* does not persist in Northern Europe and Northern America because temperatures are too cold to allow winter emergence of this species (Fernandez-Quinantilla *et al.*, 1990).

Soil moisture is another factor which affects the distribution of *A. sterilis* and *A. fatua*. *A. sterilis* germinates better in Mediterranean climates where moisture is usually a limiting factor but *A. fatua* is mostly found in soils with higher water levels (Thurston and Phillipson, 1976).

Fertiliser is another factor influencing wild oats germination and establishment. Agenbag and de Villiers (1989) demonstrated that germination and establishment of semi-dormant seeds of *A. fatua* was significantly increased by 25 to 32% by limestone, ammonium nitrate and liquid ammonium nitrate in a sandy loam and loam soil.

2.2.5.2. Wild oats and crop yield reduction

In many countries wild oats is one of the most important weeds of cereals (Combellack, 1992b; Holm *et al.*, 1977; Thurston and Phillipson, 1976). In most cereal cropping areas, where farming relies on winter rain, wild oats is commonly a dominant grass weed. Wild oats can usually be accompanied by dicot weeds such as wild radish (*Raphanus raphanistrum*), black mustard (*Brassica nigra*), wild rapeseed (*Eruca sativa*), etc. In a comparative study between twelve winter weed species in England, wild oats were found to be the most competitive weed (Wilson and Wright, 1990). Wild oats decreases crop yields drastically throughout the world. During 1976, crop losses due to wild oats in the USA were estimated at $150 to 200 million and around $200 million in Canada (Dew, 1978). It was estimated that in New Zealand, crop loss in wheat was from 6.4 to 4.1% and for barley 9.1 to 20.2% based on a crop yield of 4.5 tonnes/ha (Bourdot and Saville, 1988).

Average grain yield loss of 1% per wild oats plant per m^2 have been used for defining loss threshold (Wilson and Peters, 1992).

Larger competitive effects at lower crop densities were reported by Wilson and Wright (1990) with yield losses of 1.2% for one wild oat plant m^{-2} at a wheat crop density of 134 plants m^{-2}. In contrast, with 443 wheat plants m^{-2}, 0.7% yield loss per wild oat was recorded.

From competition studies in different parts of the world Combellack (1992a) reviewed yield reductions. Table 2.2 was extracted from this review.

Table 2.2. Wild oats and crop yield reduction

Barley (*Hordeum*)		Wheat (*Triticum*)		Flax (*Linum*)		Lentil (*Lentil*)	
wild oats density /m^2	crop loss %	wild oats density /m^2	crop loss %	wild oats density /m^2	crop loss %	wild oats density /m^2	crop loss %
48.0	7.0	84.0	22.0	48.0	41.0	32.0	32-42
48.0	18.0	84.0	31.0	48.0	55.0	65.0	49-61
100.0	15.0	100.0	30.0	100.0	65.0		
135.0	8.9	188.0	55.0	192.0	86.0		
170.0	40.0	300.0	55.0				
218.0	26.0	300.0	77.0				
306.0	32.0						
415.0	47.7						

The economic response to herbicide varies with the density of wild oats. Barton *et al.* (1992) reported that with a wild oats density of 100 plants m^{-2}, net return was greatest when the herbicide was used at half the recommended rate. At 290 weeds m^{-2}, increased returns due to herbicide use were greatest when half or full recommended rate was used.

In these crops, use of fertilisers caused wild oats to grow more vigorously causing higher crop yield reduction (Combellack, 1992a). In the absence of wild oats, wheat yield was increased from 4.3 t ha^{-1} to 6.7 t ha^{-1} when

nitrogen fertilisers were applied from nil to 200 kg/ha (Wright and Wilson,1992). But in the presence of 45 wild oats m^{-2}, yield was reduced to 2.1 t ha^{-1} (51% yield loss) in the absence of fertiliser and increased to 0.7 t ha^{-1} (90% yield loss) when nitrogen was applied at 200 kg/ha (Wright and Wilson, 1992).

Crop yield loss by wild oats in the field can be dependent on growth stage. Very early wild oat competition was not significant in reducing spring barley yield until the barley had reached the four leaf stage (Chanceller and Peters, 1974).

Holroyd (1972) reported that wild oats which emerge with the crop before early November (early in autumn in the northern hemisphere) have a better chance of good establishment and of surviving the winter than those emerging later. Furthermore, their chance of survival is increased markedly if they survive past the two leaf stage. Plants which had reached 3-leaf stage by mid-December (early in winter) suffered minor mortality (<20%) compared with plants with only one leaf at this time (>70% winter mortality). Wilson and Cussans (1978) stated that autumn emerging wild oats have greater competition and seed production in comparison to those emerged in the winter and in the following spring.

It was shown in Australia (Radford *et al.*, 1980) that greater crop density reduced the effect of wild oats in decreasing yield. The time period over which wild oats were present in the field had a positive correlation with yield reduction. The longer the time period that wild oats remained in the field the more was reported to be the reduction in yield. In experiments

where wild oats were removed from the field at different times after planting, the crop yield loss increased with the period of time lapsed before removal (reviewed in Combellack, 1992b).

2.2.5.3. Distribution in Australia

Cartedge (1973), reported both *A. fatua* and *A. ludoviciana* in southern Queensland, the latter occurring more. McNamara (1966) reported that *A. sterilis* was dominant in northern New South Wales and absent from some southern areas while *A. fatua* was dominant in southern New South Wales.

Watkins claimed that *A. sterilis* ssp. *ludoviciana* constitutes up 90% of the wild oats on the heavy black soils of the Darling Down and 65% on light brown soils (Whalley and Burfitt, 1972).

2.2.5.4 Breeding system

The hexaploid *Avena* species (*A. sativa, A. sterilis and A. fatua*) are predominantly self-pollinated but may have a low rate of outcrossing. There are no detailed reports of the relative rate of outcrossing in *Avena sterilis* ssp. *ludoviciana*. However, rates of less than 1% outcrossing have been measured for *A. fatua* and *A. sativa* (Burton *et al.* 1990). Intraspecific outcrossing may also occur between *A. sterilis* and *A. sativa*. An average rate of 0.7% outcrossing between these species was measured under field conditions in Australia (Burton *et al.* 1990).

2.2.5.5 Seed dormancy

Seed dormancy can vary greatly between and within species of *Avena*. However, it is thought that of the hexaploid species *A. sativa* has the shortest period of dormancy and *A. fatua* the longest (Simpson 1990). Detailed studies of dormancy in *A. sterilis* ssp. *ludoviciana* in Australia are lacking from the literature prior to this study. Seed dormancy is genetically controlled but may vary greatly between populations of any one *Avena* species. Many factors including moisture level, light, temperature, gaseous, inorganic and organic compounds may affect the period of seed dormancy in different ways for different genotypes (Simpson 1990).

2.3. Development of herbicide resistance in wild oats

2.3.1. Definition

Several definitions of resistance and tolerance as applied to herbicide resistance in weeds have been proposed.

Tolerance is the natural and normal variability to pesticides and other agents which exist within a species. Tolerance usually refers to relatively minor or gradual differences in intraspecific variability (LeBaron, and Gressel, 1982).

Resistance, as defined by the F.A.O, is a decreased response of a population of animal or plant species to a pesticide or control agent as a result of their

application. According to Dyer *et al.* (1992), resistance describes the ability of a population of plants within a species or larger taxonomic group, or plant cells in culture, to withstand a herbicide at a dosage substantially greater than the wild type is able to withstand, with near normal life cycle. This includes pre-existing lack of susceptibility to herbicides, loss of susceptibility due to selection pressure by a herbicide, or resistance conferred by genetic manipulation, generally in crops (reviewed in Holt and Thill, 1994).

It has been found convenient to distinguish between physiological resistance and behavioural resistance. The former involves resistance in the presence of the control agent on or in the pest organism, whereas the latter involves resistance because of some behavioural factor which decreases the probability of contact between the pest and control agent (LeBaron and Gressel, 1982).

In plants, a working definition of a resistant weed is that, a resistant weed survives, grows and reproduces normally after exposure to the normally effective dose of a herbicide.

Resistant individuals are usually found in much lower frequencies than tolerant ones in natural, untreated populations (LeBaron, and Gressel, 1982).

There are also a number of instances where one weed species is resistant to a number of herbicides, giving rise to the terms "cross-resistance" and "multiple-resistance".

Cross-resistance is the phenomenon where a resistant biotype exhibits resistance, not only to herbicides from the chemical class to which it has been exposed, but also to herbicides from dissimilar classes to which it has never been exposed (Holt *et al.*, 1993).

Multiple-resistance is resistance to more than one herbicide as a result of selection pressure from each of the herbicides, either having been used in combination or sequentially.

Negative-cross resistance: Sometimes a resistant biotype may be more susceptible to one or more herbicides than the normally susceptible biotype. This phenomenon is called negative-cross resistance (see Gressel and Segel, 1989; Leroux, 1992).

2.3.2. Factors influencing the rate of development of resistance

Variations in the control of a given weed species by a particular herbicide may be due to differences in application of the herbicide, soil type, rate of herbicide disappearance from the biosphere, depth and time of seed germination, climate and many factors other than intraspecific variation in the tolerance of the weed to the herbicide. Therefore, if resistance is suspected, it is absolutely essential to compare the effect of the herbicide on both the biotype suspected to be resistant and a susceptible biotype under the same field, greenhouse or laboratory conditions (LeBaron, and Gressel, 1982).

Resistance to herbicides has appeared largely in monoculture and/or monoherbicide practices. Several factors influence the rate at which resistance evolves in a weed population. These include genetic factors such as the initial frequency of resistance genes in untreated populations, the type of herbicide and the extent of the selection pressure from its use. Aspects of the biology of the plant species such as dormancy, seedbank life and interpollination by susceptible plants and relative competitiveness and fitness of the resistant and susceptible individuals in the population will also affect the rate of development of resistance.

The many factors that influence the rate of evolution of resistance in the field have been divided by Georghiov and Taylor, (1986) for convenience, into three categories, genetic, biological/ecological and operational factors. In plants, biological and ecological factors have been particularly important in delaying the evolution of resistance to herbicides, in contrast to the rapid rate of evolution of pesticide resistance in other organisms (Holt, 1992).

One particular concern has been the rapid development of resistance to some newer herbicides. Not only are herbicide resistant weeds appearing after fewer annual applications of some of the newer herbicides, but more species appear to be capable of developing resistance. In addition, the newly evolved resistant biotypes are equally fit and competitive, unlike most biotypes resistant to triazine herbicides (Holt *et al.*, 1993).

2.3.2.1. Initial gene frequency

Darmency and Gasquez (1990) stated that genes for herbicide resistance may or may not exist in natural weed populations. Price *et al.* (1983) studying the level of genetic variation for tolerance to herbicides in populations of *Avena barbata* and *Avena fatua* reported that: "the amount of genetic variance for herbicide reaction is higher than expected on the basis of mutation alone, suggesting selection favouring genes conferring herbicide tolerance occurs in natural populations". So:" It is possible that resistant genotypes are present in plant populations in varying, but low frequencies before any exposure to herbicides (Moss and Rubin, 1993)".

Gressel and Segel (1982) proposed that the initial frequency of genes for resistance which occurs before selection, is of fundamental importance to the rate at which herbicide resistance will arise in a particular weed population. Ahrens and Stoller (1983) on the other hand, suggested that sub-lethal doses of herbicides may also be important. Fewer genes are necessary for resistance if it is controlled by dominant alleles rather than recessive alleles (Gressel and Segel, 1982). The initial gene frequency for resistance is much lower if more than one gene controls resistance since phenotypic frequencies will be multiples of those of monogenic resistance. More than one gene may be necessary for resistance to a herbicide (or mixture of herbicides) that has multiple sites of action in the plant, such as the phenoxy acid herbicide (e.g., 2,4-D). In polyploids, the initial gene frequency for resistance can be higher since the number of mutations in a population is increased and fewer of these mutations are lethal. This allows

more rapid adaptation to many factors (including herbicides), and is hence a characteristic of many common weeds (Hass and Streibig, 1982).

2.3.2.2. Seed characteristics

The persistence of a soil seedbank of the susceptible individuals in the population, at least for cross pollinated species, has a buffering effect on the rate of enrichment for resistance. This buffering effect is most pronounced in plants with seed characteristics such as dormancy and asynchronous germination which allows susceptible individuals to escape herbicide application and eventually replenish the seedbank with susceptible seed. In this way the time frame of development of resistance can be slowed (reviewed by Maxwell and Mortimer, 1994).

2.3.2.3. Field operations

Hand weeding and other kinds of mechanical soil treatments, such as cultivation, are of importance to the time frame of the onset of herbicide resistance. In non-chemical farming systems or in developing countries, where hand weeding or mechanical methods of weed control are used, there are few reports of resistance. This is because rare individuals which survive herbicide treatment due to resistance may be killed by mechanical control methods. Inversion tillage, such as mouldboard ploughing, can reduce the requirement for herbicides and thus reduce the selection pressure (Moss and Rubin, 1993).

Considering tillage methods, it is obvious that deep mouldboard ploughing of the land, buries many seeds deep in the soil. This deep burial of seeds

prevents many seeds from germinating or emerging, compared with minimum tillage or no-tillage methods in which seed is close to the soil surface. Inter-row cultivation can be an effective method of non-chemical weed control which should impose no selection pressure (Moss and Rubin, 1993).

2.3.2.4. Fitness of resistant versus susceptible biotypes

Fitness may be defined as reproductive success, or the proportion of genes an individual leaves in the gene pool of a population. Since natural selection operates by differential reproductive success, the organism that leaves the most offspring is the one whose genes come to dominate the gene pool (Holt, 1988).

Natural selection for a particular trait may incur a cost to the organism in terms of fitness, or its ability to survive and reproduce. This holds true for weed biotypes possessing the maternally inherited trait of triazine resistance. This mutation has a detrimental effect on photosynthesis that results in decreased yield and reproductive output (Holt, 1988). When one gene replaces another in a population, the new gene may be less adaptive and, thus, has some physiological disadvantages relative to the original gene. Eventually, if changes in the environment or in the residual genotype occur, the new genotype may increase fitness over a few generations. An increase in the fitness of the new type may occur when changes in the selective agents in the environment take place that favour the new type. In addition, any physiological disadvantages of the new gene may be gradually compensated by the selection of modifiers. Until

the new gene is established in the population, however, increased mortality or reduced fertility of the organism may occur. Thus there may be a cost that accompanies a mutation and that cost is decreased fitness (Holt, 1988). In the case of resistance to pesticides, a new resistant genotype will be favoured by the presence of the chemical selective agent in the environment. In the absence of the pesticide however, the resistant genotype may suffer some cost in fitness relative to the original susceptible genotype that dominated the population. Since selection of compensatory, or modifying, traits is an evolutionary process that occurs over a long period of time, a recently selected herbicide resistant genotype may posses some physiological disadvantages over the short term that are expressed as reduced fitness (Holt, 1988). Over the long term, in the absence of the pesticide selector, the resistant genotype would be replaced by the susceptible one, due to decreased fitness (Holt,1988); if the resistant biotype is less fit than the susceptible one(s).

While fitness or reproductive success, is essential for a species to be advantaged by natural selection, many separate characteristics of a plant combine to determine its fitness. These characteristics include seed germination and dormancy, establishment, the physiological processes that determine growth rate, and seed size and yield per plant.

Fitness is mediated by the interactions of the phenotype of an organism with its abiotic and biotic environment. Therefore, both adaptation, or conformity between the plant and its environment, and competition with neighbouring plants will affect fitness. Complicating factors that may also determine fitness include other herbicides or weed control measures that

may affect resistant and susceptible genotypes differently. All of these factors combine to determine the relative fitness of resistant and susceptible plants, and thus, their frequency in a mixed population (Holt, 1988).

Of the many attributes that comprise the overall fitness of an individual plant, biomass production is often assumed to be a good indicator of fitness. While this assumption may not hold true under all conditions, high crop yield is one of the most desirable plant attributes in agricultural systems where short-term productivity is the goal. Highly productive weeds are often the most competitive, and therefore, successful or fit in agricultural systems. Thus an understanding of the extent to which herbicide resistance may be correlated with reduced fitness in the form of reduced yield is essential for the development of herbicide resistant crops, as well as for the prediction of the appearance of resistant weeds (Holt, 1988).

From Table 2.3. (Holt, 1988), it can be seen that dry weight difference between triazine resistant and susceptible biotypes in different genera/species/biotypes varies. But in general the resistant individuals are less fit. This general relationship may hold for triazine resistance but may not be so for other cases of resistance (see Section 2.3.2.4).

Table 2.3. Whole-plant differences between triazine resistant and susceptible biotypes[a] (extracted from Holt, 1988).

Species	Total dry weight		Reproductive organs	
	R[b]	S[c]	R	S
Senecio vulgaris	2.9	3.9		
Amaranthus retroflexus	9.8	16.1		
Senecio vulgaris	2.0	6.1	0.6	1.3
Senecio vulgaris	0.7	1.1	0.1	0.2
Senecio vulgaris	0.9	1.4	0.2	0.3
Amaranthus hybridus	373.3	653.3		
Amaranthus retroflexus	63.1	74.3		
Brassica campestris	1.6	2.5		
Chenopodium album	28.3	40.8	11.6	15.6
Chenopodium strictum	21.7	24.6	7.4	7.4

a. Plant age at the time of harvest varied from 4 to 11 weeks among these references; in each case, the maximum dry weight reported (g) is presented in the table.

b&c. R and S stand for Resistant and Susceptible biotypes, respectively.

2.3.2.4.1. Effects of environment on fitness

There is increasing evidence that the relative productivity of susceptible and resistant biotypes of a single species may depend on environmental conditions, in particular, temperature. For example, triazine resistant biotypes of *Setaria spp.* produced more biomass under cooler -v- warmer temperatures and yield was inversely related to increasing temperature, while susceptible plants grew best at higher temperatures (Ricroch *et al.*, 1987). Similarly, *Brassica napus* and *Phalaris paradoxa* showed different responses according to changes in temperature (Beversdorf and Hume,1984; Schonfeld *et al.*, 1987). Changes in response to environmental factors occurring in herbicide resistant biotypes may be either favourable or unfavourable. For example, a resistant biotype may exhibit a greater increase in germination in response to vernalisation than that of the original, susceptible biotype. This differential response may increase the competitiveness of the resistant biotype in colder climates but could decrease its fitness in a warmer environment. Changes in membrane lipid composition observed in the chloroplasts of resistant plants may be responsible for these differences in temperature sensitivity between susceptible and resistant biotypes of *Polygonum lapathifolium* (Holt, 1988).

It has been suggested that most of the resistant biotypes have been selected from pre-existing mutated biotypes within populations. Holt, *et al.*, (1993) stated that there are no data to suggest that mutations resulting in herbicide resistance are caused directly by the herbicide.
On the other hand, mutation is a phenomenon which may occur at any time. Therefore, there is a fair possibility for new mutations to occur

throughout a period of herbicide selection. There are many factors which may effect the rate of mutation. Such factors include use of chemical fertilisers, use of different pre and post emergent herbicides, use of other pesticides in the field and chemical seed treatment before planting. Gross environmental changes may also contribute to the rate of mutation. For example, altered ecological components such as CO_2 and other gases caused by an increased human population, petroleum combustion and ozone layer depletion.

If a population contains individuals with genes for resistance then selection pressure from repeated applications of the same herbicide will result in the enrichment of the resistant population. This enrichment process is influenced by the type of herbicide and the degree to which it is used. A number of factors operate to affect the selection pressure for herbicide resistance in weeds.

2.3.2.5. Type of herbicide

Selection pressure on weeds can be greatest if a herbicide is residual and soil active (Gressel and Segel, 1990; LeBaron and MacFarland, 1990). Such herbicides effect seedling germination throughout the growing season and therefore control late germinating susceptible plants which might have contributed to pollen flow. Resistance to non-persistent herbicides also occurs where the herbicide has been applied repeatedly through the growing season or after many years of use. Resistance to bipyridyl herbicides is an example of this (Tucker, 1989). Both the use of persistent herbicides and the repeated use of non-persistent herbicides increase

selection for resistance by reducing the survival of susceptible plants. Hence, repeated use of the same, or chemically similar herbicides, will lead to development of resistant weed populations.

2.3.2.6. Effective kill by the herbicide

Selection pressure for resistance is increased when a very high percentage of target weeds are killed at least for cross pollinated species. If only resistant individuals survive herbicide treatment, the rate of enrichment of resistant individuals in the population will be great.

LeBaron and MacFarland (1990) listed the following characteristics of herbicides (in approximate order of importance) which contribute to a high risk of resistance:

1) A single target site and specific mode of action
2) Extremely active and effective in killing a wide range of weed species
3) Provide long soil residual and season-long control of germinating weeds
4) Applied frequently and over several growing seasons without rotating, alternating, or combining with other types of herbicides (or mechanical means of control).

Other factors may be important in some cases. LeBaron and MacFarland (1990) listed some of the more common herbicides that are considered low risk for weed resistance in Table 2.4 and high risk for weed resistance in Table 2.5.

Table 2.4. Herbicides with low risk for weed resistance

Herbicide classification	example	
	Common name	Trade name
Acetanilides or Amides	alaclor	Lasso®
	diphenamid	Enidee
	metalachlor	Dual®
	napropamide	Derinol
	propachlor	Ramrod
	propanil	Stam
Aliphatics	dalapon	Dowpon
	glyphosate	Round up®
	TCA	Sodium TCA
Benzoics	chloramben	Amiben
	dicamba	Banvel
Carbamates	asulam	Asulox
	barban	Carbyne
	chlorpropham	ChloroIPC
	phenmedipham	Betanol
Nitriles	bromoxynil	Brominal
Organic Arsenicals	cacodylic acid	Rad-E-Cate
	DSMA	several
	MSMA	Ansar,Daconate
Phenoxys	2,4-D	several
	MCPA	several
Thiocarbamates	butylate	Sutan
	diallate	Avadex
	EPTC	Eptam
	molinate	Ordram
	overnolate	Vernam

(source: LeBaron and MacFarland, 1990)

Table 2.5. Herbicides with high risk for weed resistance

Herbicide classification	example	
	Common name	Trade name
Bipyridylium	diquat	Diquat
	paraquat	Paraquat
Dinitroanilines	benefin	Balan
	oryzalin	Surflan
	pendimethalin	Prowl
	trifluralin	Treflan
Diphenyl Ethers	acifluorfen	Blazer
	diclofop-methyl	Hoelon
	lactofen	Cobra
	oxyfluorfen	Goal
Imidazolinones	imazapyr	Arsenal
	imazaquin	Scepter
Sulfonylureas	chlorsulfuron	Glean
	chlorimuron	Classic
	sulfometuron-methyl	Oust
	triasulfuron	Amber
Triazines	atrazine	AAtrex®
	cyanazine	Bladex®
	mertibuzin	Sencor,Lexon
	prometron	Pramitol®
	prometryn	Caparol®
	simazine	Princep®
Uracils	bromacil	Hyvar
	terbucil	Sinbar
Ureas	diuron	Karmex
	fluometuron	Cotoron®
	linuron	Lorox
	tebuthioron	Spike

(source: LeBaron and MacFarland, 1990)

2.4. Wild oat control by herbicides

2.4.1. Introduction

The earliest chemicals used for wild oat control were non-selective graminicides such as TCA, dalapon, propham, chlorpropham and maleic hydrazide. These chemicals killed grass weeds including wild oats, but because of a lack of selectivity they could not be used in cereals. In the late 1950s, diallate and barban were introduced and these materials were able to selectively control wild oats in cereals. All varieties of oats were found to be susceptible, whereas wheat and barley were tolerant (Huston and Roberts, 1987). However, barban had a narrow window of application and not all barley varieties could tolerate it. Diallate is a root uptake herbicide and suffers from vagaries of a soil-applied herbicide; in particular, its activity varies with soil type, condition and placement. Triallate proved to be better than diallate for control of wild oats in wheat and barley (both species more tolerant to triallate) and the use of diallate for wild oat control was eventually discontinued (Hutson and Roberts, 1987).

A more recent discovery, chlorfenprop-methyl, has greater selectivity and can even control wild oats in some varieties of cultivated oats. Certain biotypes of wild oat are not controlled, however, at normal field application rates (Huston and Roberts, 1987). Chlorfenprop-methyl can be

used in a mixture with other herbicides without reducing its effect on wild oats (Huston and Roberts, 1987).

A group of aminopropionic acid derivatives (the alaninopropionates) introduced between 1969 and 1972, are specific post-emergence herbicides for the control of all species of wild oat in cereals. Benzoylprop-ethyl is selective in wheat but is not recommended for barley, whereas flamprop-isopropyl was introduced in 1972 for use in barley. Flamprop-methyl is more active than benzoprop-ethyl and can therefore be used at lower dose rates. The last introduction in the series, (R) flamprop-isopropyl can be used both in wheat and for most barley varieties. Difenzoquat is a specific wild oat herbicide introduced in 1973 primarily for use in barley.

The aryloxyphenoxypropionate diclofop-methyl, introduced in 1975, is applied post-emergence for wild oat control in a variety of crops (Huston and Robert, 1987). This herbicide has become very successful and widely used for wild oat control. Since the aryloxyphenoxypropionates, a second major group, the cyclohexanedione chemicals, have been discovered and have a similar mode of action. The enzyme Acetyl coenzyme A carboxylase (ACCase) is the target site of these two herbicide groups (Shimabukuro and Hoffer, 1989). They also affect the proton gradient at the plasmalemma of plant cells (Huston and Robert, 1987; Shimabukuro and Hoffer, 1989).

Among aryloxyphenoxypropionate herbicides, diclofop-methyl was the first compound commercialised and is highly effective against wild oats

and yet selective in wheat and barley crops. It has became one of the most widely used herbicides in Australia since its introduction in 1978. As a result of the long term, widespread use of diclofop-methyl in this country, the majority of cases of resistance detected in wild oats in Australia have come from fields with a history of diclofop-methyl treatment.

2.4.2. Aryloxyphenoxypropionates and cyclohexanediones

Since the early 1970s many herbicides of varying chemistries have been patented for control of grass weeds. Many of these herbicides have almost identical selectivities and produce similar phytotoxic symptoms in the field, despite their different chemistries. There is also evidence that many of these compounds have similar, if not identical, mechanisms of action (Fedtke, 1982).

One of the largest groups of these new herbicides, with a common structural identity, are the aryloxyphenoxypropionates (AOPPs). The nomenclature of these herbicides has changed during their development. The earlier herbicides were classified as diphenyl ethers (see Ashton and Crafts, 1981). Later they were called polycyclic alkanoic acids (PCA) (see Kearney and Kaufman, 1988). Duke and Kenyon (1988) discussed polycyclic alkanoic acids (PCAs) as comprised of an alkanoic acid with a group containing more than one ring structure, (one usually a phenyl ring) attached to an asymmetric non carbonyl carbon of the alkanoic acid. They stated that many PCA compounds had been termed diphenyl ethers or phenoxyphenoxy herbicides, even though few chemically fit these

categories. PCAs are divided into the oxyphenoxy alkanoic acids (generally termed the **fops**, eg, diclofop) and the benzoyl-N-phenyl phenoxy propanoic acids (often termed **props**, eg, flamprop).

The first group of PCAs to be developed as herbicides were the benzoyl-N-phenyl propanoic acids (Banting, 1984) which were developed for wild oats control. After long term spraying of phenoxyalkanoic acid herbicides, wild oats became a serious problem in small grain crops (Kearney and Kaufman, 1988). Benzoylprop-ethyl was used since the early 1970s, but has been replaced by flamprop-methyl. The phenoxyphenoxy propanoic acid herbicide diclofop-methyl (methyl ester of (±)-2-[4-(2,4-dichlorophenoxy) phenoxy] propanoic acid) was found to have excellent herbicidal selectivity for wild oats in several monocot crops and was first used in 1976 (Kearney and Kaufman, 1988). Diclofop-methyl was registered in Australia and used since 1978. Since then, usage of diclofop-methyl against wild oats and ryegrass (*Lolium spp.*) has increased and currently 4,000,000 ha are being annually treated in Southern Australia.

Currently, in crops, *Avena* species can be well controlled by chemical means. Diclofop-methyl, barban, tralkoxydim, diallate, and triallate are used for selective wild oat control in wheat, barley, rye and many dicot crops. Haloxyfop-ethoxyethyl, fluazifop-butyl, sethoxydim, or fenoxaprop-ethyl are widely used as selective post-emergent herbicides in dicot crops (Mansooji *et al.*, 1992; Kearney and Kaufman, 1988). All these herbicides, including diclofop-methyl, have soil activity, but higher rates are required to provide the same control as that obtained with post-emergence treatments. Although the soil life of these compounds is not long (the half

life of sethoxydim varies from 2-13 days, depending on environmental conditions), the soil residual could be helpful in controlling late germinating grass seeds in some circumstances (Duke and Kenyon, 1988).

2.4.3. Physical and chemical properties

The physical and chemical properties of many of several PCAs are presented in Tables 2.6. All commercialised versions of the PCAs are propanoic acid esters. Thus, virtually all research on PCA herbicides has been done with PCA esters. The PCA ester is rapidly hydrolysed in the plant to the herbicidal PCA (acid form). Distinguishing between the effects of the ester and acid is often difficult (reviewed in Duke and Kenyon, 1988). From a biological and environmental standpoint, the most important physical difference between the acid and ester of a PCA herbicide is probably solubility in water. The acid is more soluble than the ester, but not necessarily drastically so (Kearney and Kaufman, 1988). For example, the solubilities in water at 20 ^{o}C of quizalofop and its ethyl ester are 10.2 and 3 ppm, respectively.

Table 2.6 Physical and chemical properties of several AOPP herbicides an Flamprop-methyl. (from Ducke and Kenyon, 1988).

Chemical group	Phenoxyphenoxy	Pyridinyloxyphenoxy	Quinoxalinyloxphenoxy	Benzoyl-*N*-phenyl
Herbicide	Diclofop-methyl	Fluazifop-butyl	Quizalofop-ethyl	Flamprop-methyl
Trade name of formulated product	Hoelon	Fusilade	Assure	Mataven
Molecular wt.	341	383	372.8	335.5
Water (g/l) solubility	3 at 22°	0.2×10^{-3} at ambient	3.0×10^{-3} at 20°	3.5×10^{-2} at 20°
MP, BP, decomposition point (°C)	39–41, 175–175, 288.4	−20, None at atmospheric pressure, 210	91.7–92.1, 220, ?	81–82, ?, 300
Vapor pressure (mmHg)	0.258×10^{-6} at 20° 0.113×10^{-5} at 30°	4.1×10^{-7} at 20°	3×10^{-7} at 20°	3.5×10^{-7} at 20°
Color	None	Light straw	White	Light tan
Odor	None	None	?	Mild
Physical state	Solid	Liquid	Crystalline solid	Crystalline solid
Specific gravity (g/cm^3)	1.3 at 40°	1.21 at 20°	1.35 at 20°	0.44

2.4.4. Toxicological properties

Short term and acute toxicological properties of several of the commercialised PCA esters are listed in Table 2.7. None of the commercialised PCAs are very acutely toxic, except to aquatic organisms. However in the acid form, the acute toxicity of PCAs to aquatic life may be considerably reduced. For instance, haloxyfop is more than 3000-fold less toxic to minnows in the acid form (Dow Chemical Co., 1983). This may be because of better absorption of the more lipophilic ester.

Several effects of PCAs have been reported on mammalian physiological and biochemical systems. For instance, at a dose of 100 μM diclofop-methyl was found to stimulate calmodulin-dependent cyclic nucleotide phosphodiesterase from bovine brain twofold (Hertel and Marme, 1983a). In vitro assembly of brain tubulin into microtubules was inhibited by 25% at 100 μM diclofop-methyl (Hertel and Marme, 1983b). However, such a small effect at such a high dose is not likely to be important (Kearney and Kaufman, 1988). Benzoylprop-ethyl and flamprop-isobutyl interfered with energy transfer and inhibited electron transfer in rat liver mitochondria (Gauvrit, 1984). The effects were thought to be secondary effects of decreasing membrane fluidity. The free acids were far less effective than the esters. Since animals readily metabolise PCA esters to acids, little toxicity could be expected from these mechanisms.

Table 2.7 Toxicoligical properties of several AOPP herbicides (From Duke and Kenyon, 1988).

Herbicide	Test	Species	LD_{50} (mg/kg)	LC_{50} (ppm)
Haloxyfop-methyl	Acute oral	Rat (male)	393	—
		Rat (female)	599	—
		Mallard duck	>2150	—
	Acute dermal	Rabbit	>5000	—
	Dietary (8 days)	Bobwhite quail	—	>5620
	Fresh water	Fathead minnow	—	0.3
	(96 hr exposure)	Bluegill	—	0.2
		Rainbow trout	—	0.4
	(48 hr exposure)	*Daphnia* spp.	—	6.2
Diclofop-methyl	Acute oral	Rat	557–580	—
		Dog	>1600	—
		Bobwhite quail	4400	—
	Acute dermal	Rat	>5000	—
		Rabbit	180	—
	Dietary (8 days)	Bobwhite quail	—	13,000
		Mallard ducks	—	20,000
Flamprop-methyl	Acute oral	Pheasants, domestic fowl, mallards	>1000	—
		Pigeons, bobwhite quail	4640	—
		Rat	1210 in DMSO	—
		Mouse	720 in DMSO	—
		Dog	>2000	—
	Acute IP	Rat	—	350–500 in DMSO
Fluazifop-butyl	Acute oral	Rat (male)	4096	—
	Acute oral	Mallard duck	>17,000	—
	Acute dermal	Rabbit	>2420	—
	Fresh water			
	(4 days)	Rainbow trout	—	1.6
	(2 days)	*Daphnia magna*	—	10
Quizalofop-ethyl	Acute oral	Rat (male)	1670	—
		Mallard duck	>2000	—
		Bobwhite quail (8-day dietary)	—	>5620
	Fresh water			
	(96 hr exposure)	Rainbow trout	—	10.7
		Bluegill sunfish	—	0.46–2.8
	(48 hr exposure)	*Daphnia*	—	6.4

Kearney and Kaufman (1988) reviewed the results of mutagenicity tests of long term sublethal doses of several PCAs. No significant mutagenic or sublethal effects of commercialised PCAs had been reported. The tolerance limit for diclofop-methyl and/or its acid under the Federal Food, Drug, and Cosmetic Act of USA is 0.1 ppm in flax (*Linum usitatissimum* L.), soybean (*Glycine max* (L) Merr.), wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) seed. The tolerance for fluazifop in soybean and cotton (*Gossypium hirsutum* L.) seed oil are 2.0 and 0.2 ppm, respectively.

Considerable information exists on the compatibility of other herbicides, adjuvants, and surfactants with PCAs (reviewed in Kearney and Kaufman, 1988).

2.4.5. Degradation pathway

The first chemical reaction of PCA ester in most species is hydrolysis to the parent acid. It is assumed that, in plants, the hydrolysis of the ester is accomplished by an esterase, specifically a carboxylesterase. This enzyme has been partially purified and characterised from oat (*Avena sativa* L.) and wild oat (reviewed in Kearney and Kaufman, 1988). After hydrolysis, a number of chemical reactions can occur. These include conjugation to another ester conjugate in susceptible species, or aryl-hydroxylation and phenolic conjugations in resistant species.

2.4.6. Diclofop-methyl

Diclofop-methyl is probably the most commercially successful and thoroughly investigated post-emergence grass herbicide to selectively control annual grass weeds in wheat, barley and dicotyledonous crops, being especially effective against wild oats (*Avena sp.*) and other grasses such as foxtail (*Setaria sp.*), ryegrass (*Lolium spp.*) and volunteer corn (*Zea mays*) (Shimabukuro, 1991). It is soluble in organic solvents but has low solubility in water (22 ^{o}C) of 0.3 mg l^{-1}. Hydrolysis is an important feature of its mode of action, metabolism and environmental fate (Huston and Roberts, 1987). Diclofop-methyl follows the classic phase-I metabolism (hydrolysis, oxidation) which predisposes the compound to phase II metabolism (conjugation) and loss of phytotoxic activity (Shimabukuro and Hoffer, 1989). Hydroxylated diclofop is conjugated rapidly to the aryl-O-glucoside metabolite with almost no free phenolic forms (hydroxy-diclofop) remaining in plants (Shimabukuro *et al.*, 1978). Diclofop is also conjugated rapidly to the glucose ester, leaving almost no free acid (diclofop), in either resistant and susceptible plants (Jacobson and Shimabukuro, 1984).

2.4.7. Fenoxaprop-ethyl

Fenoxaprop-ethyl has been formulated for use in wheat. This herbicide is also absorbed through leaves of plants and inhibits ACCase resulting in inhibition of fatty acid synthesis. The active ingredient has limited movement in the xylem and phloem. Good growing conditions favour the effectiveness of fenoxaprop-ethyl, while the speed of action is slowed at low temperatures and low humidity or moisture (Bieringer *et al.*, 1982; Anderson and Howat, 1990).

2.4.8. Fluazifop-butyl

Fluazifop-butyl is a graminicide which is not selective in cereal crops It is a selective herbicide used to control grass weeds in dicot crops (Plowman *et al.*, 1980). Absorption and translocation of this herbicide increases with increased temperature. Harker and Dekker (1984) showed that absorption and translocation were greater at 15-20°C than at 5-10 °C. Absorption and translocation were also increased when petroleum oil was added (Graftsorm and Nalewaja, 1988). Absorption and translocation were also greater when soil moisture was adequate (Kells *et al.*, 1984). It has been shown that in soybean fluazifop-butyl was rapidly translocated from the leaves to the roots, but the concentration in the leaves remained about 10-fold higher than that in the roots (Balinova and Laova, 1992). Increase of temperature from 15 °C to 20 °C resulted in more rapid hydrolysis of herbicide (Balinova and Lalova, 1992). These results are similar to those obtained by Kells *et al.*, (1984) and Grafstorm and

Nalewaja (1988) with the rapid basipetal translocation of this herbicide in other plant species.

De-esterification is the first metabolic process for fluazifop-butyl as with other PCA esters. In *Elymus repens* (L.), Coupland (1985) found metabolism and distribution of ^{14}C labelled fluazifop-butyl to be characterised by: (a) relatively high amounts of radioactivity remaining in tissue residues after extraction; (b) small amounts of polar and nonpolar conjugates; and (c) relatively high levels of the PCA acid. Warmer temperatures favoured de-esterification of fluazifop-butyl in rhizomes.

2.4.9. Haloxyfop-methyl

Haloxyfop-methyl is rapidly hydrolysed to haloxyfop in plant tissues and then is more slowly metabolised to more polar metabolites (Kearney and Kaufman, 1988). The formulation used in experiments in this thesis was haloxyfop-ethoxyethyl.

2.4.10. Paraquizafop

Paraquizafop (Correct^{TM}) is a systematic post-emergence herbicide for the selective control of grass weeds in dicot crops. Paraquizafop at rates of 0.02 and 0.045 kg ai ha-1 provided effective control of a range of grass weeds, when applied prior to the end of tillering (Sumner *et al.* 1990). It is mostly foliar absorbed and is translocated through the plant (Sumner *et al.* 1990). Paraquizafop prevents regrowth from rhizome of perennial grasses. Paraquizafop is readily absorbed by the leaves, rainfall occurring one hour or more after treatment does not affect the efficacy of the product.

2.4.11. Flamprop

Flamprop-methyl is closely related to flamprop-isopropyl and is similar in its chemical and biological properties. The major difference is that the methyl group is more chemically and biochemically labile than is the (secondary) isopropyl group. While flamprop-isopropyl is soluble in water to the limited extent of 18 mg L^{-1}, flamprop-methyl is twice as soluble in water, but like the isopropyl, is soluble in organic solvents. While flamprop-isopropyl is recommended to be used in barley crops, flamprop-methyl can control *A. fatua* and *A. ludoviciana* and other species in wheat. As with benzoylprop-ethyl, competition from the crop is essential to the effective control of wild oats with flamprop-isopropyl. Flamprop-methyl is less dependent than benzoylprop-ethyl and flamprop-isopropyl on crop competition for its effect (Huston and Roberts, 1987).

Both the methyl and isopropyl esters of flamprop have been studied. Both are hydrolysed to flamprop, but the methyl ester is hydrolysed somewhat faster (Jeffcoat *et al.*, 1977). Pillmoor *et al.* (1982) found flamprop to be rapidly metabolised in oats, with up to 85% degradation within 24 h. The major metabolic fraction appeared to be sugar conjugates of flamprop (Kearney and Kaufman, 1988).

Environmental degradation: Most of the loss of PCAs from soil is through biological degradation, rather than leaching or volatilisation. For example, neither diclofop-methyl nor diclofop acid leaches significantly and no loss of diclofop-methyl from a wheat field could be accounted for by volatilisation over a 7-day period (Grover, 1984). An exception may be haloxyfop, which is more volatile than other commercially available PCAs. As in plants, degradation in soil usually begins with rapid hydrolysis of the ester to the PCA acid, followed by slower degradative steps (Kearney and Kaufman, 1988).

Early work with a series of PCAs (benzoylprop-ethyl, flamprop-methyl, flamprop-isopropyl) in animals showed that these herbicides were rapidly excreted by cattle, pigs, and poultry after metabolic de-esterification (Crayford, *et al.* 1976). They concluded that the parent acids have ideal physical properties for excretion via urine and faeces and were chemically unsuited for transport to milk and eggs. De-esterification in mammals is mediated via a mono-oxygenase (Bedford, *et al.* 1978).

According to Kearney and Kaufman (1988) photodecomposition of PCAs is not likely to be an important mechanism of dissipation in field situations

when these herbicides are soil incorporated, however, photodecomposition may be a problem with foliar application.

In general, PCAs are strongly adsorbed to soil and have a very low solubility in water. As a result, in agricultural situations very little PCA herbicides enter ground water, streams, or other aquatic environments through leaching or runoff of soil applied PCAs or PCAs washed off foliage. Diclofop is relatively soluble in water, making it an exception. Nevertheless, even with diclofop, less than 10% of soil applied herbicide was found to be leached to greater than 10 cm depth in a variety of soils by 10 cm of water (Mulder and Nalewaga, 1979).

2.4.12. Absorption and translocation

PCA herbicides are readily absorbed by root and leaf tissues. Although absorption varies with species and with PCA herbicide studied, it can be generalised that PCA herbicides are relatively well absorbed. Nevertheless, adjuvants and environmental parameters can strongly alter the rates and, to a lesser extent, the final level of absorption (Kearney and Kaufman, 1988). Although in some weeds, absorption of some PCAs did not show any difference at different relative humidity, for most PCA herbicides absorption is positively correlated with relative humidity. Within metabolic ranges, increased temperatures generally increase absorption of PCAs. Thus humid warm conditions generally favour PCA uptake (Kearney and Kaufman, 1988).

Absorption and translocation of diclofop-methyl can significantly influence selectivity if they impact directly upon herbicide activity at the sensitive sites of action. The intercalary meristem and the apical meristems of shoots and roots are the sensitive sites in susceptible species (Brezeanu *et al.*, 1976; Hoerauf and Shimabukuro, 1979; Kocheritt and Lotzsch, 1975; Shimabukuro *et al.*, 1978; Walter *et al.*, 1980). The fully differentiated leaves are generally unaffected during early development of injury but chlorosis appears at about 3 days after treatment, especially in leaves that emerge subsequent to diclofop-methyl application. Further growth of new leaves may be inhibited totally. The absence of internodal growth further implicates the intercalary meristem as a sensitive site (Brezeanu *et al.*, 1976). Similarly, the apical root meristem is extremely sensitive with complete inhibition of root growth occurring at 10 to 100 μM diclofop-methyl in oat seedlings (Donald *et al.*, 1982; Kocher and Lotzsch, 1975; Shimabukuro *et al.*, 1978).

Diclofop-methyl is not generally translocated from the site of application. Of the total ^{14}C activity recorded, 96% of diclofop-methyl, was in the treated zone of both wheat and wild oat leaves and only 1% and 0.3% of the radioactivity was translocated out of the treated leaves in wheat and barley, respectively (Brezeanu *et al.*, 1976). Hall *et al.* (1982) reported that only 4% of the absorbed radioactivity was translocated basipetally from oat leaves with most of the ^{14}C activity remaining in the intercalary meristem zone. In five species, less than 2% of the ^{14}C from [^{14}C] diclofop in a treated leaf was translocated over a five-day period. Most of the translocated ^{14}C is moved acropetally (reviewed by Gronwald, 1991). Approximately equal amounts of herbicide were translocated to roots, to

the shoot above the treated leaf, and to the shoot below the treated leaf. Development of chlorosis and leaf necrosis were independent of herbicide placement whereas growth inhibition required placement near the meristematic region (Hoerauf and Shimabukuro, 1979). Sensitive plants will recover when chlorosis is the only injury but not when chlorosis is combined with an inhibition of growth (Hoerauf and Shimabukuro, 1979; Kafiz *et al.*, 1989).

Diclofop-methyl uptake by shoot and root zone of emerging seedlings was investigated using placement of the herbicides in soil (Crowley *et al.*, 1978). When placed in the root zone it was more toxic to all parts of the plants than when applied to the shoot zone.

Other compounds can reduce absorption of PCAs. For example, the herbicide MCPA significantly reduces uptake of diclofop-methyl by wild oat leaves (Qureshi and Handen Born, 1979). This inhibition of uptake is apparently due to chemical interactions outside the plant because in MCPA-pretreated plants, no effect was found on diclofop uptake (Olson and Nalewaja, 1982).

2.4.13. Mode of action

In post-emergence application, the herbicide contacts the leaves and most is absorbed. A significant percentage of the absorbed herbicide is hydrolysed into the parent acid which is phloem and xylem mobile. The acid moves towards and accumulates in meristematic regions. Disruption of cell division and cell elongation in the meristems results in stunted

growth, presumably through membrane alterations and/or inhibition of fatty acid synthesis. When sufficient herbicide has accumulated in the meristematic regions, the occurrence of wholesale membrane disruption and cellular autolysis eventually results in plant death or the inability of the plant to compete with any tolerant or resistant plant species (Ashton and Crafts, 1981). Usually plants die within 2-4 weeks depending on ecological conditions.

The rapidly developing and differentiating cells in the meristematic regions are the primary sensitive sites to aryloxyphenoxypropionate herbicides. Growth of leaves and roots are inhibited shortly after contact with diclofop. A characteristic property of diclofop-methyl and closely related analogs is its antagonistic interaction with IAA and other auxin compounds such as 2,4-D, MCPA and dicamba (Hall *et al.*, 1982; Kafiz *et al.*, 1989).

The herbicidal action of diclofop-methyl and other graminicides are readily reversed by auxin-like compounds. The mechanism of action have been proposed for diclofop-methyl. One is a biophysically based mechanism involving the dissipation of the transmembrane proton gradient in plant cells (Lucas *et al.*, 1984; Ratterman and Balke, 1988; Wright and Shimabukuro, 1987) and the second is a biochemically based mechanism involving the inhibition of fatty acid biosynthesis (Hoppe and Zacher, 1985; Kobek *et al.*, 1988).

The effect of herbicides such as diclofop-methyl and haloxyfop-methyl on acetate and sucrose uptake by plant cells is due to their perturbation of

the transmembrane proton gradient. Young actively growing cells in the meristematic regions may be seriously damaged if their ability to import organic solutes and inorganic ions is impaired (Shimabukuro, 1991).

The inhibition of lipid biosynthesis by PCA herbicides has been investigated extensively (Fedtke, 1982). Lipid metabolism is critical to the maintenance of membrane integrity. The incorporation of [^{14}C] acetate and [^{14}C] malate into lipids, the inhibition of these reactions by herbicides, and qualitative and quantitative changes in plant lipid fractions and their physiological effects have been reported for several herbicides.

However, diclofop-methyl and other analogs are probably the first herbicide compounds where the inhibitory site (acetyl-CoA carboxylase) for lipid biosynthesis has been identified (see Shimabukuro, 1991). ACCase is a key regulatory enzyme, containing biotin, which is found in plastids. ACCase catalyses ATP- and HCO_3^- dependent conversion of acetyl-CoA to malonyl-CoA. Malonyl-CoA is required for synthesis and elongation of fatty acids and for synthesis of secondary compounds (Shimabukuro, 1991; Holt *et al.*, 1993; Walker *et al.*, 1989).

From reports on the inhibition of ACCase by the post-emergence graminicides, haloxyfop-methyl, fluazifop-butyl, and sethoxydim and analogs, ACCase is a sensitive site of inhibition. The enzymes isolated from susceptible grasses are strongly inhibited whereas the enzymes from resistant dicotyledonous plants are unaffected by the different graminicides. The inhibition of ACCase resulting in decreased fatty acid biosynthesis has been concluded to be the primary mechanism of action

and the basis for selectivity by diclofop-methyl and related graminicides (see Shimabukuro, 1991).

This thesis examines the development of resistance to aryloxyphenoxy-propionate and cyclohexanedione herbicides in wild oats in Australia. A biotype of *Avena sterilis* ssp. *ludoviciana* from near Bordertown in South Australia is examined in detail. The mode of inheritance, the life of seed in the seedbank, the relative productivity and competitiveness of the biotype are compared to susceptible oat and wild oat populations. The implications for agriculture of the development of herbicide resistance in wild out are discussed.

Chapter 3

General materials and methods

Some general aspects of different methods are described in this chapter. Specific materials and methods are described within relevant chapters.

3.1. Plant material

Seeds of wild oats biotype SAS1 which survived haloxyfop-ethoxyethyl in 1989 were collected in hessian bags in November 1989. Susceptible seeds, of biotype SAS2, were collected in an adjacent field from an area with almost the same herbicide history (Tables 3.1 and 3.2). Wild oat seeds in hessian bags were left outdoors to dry for 2-3 days. Later, seeds were scattered on hessian bags indoors for 1.5 months to dry further. They were returned back into the hessian bags and stored indoors. Temperatures in the store varied between 5-40 ^{o}C during different times of the year.

Resistant and susceptible seeds harvested during November 1990 were dried and stored either in a glasshouse with temperatures from 5-40 ^{o}C, or in a cold-room (3-4 ^{o}C) to investigate the effects of different storage temperatures on seed germination.

Table 3.1. Resistant, SAS1, paddock history

year	crop	herbicide (s)
1981	wheat	diclofop-methyl
1982	pasture, medic, hay	cut
1983	medic seed	trifluralin + fluazifop-butyl
1984	rapeseed	trifluralin + fluazifop-butyl
1985	wheat	triallate (fail*)+ diclofop-methyl
1986	wheat	diclofop-methyl
1987	clover	trifluralin + fluazifop-butyl (74.2 g/ha)
1988	wheat	glyphosate (sown August, no spray)
1989	clover	trifluralin+haloxyfop-ethoxyethyl 52 g fail haloxyfop-ethoxyethyl 31.2 g fail Lack of control first reported

* fail, indicates that herbicide provided unacceptable wild oat control.

Table 3.2. Susceptible, SAS2, paddock history

Year	crop	herbicide (s)
1982	wheat	------
1983	wheat	diclofop-methyl
1984	pasture	none
1985	wheat	diclofop-methyl
1986	wheat	diclofop-methyl
1987	beans	simazine(1056 g ha^{-1}) + fluazifop-butyl
1988	clover	trifluralin(0.4 kg ha^{-1}) + haloxyfop-ethoxyethyl (.72.8 g/ha)
1989	rape	trifluralin(0.4 kg ha^{-1}) + haloxyfop-ethoxyethyl (72.8 g/ha)
1990	wheat	trifluralin(0.48 kg ha^{-1}) + Glean (6.75 g ha^{-1})

Some additional populations were collected from farms where farmers suspected herbicide resistance. Biotypes were also kindly sent, from different states of Australia, by individuals from Departments of Agriculture, representatives of the chemical companies and seed cleaners. Some other biotypes were sent to us in response to request for susceptible biotypes. These biotypes were screened for resistance to certain aryloxyphenoxypropionate and cyclohexanedione herbicides.

Biotype SAS1 was the main resistant biotype used for experiments in this thesis including: Bioassay, Breeding, Seedbank, and Competition experiments. The biotype SAS2 was used as the comparative susceptible biotype in all experiments.

3.2. Coding of biotypes

A coding system was used to identify the different wild oats seed samples. Different wild oats seeds received were coded according to 4 major criteria. These are:

1) The origin of biotypes for the states of South Australia, New South Wales, Victoria, Western Australia and Queensland, were indicated by the use of the letters S, N, V, W and Q at the beginning of the code name.

2) The second letter of the code, 'A' represents the genus of the weed, (*Avena*).

3) The third letter of the code indicates the species. Thus S, F or B indicate the species *sterilis*, *fatua* or *barbata*, respectively.

4) The final element of the code is an arbitrary number given to each biotype in their order of collection from each state.

For example SAS1 stands for a biotype from South Australia with the genus and species names of *Avena sterilis* and is the first (1) *sterilis* biotype noticed in this state by the author.

3.3. Soil mixtures and pots used

Of over 90 experiments used to determine the response of wild oats to herbicides, more than 90% were conducted using plants transplanted into pots maintained in the field at the Waite Institute.

The soils used in pot experiments were prepared at the Waite Institute. These are the U.C. and the recycled soil, R.S., as described in appendices 1

and 2. The blood meal in the recycled soil contained 16% nitrogen. The U.C. and the R.S. soils had 60% and 40% organic matter, respectively.

The R.S., with 40% organic matter, was the main soil used through all post-emergence herbicide experiments. In preliminary experiments the R.S. soil proved to be unsuitable for pre-emergence herbicide experiments. A specific soil mix was prepared for pre-emergence herbicide experiments. This soil had 97% sand and 3% organic matter, and no other additives such as fertiliser. To show the role of organic matter in pre-emergence herbicide use the U.C. soil was used as a comparison in this experiment.

Pots of 17.5 cm diameter containing two litres of soil were used for all experiments.

3.4. Seed germination and germination media

Seed germination is the first step in the execution of most experiments in this thesis. Different media and containers may be used for seed germination. Some of these are: seeds on Putnam papers in petri dishes, seeds wrapped in Putnam papers in post dishes, seeds on Agar media in post dishes or larger and taller plastic containers, 18 X 12 X 4 cm, or seeds in vermiculite media in even larger 45 X 30 X 12 cm rectangular plastic trays.

Germination media were moistened using water alone or solutions containing germination stimulants (eg. KNO_3 or GA_3 at various concentrations) or solutions of herbicides.

In Chapter 5 (Competition) the large scale mass seedling production for competition experiments is described.

The results of experiments on germination led to the adoption of a basic germination procedure which was used for all subsequent experiments (unless otherwise stated).

Seeds were first dehusked and the naked seeds (caryopses) were pricked with a metal needle at the middle of the dorsal side. The pricked seeds were put on media, usually Agar (0.6%). In closed containers they were kept at 3-4 °C for 12 days in the dark. After 12 days trays were transferred to an incubator under a 12 h 20 °C light/12 h 16 °C dark regime. Photon flux density was 19 µmol photons $m^{-2}s^{-1}$.

3.5. Seedling establishment and herbicide treatment

3.5.1 Timing of planting and herbicide treatment

In order to achieve results similar to those obtained in the field, experiments were conducted during the normal cropping season. Therefore, experiments were performed from early in the autumn (April) up to late in the winter (September) unless otherwise stated. Experiments contrasting warm season response with the normal season (autumn - winter) response are described in Chapter 8 (other wild oats populations).

3.5.2. Planting

Seedlings were transplanted from 5 days after placing of seed in the incubator up until 9 days. Thus, seedlings were transplanted from the time when they had reached the one leaf stage, from 4 cm in height, up until the time they had reached the 2 leaf stage and up to 10 cm in height.

Seedlings were transplanted at a density of five plants per pot into moistened soil, except for preliminary experiments in which seedlings were transplanted at the density of one plant per pot. Transplanted seedlings were immediately and carefully watered with a fine spray to avoid damage. Pots were kept in the open and watered as necessary. Plants were treated from 7 to 14 days after transplanting at the 2 to 3 leaf growth stage. Appendix 3 gives rainfall at the Waite Institute for the experimental period.

3.6. Herbicide treatment by foliar spray application

Foliar spray applications were applied to plants at the two to three leaf stage. Commercial formulations of herbicides were used together with the surfactant Agral 600 (0.2%, v/v). Herbicides were applied using a spray cabinet or a portable spray unit, for pot and field experiments, respectively (described below).

3.6.1 Cabinet sprayer

For pot experiments the herbicides were applied in a herbicide spray cabinet through two flat-fan hydraulic nozzles (Tee Jet, 0011) suspended 40 cm above the plants; using a pressure of 250 KPa and a boom speed of 1.0 m s^{-1}. The sprayer output was 113 L ha^{-1}.

When necessary, plants were left indoors overnight and then transferred outdoors the following morning to prevent exposure to rain during the rainfast period.

3.6.2 Portable sprayer

For field experiments herbicides were applied by a portable sprayer, on clear days. The sprayer was fitted with four flat-fan hydraulic nozzles (Tee Jet, 0011), 50 cm apart. Gas pressure was provided by compressed CO_2. A pressure of 250 KPa and boom speed of 1.0 m s^{-1} gave an sprayer output of 126-129 L ha^{-1}. The herbicide was applied 40 cm above the plant canopy using a 40 cm chain hanging from the sprayer boom to ensure constant boom height.

3.7. Statistical designs

Most of the experiments were conducted in Randomised Complete Block Designs (RCBD). In some of them the plots were split into two sub-plots (split plots in RCBD). One germination experiment was conducted as a factorial experiment (2^5) in a RCBD. Duncan's Multiple Range Test method, or LSD method were used for mean comparisons. In breeding experiments Chi squared analysis was used.

3.8. Measurements

Soon after spraying all early effects of herbicides such as change of colour, inhibited growth, stunted growth, chlorosis or necrosis and death of plants or plant organs were recorded. The aboveground, green parts of surviving plants were harvested from 45 days after spraying. For those surviving, the numbers of tillers, plant height and date of flowering were measured. Dry weights for above ground, green plant tissues were recorded following drying for 3 days at 85 ^{o}C.

Some measurements specific to certain experiments are mentioned in the relevant chapters.

Chapter 4

Dose response to herbicide

4.1. Effects of herbicides on survival of SAS1

4.1.1. Introduction

Until 1989 the only confirmed case of herbicide resistance in wild oats was reported from York, 60 km east of Perth, Western Australia by Piper (1990) and Boutsalis (1990). This biotype, *A. fatua* L. (WAF1) exhibited 33% survival at the recommended rate of diclofop-methyl (Boutsalis, 1990). However, late in 1989 growing season, a failure of haloxyfop-ethoxyethyl (Verdict) to control wild oats, *Avena sterilis* ssp. *ludoviciana* biotype SAS1, was reported from a field near Bordertown, 280 km east of Adelaide, South Australia (Latitude 36 ^{o}S; Longitude 141 ^{o}E). Between 1981 and 1988 this field had been in a wheat, legume, rapeseed rotation and had received 3 applications of diclofop-methyl, 3 applications of trifluralin, 3 applications of fluazifop-butyl, one application of triallate and one application of glyphosate (Table 3.1). In 1989 the field had been treated with haloxyfop-ethoxyethyl. Seed samples collected from the field following haloxyfop-ethoxyethyl failure in 1989 were designated as population SAS1. Subsequently, the response of population SAS1 to a range of herbicides used for wild oat control was tested (Table 4.1)

In a preliminary experiment it was found that 100% of individuals from biotype SAS1 survived up to 78 g ha^{-1} of haloxyfop-ethoxyethyl.

Table 4.1. Herbicides tested against population SAS1.

Herbicide group	Retail name
ARYLOXYPHENOXYPROPIONATES	
-haloxyfop-ethoxyethyl	(Verdict)
-fluazifop-butyl	(Fusilade)
-diclofop-methyl	(Hoegrass)
-quinfurop	(Pantera)
-propaquizafop	(Correct)
-quizalofop-ethyl	(Assure)
-fenoxaprop-ethyl	(Puma)
-flamprop-methyl	(Mataven)
CYCLOHEXANEDIONES	
-sethoxydim	(Sertin)
-tralkoxydim	(Grasp)
-cycloxydim	(Focus)
TRIAZINES	
-simazine	(Simazine)
-atrazine	(Atrazine)
DINITROANILINE	
-trifluralin	(Tridan)
THIOCARBAMATES	
-triallate	(Avadex)
OTHER HERBICIDES	
-glyphosate	(Round Up)
-oxyfluorfen	(Goal)

Recommended rates of all AOPPs and CHDs in Table 4.2 were tested on this biotype and it was found that biotype SAS1 exhibits 100% survival when treated with AOPP herbicides, but is controlled by CHDs, at their

recommended rates. In contrast it was found that SAS1 exhibited susceptibility to flamprop-methyl.

4.1.2. Materials and methods

For almost all experiments in this chapter, seeds were pricked, incubated on 0.6% agar media and cold treated (as described in Chapter 3). Five seedlings were planted in each pot. For preliminary experiments (see section 4.3) seeds were pricked, incubated on Putnam No.1 papers in post dishes. The resultant seedlings were planted one in each pot. All plants were sprayed using a laboratory cabinet sprayer as described in Chapter 3.

Many herbicides from different chemical classes were tested (Table 4.1). These included three recently introduced products, the AOPPs quinfurop (Pantera) and paraquizafop (Correct) and a CHD, cycloxydim (Focus).

Several experiments during two growing seasons were used to test a wide range of several AOPPs on biotype SAS1 in order to find the application rate of each herbicide causing 50% mortality of SAS1. For some herbicides, like diclofop-methyl or fluazifop-butyl, LD_{50} values were not detected at rates up to 30 and 17 kg ai ha^{-1} for diclofop and fluazifop, respectively.

As CHD herbicides killed both the resistant biotype (SAS1) and the susceptible (SAS2) at the recommended rate, wide dose response experiments were conducted at doses below the recommended rates.

The experiments were designed to detect whether the resistant biotype SAS1 was as equally sensitive to CHD herbicides as the susceptible.

The experimental designs were usually factorial experiments in CRDs or RCBDs with at least 4 replications for each treatment. When 1st and 2nd seeds of spikelets were to be compared for germination or response to herbicides each treatment was split into two subplots, with respect to seed type, using a split plot in CRD or RCBD.

4.1.3. Results

4.1.3.1. Preliminary experiments

As the putative resistant population had been reported following haloxyfop failure in the field, response of the population to this herbicide was first investigated. The first experiment was conducted as a dose response curve with different rates of haloxyfop-ethoxyethyl up to 0.078 kg ai ha^{-1} (750 ml Verdict). The susceptible seedlings were well controlled by 0.013 kg ai ha^{-1} haloxyfop-ethoxyethyl, a rate which is well below the recommended rate for *A. sterilis* control, 0.054 to 0.158 kg ai ha^{-1}. In contrast, the resistant biotype exhibited no mortality even at the highest rate used in this experiment (0.078 kg ai ha^{-1}).

The next experiment was conducted in 40 X 25 X 12 cm trays with six rows, each containing five wild oats plants. The rate of the herbicide was increased up to a maximum of 0.416 kg ai ha^{-1} of haloxyfop-ethoxyethyl. Again, biotype SAS1 showed very high resistance to haloxyfop, this time

exhibiting no mortality even at 0.416 kg ai ha^{-1}. The next preliminary experiment used other AOPP herbicides in successive experiments at recommended rates and up to four times the recommended rates to investigate the response of biotype SAS1. As a result it was found that biotype SAS1 exhibits resistance to AOPP herbicides diclofop-methyl, fluazifop-butyl, haloxyfop-ethoxyethyl, fenoxaprop-ethyl and quizalofop-ethyl.

The biotype SAS1 was also tested against CHD herbicides at the recommended rates. Sethoxydim, tralkoxydim and cycloxydim controlled SAS1 at the recommended rates.

Therefore, it was concluded that wild oats biotype SAS1 was highly resistant to all aryloxyphenoxypropionate herbicides tested. However, SAS1 is susceptible to cyclohexanediones at the recommended rates. Based on these results further rates of these herbicides were tested on biotype SAS1 to find those rates which cause fifty per cent mortality. Higher rates of AOPP herbicides were tested while lower rates of CHD herbicides were used.

4.1.3.2. Effects of aryloxyphenoxypropionate herbicides on wild oats, biotype SAS1.

In order to find the 50% mortality of the resistant wild oats, biotype SAS1, different rates of the aryloxyphenoxypropionate herbicides were tested. The results of some of these experiments are shown in Figures 4.1. to 4.7. For these experiments, there were four replications (pots) with five plants in each pot.

The dose response to haloxyfop-ethoxyethyl is shown in Figure 4.1. Susceptible wild oats are normally controlled by the field application rates of 0.052 to 0.156 kg ai ha^{-1} as can be seen by the response of biotype SAS2. In fact biotype SAS2 was completely controlled by 0.013 kg ai ha^{-1} in these experiments (Figure 4.1.A). In contrast, biotype SAS1 exhibits almost no mortality at treatment rates of up to 0.5 kg ai ha^{-1} and rates greater than 2.0 kg ai ha^{-1} are required to achieve 50% mortality (Figure 4.1.B). Thus, the LD_{50} for haloxyfop-ethoxyethyl for SAS1 is >180 times that of the susceptible biotype SAS2 (Table 4.2).

Similarly, a large difference between the biotypes is observed in the response to fluazifop-butyl, where 0.016 kg ai ha^{-1} is enough to kill >50% of susceptible biotype, SAS2, while almost no mortality is detected at 17 kg ai ha^{-1} (80 lit. of product ha^{-1}) for the resistant biotype, SAS1, (Figures 4.2.A and 4.2.B). The recommended rate of this herbicide for wild oats control is 0.125 kg ai ha^{-1}. Thus the LD_{50} of SAS1 for fluazifop-butyl is >135 times the recommended rate for this biotype, and >1000 times the LD_{50} for SAS2.

When the two resistant and susceptible biotypes were tested with fenoxaprop-ethyl the susceptible biotype SAS2 was controlled at the recommended rate of from 0.120 to 0.150 kg ai ha^{-1} (Figure 4.3.A). The susceptible biotype, SAS2, showed more than 50% mortality at 0.030 kg ai ha^{-1}, (Figure 4.3.A). The resistant biotype did not show significant mortality up to 1.2 kg ai ha^{-1} (20 L of registered product ha^{-1}) and 50% mortality was achieved at 4.8 kg ai ha^{-1} (Figure 4.3.B). Thus the LD_{50} for SAS1 for fenoxaprop is >30 times the recommended rate.

The recommended rate for diclofop-methyl is 0.375 to 0.563 kg ha^{-1}. The dose of 0.563 kg ai ha^{-1} of this herbicide caused a 50% mortality in the susceptible biotype, SAS2, (Figure 4.4.A). Similar to the case with fluazifop-butyl, SAS1 did not show any significant mortality due to diclofop-methyl, even at the highest rate of 30 kg ai ha^{-1}, which is equivalent to 80 L. of registered product ha^{-1} (Figure 4.4. B).

With quizalofop-ethyl the susceptible biotype was 100% controlled at a very low rate of 0.006 kg ai ha^{-1}. The resistant biotype also exhibited 50% mortality at a low rate of 0.350 kg ai ha^{-1}, (Figure 4.5). Thus both resistant and susceptible wild oats populations, SAS1 and SAS2, are controlled by lower doses of quizalofop-ethyl than the other AOPPs tested. The recommended rate of this herbicide is 0.048 kg ai ha^{-1}. A 100% mortality of SAS1 against quizalofop-ethyl was achieved using the dose of 0.862 kg ai ha^{-1}, (Figure 4.5). Thus although controlled by lower rates of quizalofop than other AOPPs, SAS1 is resistant to quizalofop at the recommended rate. Biotype SAS1 was also less sensitive to the

aryloxyphenoxypropionate herbicides Quinfurop and Paraquizafop (Figure 4.6).

The final aryloxyphenoxypropionate tested, flamprop-methyl, controlled this resistant biotype at the recommended rate, 0.450 kg ai ha^{-1} (Figure 4.7; Table 4.3)

4.1.3.3. Effects of cyclohexanedione herbicides on wild oats, biotype SAS1

Three cyclohexanedione herbicides, tralkoxydim, sethoxydim and cycloxydim, were tested on biotype SAS1. Since, in preliminary experiments, the biotype SAS1 did not show any survival at the recommended rates of these herbicides, experiments were conducted at rates below the recommended rates. Results of the effects of CHDs on comparative survival of SAS1 and SAS2 are shown in Figure 4.8. The doses of tralkoxydim required to cause 100% mortality for SAS1 and SAS2 were 0.9 and 0.06 kg ai ha^{-1}, respectively, (Figure 4.8.A). With sethoxydim the rates which caused 100% mortality in SAS1 and SAS2 were 0.094 and 0.024 kg ai ha^{-1}, respectively (Figure 4.8.B). Considering cycloxydim, the rates causing 100% mortality in resistant and susceptible biotypes were .021 and .011 kg ai ha^{-1}, respectively, (Figure 4.8.C). So at 60%, 62% and 42% of the recommended rates of tralkoxydim, sethoxydim and cycloxydim, respectively, 100% mortality was observed for the resistant biotype SAS1. These values were 40%,16% and 22% for SAS2 for those herbicides respectively. Recommended rates of these herbicides are presented in Table 4.2.

4.1.4. Discussion

4.1.4.1. Growth responses

Seedlings of the resistant biotype, SAS1, and the susceptible biotype, SAS2, could be distinguished 24 h after treatment with most AOPPs. Although the susceptible seedlings continued to exhibit leaf elongation no new leaves emerged. In contrast, the new leaf emerged in resistant plants within 24 hours. Growth responses of susceptible plants were adversely affected before chlorosis and other symptoms of damage were observed. These early growth effects are typical of responses of susceptible grasses to AOPP herbicides (Shimabukuro *et al.*, 1989) and have previously been observed for resistant and susceptible biotypes of annual ryegrass (Heap, 1988).

Growth responses of the resistant SAS1 were not the same for all the AOPP herbicides. When exposed to diclofop and fluazifop, resistant plants remained green. At very high rates, such as 40 or 80 L. of formulated product ha^{-1} (15 to 30 kg ai diclofop ha^{-1} and 8.48 to 16.96 kg ai fluazifop ha^{-1}) transient yellowing, consistent with that caused by solvent burn, was observed. However, when subjected to high rates of herbicides, for example to three or four times the rates proposed for paraquizafop or quinfurop, tops of the resistant plants died but the plants subsequently regrew from the meristematic region (see Figure 7.1 for die-back phenomenon).

4.1.4.2 Survival

4.1.4.2.1. Aryloxyphenoxypropionates

Avena sterilis ssp. *ludoviciana*, biotype SAS1 exhibited resistance at the registered or proposed rate of the AOPP and CHD herbicides tested. Rates of AOPP and CHD herbicides required to kill 50% of the individuals of biotype SAS1 populations are given in Table 4.2.

It is clear from the studies reported here that the resistant biotype, SAS1, exhibited resistance to AOPP herbicides. There is resistance to all AOPP herbicides which had been used against SAS1, diclofop, fluazifop and haloxyfop. These studies also revealed resistance to several AOPP herbicides, to which the population had not been exposed in the field (fenoxaprop, quizalofop, paraquizafop and quinfurop). This phenomenon supports the possibility of a common mechanism of resistance operating for these herbicides in this biotype.

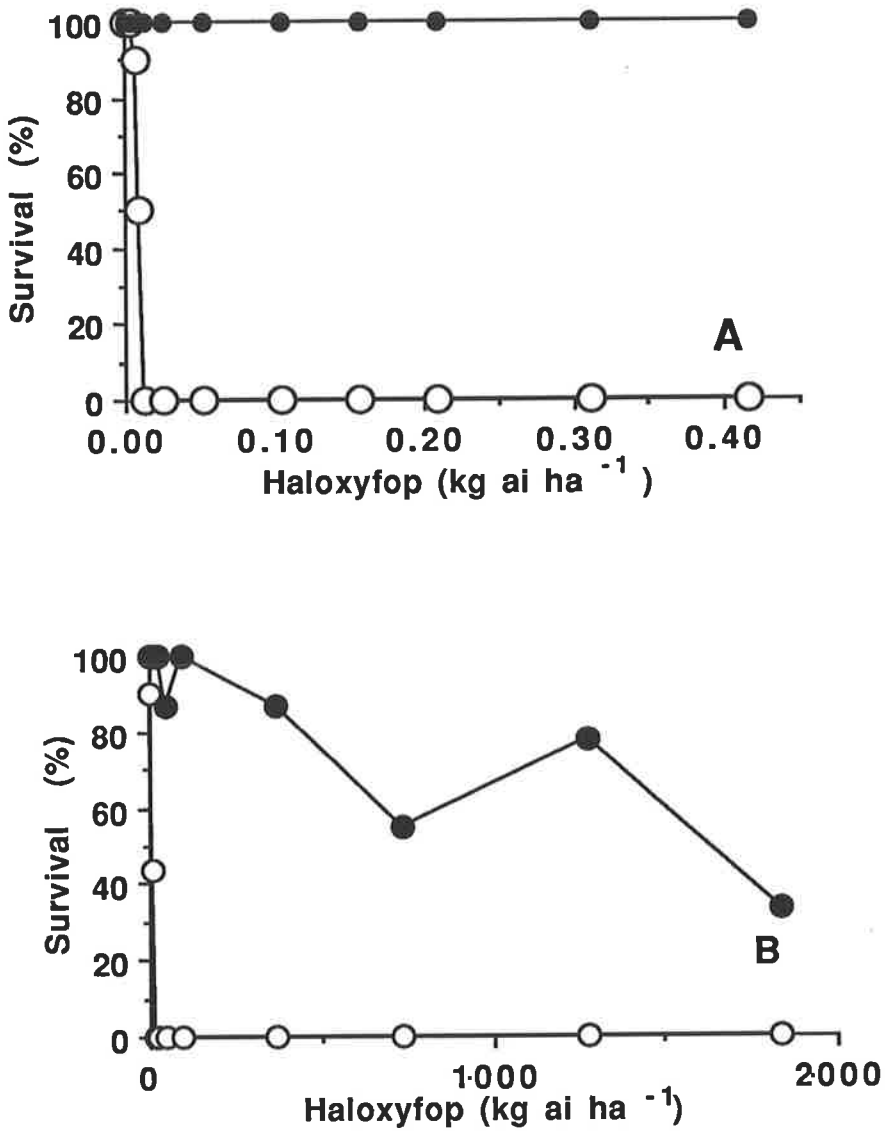


Figure 4.1. Survival of *A. sterilis* ssp. *ludoviciana* biotypes SAS1 (●) and SAS2 (O) following treatment with haloxyfop-ethoxyethyl at les than o.4 kg ai ha^{-1} (A) and less that 2 kg ai ha^{-1} (B). Each point is the average of four replications, and the vertical bars are standard errors of the means. When the bars are not seen, they are contained within the symbols. The recommended rate for the herbicide is 0.052-0.154 kg ai ha^{-1}.

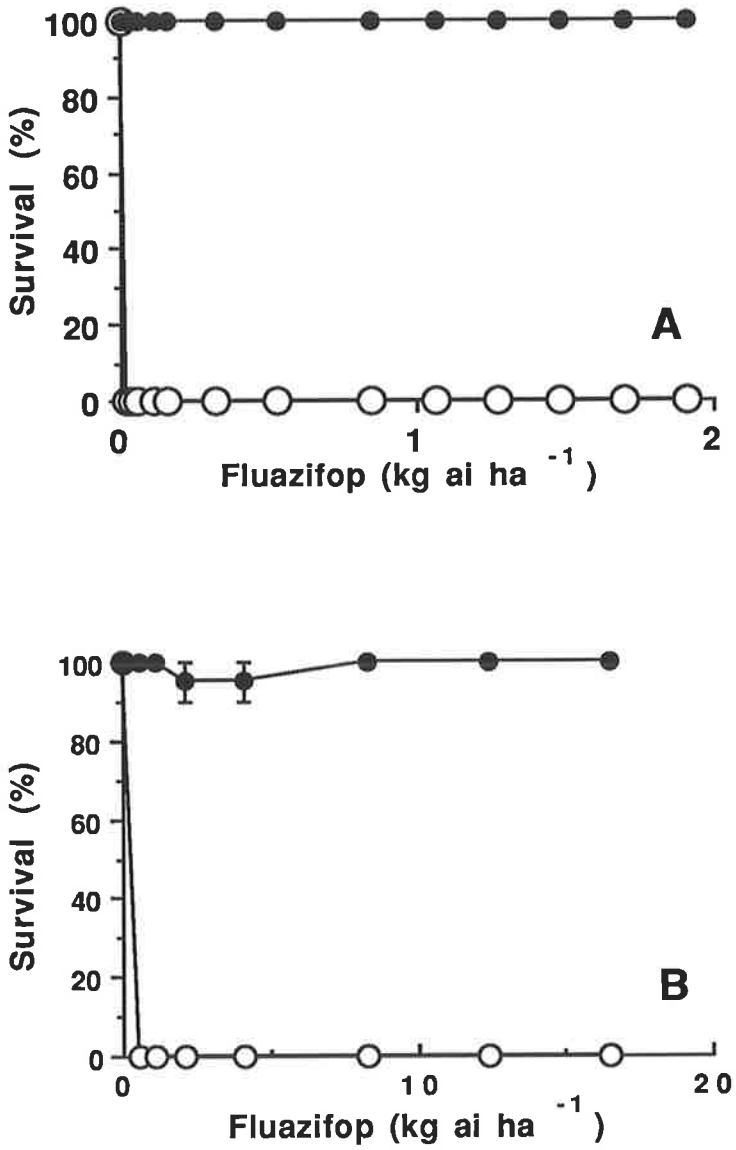


Figure 4.2. Survival of *A. sterilis* ssp. *ludoviciana* biotypes SAS1 (●) and SAS2 (○) following treatment with fluazifop-butyl at less than 2 kg ai ha^{-1} (A) and at less than 20 kg ai ha^{-1} (B). Each point is the average of four replications, and the vertical bars are standard errors of the means. When the bars are not seen, they are contained within the symbols. The recommended rate for the herbicide is 0.125 kg ai ha^{-1}.

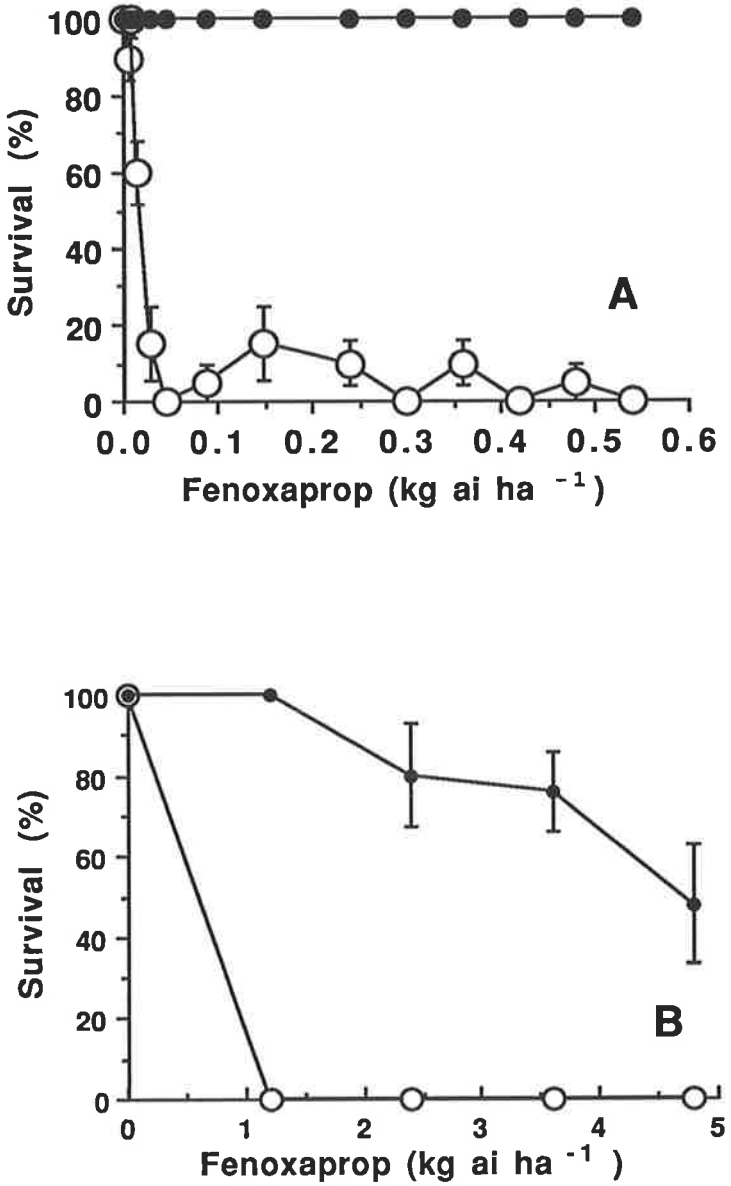


Figure 4.3. Survival of *A. sterilis* ssp. *ludoviciana* biotypes SAS1 (●) and SAS2 (○) following treatment with fenoxaprop-ethyl at less than 0.6 kg ai ha^{-1} (A) and less than 5 kg ai ha^{-1} (B). Each point is the average of four replications, and the vertical bars are standard errors of the means. When the bars are not seen, they are contained within the symbols. The recommended rate for the herbicide is 0.090-0.180 kg ai ha^{-1}.

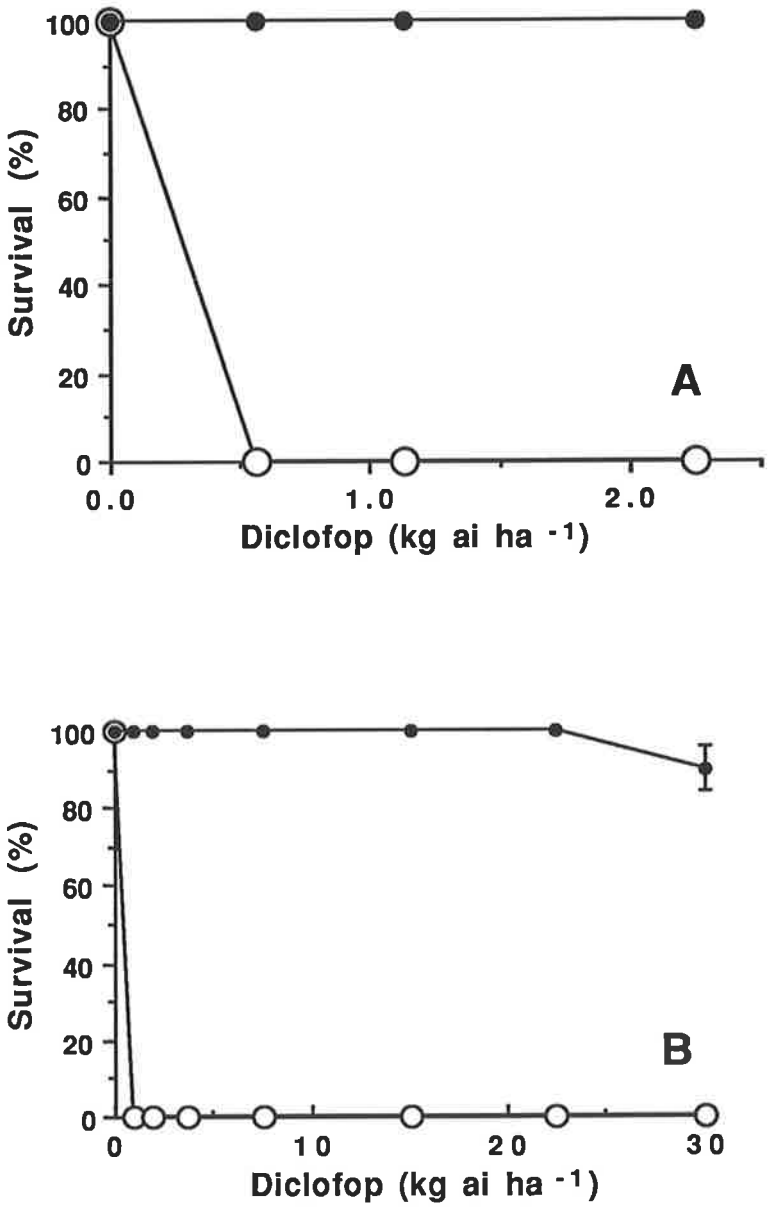


Figure 4.4. Survival of *A. sterilis* ssp. *ludoviciana* biotypes SAS1 (●) and SAS2 (O) following treatment with diclofop-methyl at less than 2.5 kg ai ha^{-1} (A) and less than 30 kg ai ha^{-1} (B). Each point is the average of four replications, and the vertical bars are standard errors of the means. When the bars are not seen, they are contained within the symbols. The recommended rate for the herbicide is 0.563 kg ai ha^{-1}.

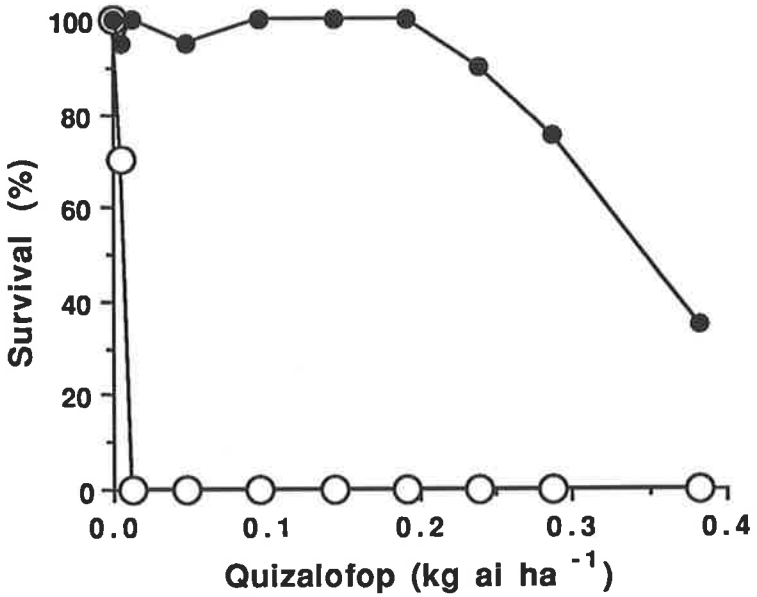


Figure 4.5. Survival of *A. sterilis* ssp. *ludoviciana* biotypes SAS1 (●) and SAS2 (O) following treatment with quizalofop-ethyl. Each point is the average of four replications, and the vertical bars are standard errors of the means. When the bars are not seen, they are contained within the symbols. The recommended rate for the herbicide is 0.030 kg ai ha^{-1}.

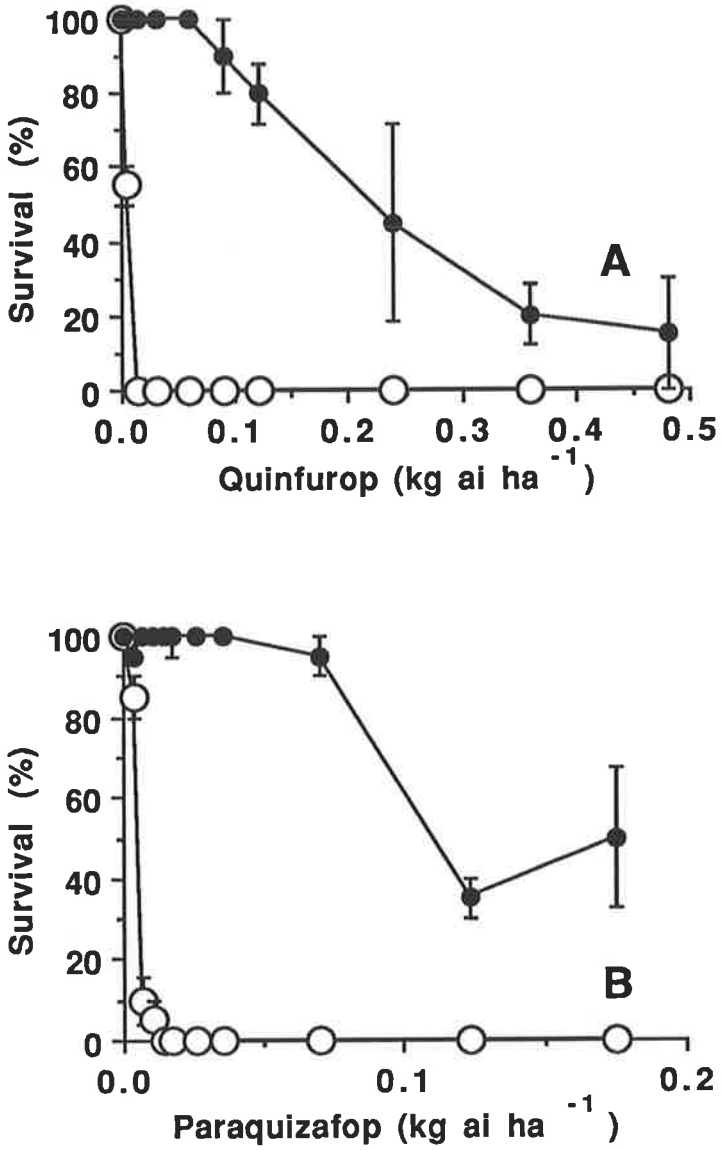


Figure 4.6. Survival of *A. sterilis* ssp. *ludoviciana* biotypes SAS1 (●) and SAS2 (○) following treatment with quinfurop and paraquizafop. Each point is the average of four replications, and the vertical bars are standard errors of the means. When the bars are not seen, they are contained within the symbols. The recommended rates for these herbicides are 0.030 and 0.020 kg ai ha^{-1}, respectively.

Table 4.2. Amounts of herbicides, in kg ai ha^{-1}, required to kill 50% of population of two biotypes of *Avena sterilis* spp. *ludoviciana*.

Herbicide[a]	LD_{50}		LD_{50} ratio
	SAS2	SAS1	SAS1/SAS2
	kg ai ha^{-1}		
Aryloxyphenoxypropionate graminicides:			
Diclofop	0.141	>30.000[b]	>213
Fluazifop	<0.016	>16.960[b]	>1067
Fenoxaprop	0.017	4.620	275
Haloxyfop	0.010	1.768	181
Quizalofop	0.004	0.148	39
Quinfurop	0.008	0.218	29
Paraquizafop	0.010	0.209	20
Flamprop	0.400	0.300	0.75
Cyclohexanedione graminicides:			
Sethoxydim	0.012	0.029	2.5
Tralkoxydim	0.013	0.034	2.7
Cycloxydim	0.003	0.009	3.0

[a](lower) Registered rates in kg ai ha^{-1} were: diclofop, 0.563; fluazifop, 0.125; fenoxaprop, 0.090; haloxyfop, 0.056; quizalofop, 0.048;quinfurop, 0.030; paraquizafop, 0.020;flamprop, 0.450; sethoxydim, 0.150; tralkoxydim, 0.150; cycloxydim, 0.050.

[b]50% mortality was not achieved in the presence of the highest herbicide application rate of 80 L of formulated product per ha (30 kg and 16.960 kg ai ha^{-1} for diclofop and fluazifop, respectively).

Since the susceptible biotype, SAS2, had not been exposed to fenoxaprop, the incomplete susceptibility of the susceptible biotype (Figure 4.3.A) because of use of other AOPP herbicides than fenoxaprop-ethyl could be

of special interest. This phenomenon may lead to the conclusion that some other factor is active for this response. These factors could be a special mechanism of resistance which works only for this member of AOPPs. The effect of fenoxaprop on resistant biotype, SAS1, was evident up to 80 L. of registered product, similar to diclofop and fluazifop. Dissimilar to these two herbicides, which did not achieve any significant control of SAS1, fenoxaprop caused 50% mortality of the resistant biotype SAS1 at 80 L of registered product ha^{-1}. The partial effect of fenoxaprop could be due to a) an effect of solvent at these very high rates, b) the effect of the crop safener present in commercial fenoxaprop formulations and/or c) may be induction or selection of resistant biotype(s) in susceptible field. The final possibility d) is the point that the mode of action of fenoxaprop-ethyl may be slightly different from other AOPPs to which SAS1 is resistant. Using pure fenoxaprop could clarify the possibilities a, b, and c.

4.1.4.2.2. Flamprop-methyl

The resistant biotype had never been exposed to flamprop-methyl and was well controlled by this herbicide (Figure 4.7. and Table 4.3).

As mentioned earlier, polycyclic alkanoic acid herbicides are divided into two major groups of **fops** and **props,** standing for oxyphenoxy alkanoic acids and benzoyl-N-phenyl phenoxy propionic acids, respectively, see 2.3.2., (Duke and Kenyon, 1988). While fops kill susceptible weed species, prop herbicides do not usually kill wild oats but impair their growth and development so that their final effects are dependent on crop growth and competition (Huston and Roberts, 1987). Because of this property it was seen that the wild oat biotypes SAS1 and SAS2 were still surviving even though they proved to be susceptible to flamprop-methyl (Figure 4.7). On the other hand, flamprop-methyl is less dependent than some other props like benzoylprop-ethyl and flamprop-isopropyl on crop competition for its effect (Huston and Roberts, 1987).

Flamprop-methyl, although structurally related to AOPP herbicides, has a different mode of action and crucial differences in chemistry from the other AOPP herbicides tested. The expression of susceptibility of biotype SAS1 to flamprop-methyl suggests that the mechanism of resistance is not effective against this herbicide.

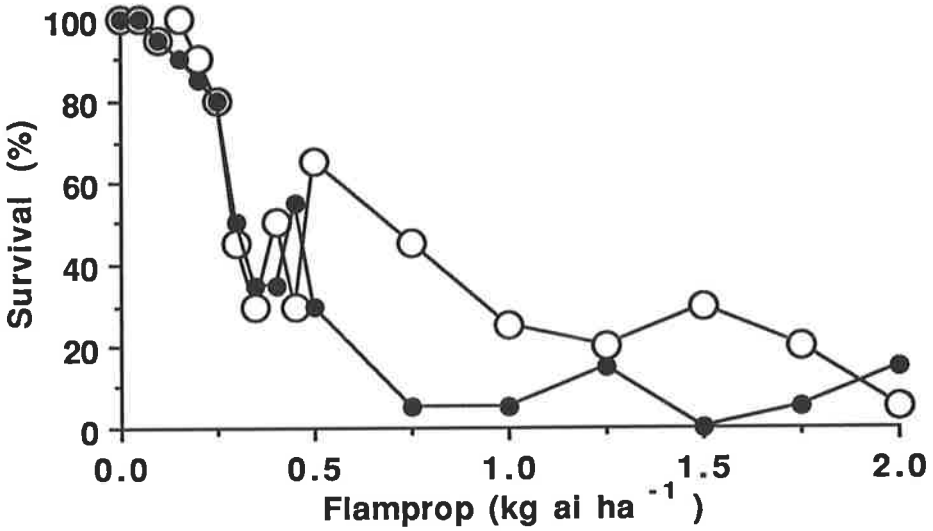


Figure 4.7. Survival of *A. sterilis* ssp. *ludoviciana* biotypes SAS1 (●) and SAS2 (O) following treatment with flamprop-methyl. Each point is the average of four replications, and the vertical bars are standard errors of the means. When the bars are not seen, they are contained within the symbols. The recommended rate for the herbicide is 0.450 kg ai ha^{-1}.

Table 4.3. Effect of flamprop-methyl on survival of resistant and susceptible wild oats biotypes SAS1 and SAS2, respectively[a].

Rate of herbicide (kg ai ha^{-1})	SAS1	SAS2
0.000	20[a]	20
0.050	20	20
0.100	19	19
0.150	18	20
0.200	17	18
0.250	16	15
0.300	10	9
0.350	7	6
0.400	7	10
0.450	11	6
0.500	6	13
0.750	1*	9*
1.000	1	5
1.250	3	4
1.500	0(*)	6(*)
1.750	1	4
2.000	3	1

[a] each number represents survivals out of 20 plants.
* numbers followed by star are significantly different (P=0.05)
(*) marginal significance at p=0.05 (right on p=0.052 critical value)

Because of this property of flamprop-methyl, many dead plants were observed in treated resistant and susceptible biotypes (Table 4.3). The mortality of biotype SAS1 was at least equal to SAS2 at most doses and was slightly higher than that of SAS2 at some doses (Figure 4.7 and Table 4.3)

4.1.4.2.3. Cyclohexanedione herbicides

Rates of the CHDs sethoxydim, tralkoxydim and cycloxydim required to kill 50% of the AOPP resistant *A. sterilis* were 2.5 to 3-fold those required to kill 50% of the susceptible population, biotype SAS2 (Table 4.2), although both populations were controlled by the registered rates (Figure 4.8). As neither population had been exposed to CHD herbicides, the increased tolerance to them in SAS1 indicates a potential for development of resistance.

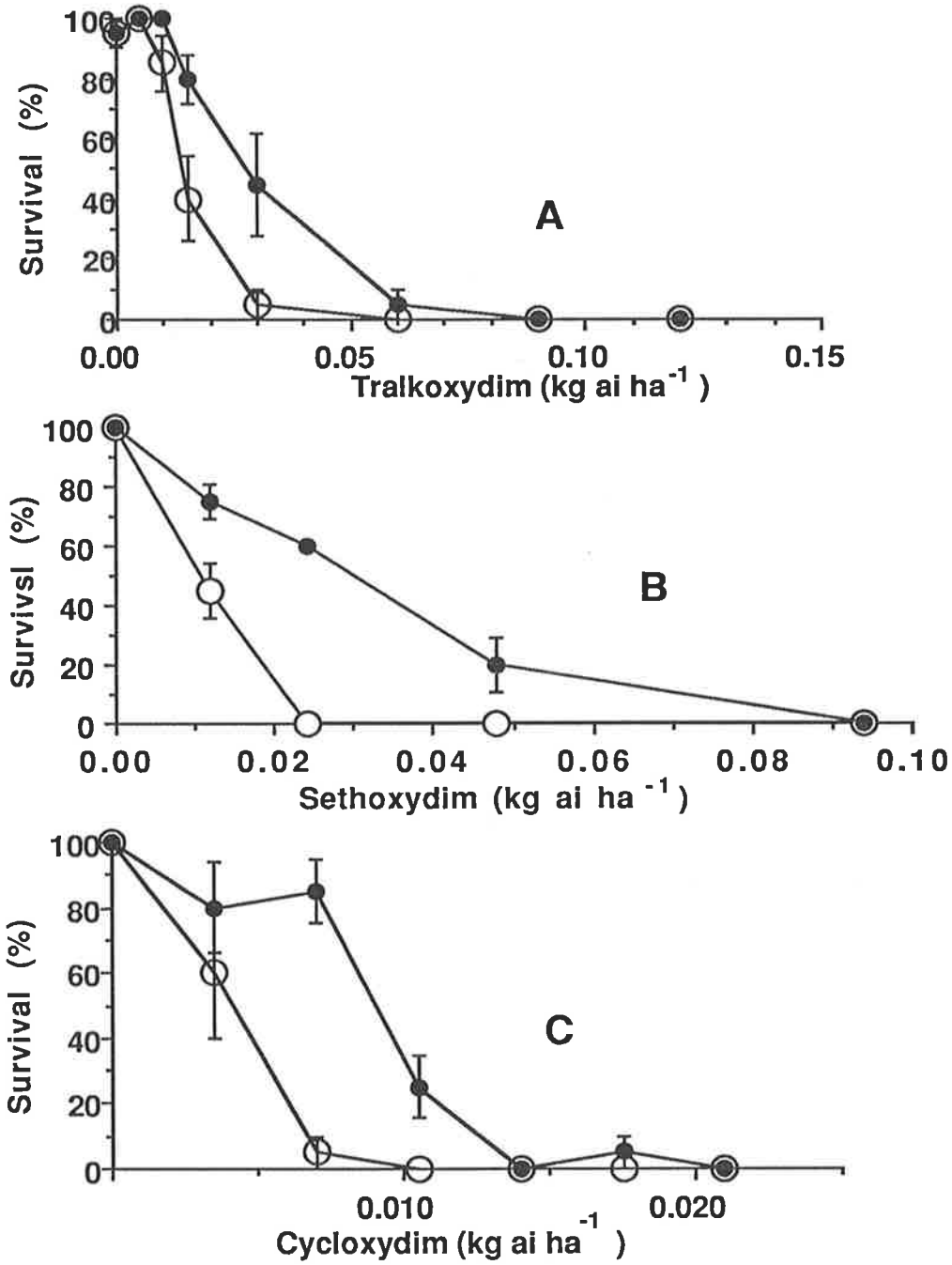


Figure 4.8. Survival of *A. sterilis* ssp. *ludoviciana* biotypes SAS1 (●) and SAS2 (O) following treatment with tralkoxydim (A), sethoxydim (B) and cycloxydim (C). Each point is the average of four replications, and the vertical bars are standard errors of the means. When the bars are not seen, they are contained within the symbols. The recommended rates for these herbicides are 0.150, 0.150 and 0.050 kg ai ha^{-1}.

4.1.5. Conclusion

Overall it can be concluded that SAS1 is highly resistant to all AOPPs to which it was exposed. The new members of AOPP and CHD herbicides are more effective against wild oats than the older ones. These conclusions can be seen since: a) new herbicides, have much lower recommended rates, see foot note of, Table 4.2 b) at lower rates new herbicides can cause more mortality in both susceptible and resistant biotypes (Figures 4.1 to 4.8 and Table 4.2). Biotype SAS1 remained susceptible to flamprop methyl, to which SAS1 was not exposed. Cyclohexanediones controlled SAS1 at the recommended rates but at lower rates of these herbicides SAS1 exhibited a higher tolerance than SAS2. SAS1 and SAS2 had no history of CHD herbicides. Therefore, the higher tolerance of SAS1 against CHDs could mean a potential for progress of resistance especially if further selection pressure from AOPP or CHD herbicides is applied to this biotype. The mechanism of resistance is suspected to be the same against AOPPs.

Chapter 5

Studies of the competitive ability of aryloxyphenoxypropionate resistant wild oat, *Avena sterilis*, biotype SAS1

5.1. Introduction

In every plant population there is competition between individual plants. Competition of plants is significant, firstly, for survival and, secondly, for maximum reproduction. A more competitive biotype can occupy more space, displace competitors and produce more seed, thus, becoming enriched in the seed-bank. "When one biotype leaves more offspring than another because of its superior ability to survive and reproduce, that phenotype has an evolutionary advantage relative to other phenotypes in that environment" (Holt and Thill, 1994). A plant may gain a competitive advantage by gaining better access to resources such as water, nutrients and light (Hickney and McNeilly, 1975). Conversely, the development of mechanisms to avoid stress factors in the environment may also provide a competition advantage. Such stress factors may include, water logging, drought, mineral deficiency or toxicity, temperature extremes, pests, diseases and herbicides.

It has been suggested that resistant (R) biotypes exist at very low frequencies in susceptible (S) populations prior to selection by herbicides (Gressel and Segel, 1978; Conard and Radosevich, 1979; Holt *et al.*, 1981). The existence of the very rare herbicide resistant genotype will become apparent only after selection by herbicides (see Chapters 2 and 7). In the case of triazine resistance, the postulated occurrence of R individuals at

low frequencies in S populations before the onset of triazine selection pressure implies that the R biotypes are competitively equal or inferior to their S counterparts (Gressel and Segel, 1978). "Reduced fitness is often the penalty incurred by an organism which has been selected for a particular trait" (Tucker, 1989). Reduced fitness in newly emerged resistant biotypes has proved to be common in cases of triazine resistant weeds. The first reported cases of herbicide resistance were cases of triazine resistance (Ryan, 1970; Holt, 1992). It was shown that triazine resistant plants were weaker than susceptible ones in both competitive ability and physiological performance (Holt, 1990; Warwick, 1991). Studying *Hordeum leporinum*, Tucker (1989) also reported that "there is reduced growth and competitiveness in the paraquat resistant *Hordeum glaucum* compared to the susceptible biotype as well as the natural paraquat susceptible *H. leporinum*." In a review, Holt and Thill (1994) concluded that dinitroaniline resistance is also correlated with reduced fitness.

Since triazine resistant biotypes are often less fit than their susceptible rivals, the view that herbicide resistant plants would be less fit than susceptible biotypes became widely accepted (Gressel and Segel, 1978; Ahrens and Stoller, 1983; Gressel and Ben-Siana i, 1985; Holt, 1988; Holt, 1990). However, this hypothesis is not confirmed in all cases (Holt and Thill, 1994) as some workers reported resistant biotypes which are equally competitive to susceptible biotypes. Purba (1993) reported that a paraquat-resistant biotype of *Hordeum leporinum*, THL1, is not less reproductive than the susceptible biotype, THL4. Warwick and Black (1981) reported that R and S biotypes of late flowering goosefoot

(*Chenopodium strictum* Roth.) were approximately equally competitive. Schonfeld *et al.*, (1987) also reported triazine resistance without reduced vigour for *Phalaris paradoxa*. However, in the case of AOPP resistance, there is little information available on differences between biotypes in relative growth, competitiveness, and fecundity, so few conclusions can be drawn regarding effects of resistance to ACCase inhibiting herbicides on fitness (Holt and Thill, 1994).

As reported in this thesis, many wild oat populations have been found to be resistant to the AOPP and CHD herbicides. (Chapter 8; Heap *et al.*, 1993). No studies of the competitive growth, productivity or competitiveness of the resistant versus susceptible wild oat biotypes have been reported. Hence, the result of studying competition between R and S wild oat biotypes in unsprayed populations is required as information on the relative fitness of R and S biotypes in the absence of herbicide is important. In the absence of herbicide, a fitter, more competitive biotype will be expected to dominate the population.

The present studies were conducted to determine productivity, competitiveness and relative fitness of wild oats *Avena sterilis* ssp. *ludoviciana*, SAS1 (R), and SAS2 (S). The productivity of resistant and susceptible wild oats in the absence of competition was determined. As part of this study the productivity of plants derived from the 1st and 2nd seeds of each spikelet were separately investigated. First or primary (bigger) and second or secondary (smaller) seeds within spikelets are different in many aspects including dry weight and dormancy (Johnson

1935; Thurston, 1963). Plants derived from these seeds may perform differently. This difference is important as most of the first seed germinates in the first year following production while much of the 2nd and 3rd seeds remain in the seedbank (author unpublished; Johnson, 1935; Rijkslandbouwhogeschool, 1960; Holm *et al.*, 1977). Guillemenet (1971) found that this was especially true of *A. sterilis* ssp. *ludoviciana*. Thus, if the resistant seedlings germinating in the first year are successfully controlled the remaining resistant seed will be largely 2nd and 3rd seeds which are smaller and less vigorous. These differences may have implications for resistance management.

Non-competitive experiments were also conducted using field grown plants of resistant and susceptible wild oats. The productivity of plants derived from 1st and 2nd seeds of R and S biotypes were measured under non-competitive conditions. The time to first inflorescence was also measured in non-competitive experiments.

Competition between resistant and susceptible wild oat biotypes was also studied using pot cultivated and field grown plants. Total aboveground dry weight, number of matured panicles and seed weight per plant in competitive situations were measured. A replacement series experiment (de Wit, 1960) was performed to determine field performance and fitness of AOPP herbicide-resistant and -susceptible biotypes of *A. sterilis* ssp. *ludoviciana* under competitive field conditions.

5.2. Materials and methods

5.2.1. Plant material

Seeds of resistant wild oat, SAS1, were collected in 1990 from a population in a clover (*Trifolium sp.*) field at Bordertown, South Australia, as described in Chapter 3. Susceptible seeds were collected at the same time from an adjacent paddock of the same farm less than 100 m from the resistant population. Herbicide histories of the R and S paddocks are presented in Tables 3.1 and 3.2. Cultivated oat, variety Coolabah, was taken from Waite Institute, and used as buffer plants around field plots.

5.2.2. Seedling emergence and establishment

Emergence of seedlings from the resistant and susceptible biotypes was similar for both field and pot experiments following the method described in Chapter 3 (Section 3.4). For field experiments, cultivated oat, variety Coolabah, was planted in 42 X 30 X 12 cm plastic containers filled with perlite. At the two leaf stage , seedlings were placed outdoors for 2 days to aclimatise the plants to field conditions before transplanting into plot borders.

For all pot experiments, seedlings were established as described in section 3.4. For non-competitive field experiments, plants were transplanted into the field 50 cm apart on the 6th of July 1992. For competition field experiments a metal grid was used to determine planting density and planting patterns. Metal grids, which had been marked using red and

green paints, were laid on the soil before transplanting to delineate individual plant positions. Mesh sizes of 5 X 5 and 10 X 10 cm were used to give planting densities of 400 and 100 plants m^{-2} respectively. Planting arrangement within the grids was such that R and S plants were evenly distributed throughout the plots. Seedlings were then planted in the centre of each grid segment according to the predicted densities and proportion on the 1st and 2nd of July 1992 (see section 5.2.3). Each plot was surrounded by 3 buffer rows of cultivated oat, Coolabah. One week after planting, a small number of failed transplants were replaced by appropriate sized seedlings. Plots were kept free of weeds by hand weeding throughout the experiment, with minimal disturbance to the plots.

5.2.3. Experimental site

The field experiments were conducted during the winter cereal growing season of 1992 in an experimental field at the Waite Agricultural Research Institute. The field was ploughed during autumn 1992. The soil on which the experiment was conducted is a red brown earth with at least 25 cm of top soil of sandy loam texture, a prismatic structured clay subsoil and a calcareous deep subsoil. The site had been sown in rotation to ryegrass, wheat (*Triticum aestivum*) and fababean (*Vicia faba*) in the previous three years.

5.2.4. Non-competitive experiments

The productivity of the two biotypes was investigated using a non-competitive field experiment. Productivity of both biotypes were thus evaluated under non-competition conditions. Plots of this experiment were located near the site of the competition experiments. The same plant materials were used as those used for competition experiments. For the non-competitive situation, seedlings were planted at 50 cm intervals on 6th July 1992. Plots were kept free of other weeds throughout the experiment by hand weeding. Fifty plants for each biotype were planted. For each biotype the seedlings were separated into those derived from 1st (bigger) seeds of the spikelets and those from 2nd (smaller) seeds of spikelets.

5.2.5. Competition experiments

A preliminary competition experiment was conducted in 1990 in plastic containers (42 X 30 X 12 cm) in a completely randomised design. The area of each container was 0.126 m^{-2}. A total of 35 seedlings were planted in each container to give a density of 277 plant m^{-2}. The proportions of the two biotypes were: 35 S; 34 S:1 R; 30 S: 5 R; 25 S: 10 R; 18 S: 17 R.

For the competition field experiment, a replacement series design (de Wit, 1960) was used where the two biotypes were grown at the two densities of 100 and 400 plants m^{-2}. The resistant and the susceptible plants were planted at the following seven relative proportions:

Resistant %	:	susceptible %
0		100
11		89
25		75
50		50
75		25
89		11
100		0

Every plot was surrounded by 3 rows of cultivated oat, variety Coolabah, as buffer plants. There were three replications arranged in a randomised block design. The data were analysed as a completely randomised block design (Purba, 1993; Ahrens and Stoller, 1983). The experiment was established in the field on 1st and 2nd of July 1991.

5.2.6. Plant harvest and measurements

In non-competitive field experiments, dates at which first inflorescence emerged out of the flag-leaf for each treatment were recorded. In competition field experiment, dates of emergence of first spikelets from flag-leaves were recorded for every biotype at every proportion in each plot. Plants of both non-competitive and competitive experiments were harvested on the 4th and 5th of November 1991, 122 and 126 days after transplanting the non-competition and the competition experiments, respectively. Most of the ripe spikelets were still green (not ripe). At harvest, some seeds had been lost, though these losses were judged to be

less than 5% and to represent roughly equal percentages for both biotypes (spikelets shedding during 1st days of beginning of shedding are usually empty spikelets). Paper envelopes (15 cm X 35 cm) were prepared for plant harvest. Plants were individually harvested by excising at the soil surface and were placed in identified individual paper bags. The number of panicle bearing tillers per plant, total aboveground dry weight and weight of seeds produced by each plant were measured individually. Dry weights were measured after drying at 80 ^{o}C for 24 hours. Data were subjected to analysis of variance. T-test was used to compare data obtained from non-competition experiments. Two way analysis of variance and the F-test were used for competition experiments and Duncan's Multiple Range test method was used to detect differences among means.

The results of dry matter production, number of panicles and seed weight per plant of field competition experiments were plotted as replacement series diagrams to illustrate the nature of the competition occurring between the two biotypes. In these diagrams, relative yields of each biotype are plotted against the relative frequencies in the mixtures (de Wit, 1960). The shape of the curves in such plots can be used to indicate the relative competitiveness of the biotypes.

5.3. Results

5.3.1. Relative weight of 1st and 2nd seeds of SAS1 and SAS2

To determine whether weight differences reported for first and second seeds of wild oat exist in biotypes SAS1 and SAS2, 1000 seed weights were measured for them. 1000 Seed weight of resistant, SAS1, and susceptible, SAS2, biotypes were measured by weighing more than 1000 seeds (in multiples of 100) of each biotype. For each biotype seeds were separated into 1st and 2nd seeds of the spikelets. For ease of discussion the first and second seeds of the resistant biotype will be called the R1 and R2 while the first and second seeds of the susceptible biotype will be called the S1 and S2, respectively Table 5.1 represents 1000 seed weight of SAS1 and SAS2 seeds harvested late in the spring 1989. For R1 and R2 seeds 16 samples of 100 seeds were weighted while for S1 and S2 15 samples of 100 seeds were weighted. Data were analysed by analysis of variance using Duncan's Multiple Range test to separate means.

Table 5.1. 1000 seed weight, in grams, of resistant, SAS1, and susceptible, SAS2, biotypes of wild oats, *Avena sterilis* ssp. *ludoviciana*.

biotype	1st seed	2nd seed	pooled
SAS1	19.518 b*	10.368 d	14.944**
SAS2	21.986 a	13.000 c	17.493

*values followed by different letters are significantly different at P=0.01. Data were compared using Duncan's Multiple Range test mean comparison method.

** pooled data significantly different at P=0.01

SAS2 plants had significantly heavier seeds than the resistant SAS1. Furthermore, 1st and 2nd seeds of SAS2 were significantly heavier than 1st and 2nd seeds of resistant SAS1, respectively. Thus, differences between weight of 1st and 2nd seed exist in both biotypes. Additionally there are differences between biotypes. Further studies were conducted to determine whether these differences can affect the productivity of the resultant plants.

5.3.2. Productivity of resistant and susceptible *Avena sterilis* ssp *ludoviciana* in the absence of competition

The result of dry matter production, number of panicles, number of seeds produced per plant and number of days to emergence of first panicles in the absence of competition are presented in Table 5.2.

Table 5.2. Dry matter productivity (g plant^{-1}), number of panicles (plant^{-1}), amount of seed produced (g plant^{-1}) and number of days to emergence of first panicle (days to heading= DTH) of resistant, SAS1, and susceptible, SAS2, wild oats, *Avena sterilis* ssp. *ludoviciana* grown in the absence of competition.

biotype	dry matter	no. panicles	seed yield	DTH
comparison A*	g plant^{-1}	plant^{-1}	g plant^{-1}	days
SAS1-1 (R1)**	76.04 a***	24.84 a	19.44 a	89.00 a
SAS1-2 (R2)	40.64 b	15.88 bc	11.58 bc	89.00 a
SAS2-1 (S1)	50.59 b	19.28 bc	14.53 bc	88.00 a
SAS2-2 (S2)	50.58 b	20.00 b	15.57 ab	88.00 a
comparison B*				
SAS1	58.34 a	20.36 a	15.12 a	89.00 a
SAS2	50.08 a	19.64 a	15.05 a	88.00 a

*R_1, R_2, S_1 and S_2 compared only with each other, independent from comparisons made in part "comparison B" which has been made on the basis of pooled data for each biotype from comparison A.

**R_1, R_2, S_1 and S_2 represent plants derived from bigger (1st) and smaller (2nd) seeds of spikelets for the resistant and susceptible biotypes, respectively.

***means followed by different letters in each column in each comparison are significantly different (t=0.05, t-test)

In the absence of competition the resistant and the susceptible biotypes were not significantly different for dry matter production, number of panicles per plant, seed yield and number of days to heading (Table 5.2). But when the R and S biotypes are separated into those derived from 1st

and 2nd seeds of spikelets some significant differences are detected. Plants derived from first seeds of resistant biotype (R_1) produced significantly more dry matter than plants derived from three other kinds of seeds (Table 5.2). Similarly, plants derived from first seeds of resistant spikelets (R_1) produced more panicles than plants derived from the three other seed sources (Table 5.2). But for the case of seed yield, although plants from first seeds of resistant biotype (R_1) produced the highest amount of seed, plants derived from the second seeds of the susceptible biotype (S_2) produced a similar seed yield while plants from first seeds of susceptible (S_1) and those produced from second seeds of the resistant biotype (R_2) produced the least weight of seed. A simple calculation reveals that although plants derived from the 1st and the 2nd seeds of the R biotype, R_1 and R_2, produced highest and lowest yields per plant, the summation of product of 1st and 2nd seeds of biotypes R and S not only compensated for this difference but resulted in very similar overall seed production for R and S (Table 5.2, comparison B versus comparison A).

5.3.2.1. Number of days to heading (DTH)

Whilst recording these values most of the plants were at the booting stage. This was true for both the non-competitive plots which were measured and the adjacent competitive experiment. Panicle emergence was recorded by the appearance of the first awn from the flag-leaf at the booting stage. The number of days for emergence of the first panicle in the resistant and the susceptible biotypes of wild oats SAS1 and SAS2, respectively, in the absence of competition are presented in Table 5.2. These results show that in the absence of competition, R and S biotypes

did not differ significantly in the number of days to the emergence of the first panicle. For non-competition experiments, planted on 6th July 1991, first panicles emerged 88 days after planting for S1 and S2 and 89 days after planting for R1 and R2 (1st and 2nd October 1991, respectively). There were no significant differences between the number of days to emergence of the first inflorescence recorded in any of the experiments (Table 5.2).

5.3.3. Productivity of resistant, SAS1, and susceptible, SAS2, wild oats, *Avena sterilis* ssp. ludoviciana when in competition

Dry matter and panicle number per plant of the preliminary experiment in plastic container are presented in Table 5.3. Neither dry matter production nor number of panicles per plant differed significantly between biotypes when planted together at different proportions (Table 5.3).

Table 5.3. Aboveground dry weight and number of panicles per plant in plastic container grown plants, using a total plant density of 277 plants m^{-2}, of resistant, SAS1 (R), and susceptible, SAS2 (S), wild oats, *Avena sterilis* spp. *ludoviciana* in various proportions.

initial proportions (%)	biotypes	dry weight (g $plant^{-1}$)	No. panicles ($plant^{-1}$)
100	S	1.855*	2.58
97	S	1.867	2.25
3	R	2.103	2.33
86	S	2.200	2.48
14	R	2.087	2.22
71	S	1.977	2.57
29	R	2.183	2.77
51	S	1.710	2.11
49	R	1.740	2.18

*in each column values were not significantly different at P=0.05 (Duncan's Multiple Range test was used for mean comparison).

For the competition field experiments, dry matter and seed yield of resistant, SAS1, and susceptible, SAS2, biotypes in monoculture and in the various proportions at a planting density of 100 plants m^{-2} are presented in Table 5.4. Dry matter production for many proportions of R and S are not significantly different between biotypes (Table 5.4).

Table 5.4. Dry weight, number of panicles and seed yield of resistant (SAS1 = R) and susceptible (SAS2 = S) wild oats, *Avena sterilis* ssp. *ludoviciana* in monocultures and in mixtures at a constant plant density of 100 plants m^{-2}.

initial proportion	biotype	dry weight	number of panicles	seed yield	DTH
%		g $plant^{-1}$	$plant^{-1}$	g $plant^{-1}$	days
100	R	8.92 bc*	3.09 bc	2.16 bc	82 a
89	R	7.86 bc	3.27 bc	2.16 bc	83 a
11	S	8.16 bc	3.13 bc	2.12 bc	82 a
75	R	9.11 bc	3.16 bc	2.13 bc	83 a
25	S	8.11 bc	3.61 b	2.37 bc	82 a
50	R	7.82 bc	2.96 bc	2.02 bc	80 a
50	S	9.38 abc	3.50 bc	2.24 bc	82 a
25	R	6.69 c	2.79 c	1.89 c	83 a
75	S	10.51 ab	3.11 bc	2.10 bc	82 a
11	R	12.26 a	4.33 a	3.32 a	82 a
89	S	9.09 bc	3.15 bc	2.33 bc	81 a
100	S	9.44 abc	3.21 bc	2.49 b	82 a

* in each column values with the same letters are not significantly different at P=0.05 (Duncan's Multiple Range test was used for mean comparison).

However, at a ratio of 25R: 75S the S biotype produced significantly more dry weight than R. At 11R: 89S, the R produced more dry matter than S.

R produced highest dry matter at 11R: 89S proportion. Dry matter production, however, by R and S biotypes in monoculture were not statistically different. Considering the number of productive tillers (panicles) per plant, again R and S are equal in monoculture and at most proportions. Again, the biotype R has highest panicle number at 11R: 89S proportion and lowest at the proportion of 25R: 75S . A similar situation occurs for biotype R concerning seed yield per plant in competition experiment at the 100 plants m^{-2} density.

For competition field experiments at an overall density of 400 plants m^{-2}, the results for production of dry matter, number of panicles and seed yield per plant of biotypes SAS1 and SAS2 are presented in Table 5.5. As seen in Table 5.5 there is no significant difference between SAS1 and SAS2 either in monoculture or in mixed culture for either dry matter production, number of panicles per plant, or seed yield.

The results of dry matter production, number of panicles and seed yield in competition between SAS1 and SAS2 are further illustrated in replacement series diagrams for plant densities of 100 and 400 plants m^{-2} in Figures 5.1 and 5.2, respectively. Dashed lines represent the theoretical expected results for biotypes having exactly equal competitive fitness. These figures show that at either density, the amount of dry matter production, number of panicles and seed yield of the two biotypes, R and S, are not significantly different from those predicted (hence the shapes of the curves are almost coincident with the dashed lines predicted for equal fitness for the two biotypes). The slight differences are not statistically significant (See Tables 5.4 and 5.5).

Table 5.5. Dry matter, number of panicles, and seed yield of resistant (SAS1 = R) and susceptible (SAS2 = S) biotypes of wild oats, *Avena sterilis* ssp. *ludoviciana* in monocultures and mixtures at a constant plant density of 400 plants m^{-2}.

initial proportion	biotype	dry weight	number of panicles	seed yield	DTH
%		g $plant^{-1}$	$plant^{-1}$	g $plant^{-1}$	days
100	R	5.20*	1.88	1.37	82
89	R	4.25	1.73	1.06	82
11	S	5.41	2.09	1.20	83
75	R	4.70	1.85	1.21	83
25	S	4.60	1.68	1.19	82
50	R	4.46	1.57	1.07	82
50	S	3.67	1.73	0.81	82
25	R	4.27	1.69	1.17	83
75	S	5.60	2.03	1.43	82
11	R	4.09	1.92	1.18	83
89	S	5.47	2.03	1.45	81
100	S	4.47	1.49	1.20	81

* in each column values did not differ significantly at P=0.05 (Duncan's Multiple Range test was used for mean comparison).

5.3.3.1. Number of days to heading (DTH)

The number of days for emergence of the first panicle in the resistant and susceptible biotypes of wild oats SAS1 and SAS2, respectively, in the presence of competition are presented in Tables 5.4 and 5.5, for experiments using planting densities of 100 and 400 plants m^{-2} densities, respectively. These results show that in the presence of competition, R and S biotypes did not differ significantly in the number of days to the emergence of the first panicle. Neither were the number of days to the emergence of the first panicle in competition experiments different for the various plant proportions either (Tables 5.4 and 5.5). For competition experiments, planted on 1st and 2nd of July 1991, first panicles emerged after 81 days, (19th and 20th September 1991).

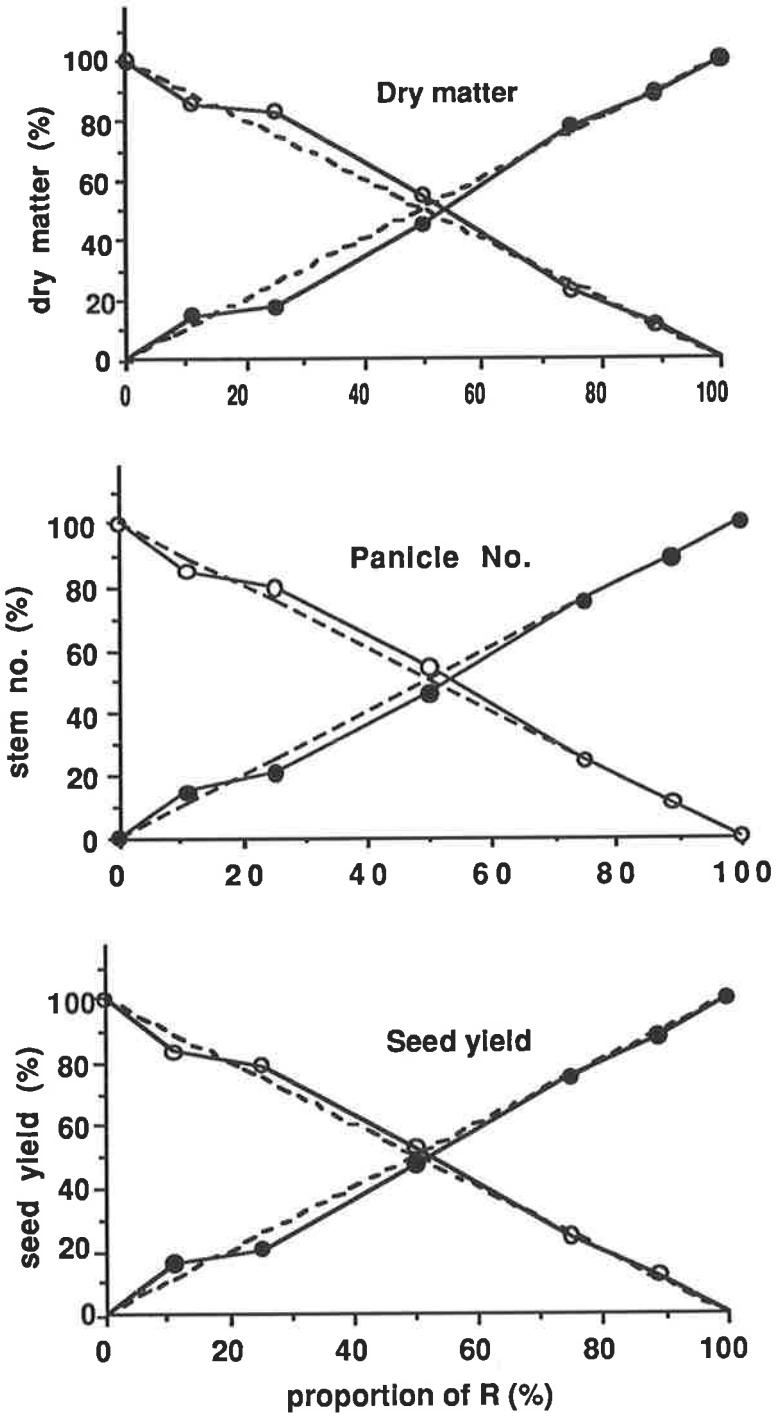


Figure 5.1. The proportion of total dry weight, number of flowering stems, and seed yield of resistant , SAS1, (●) and susceptible, SAS2, (○) biotypes of *A. sterilis* ssp. *ludoviciana* grown together in various proportions at a constant plant density of 100 plants m^{-2}. Plants were harvested 126 days after transplanting.

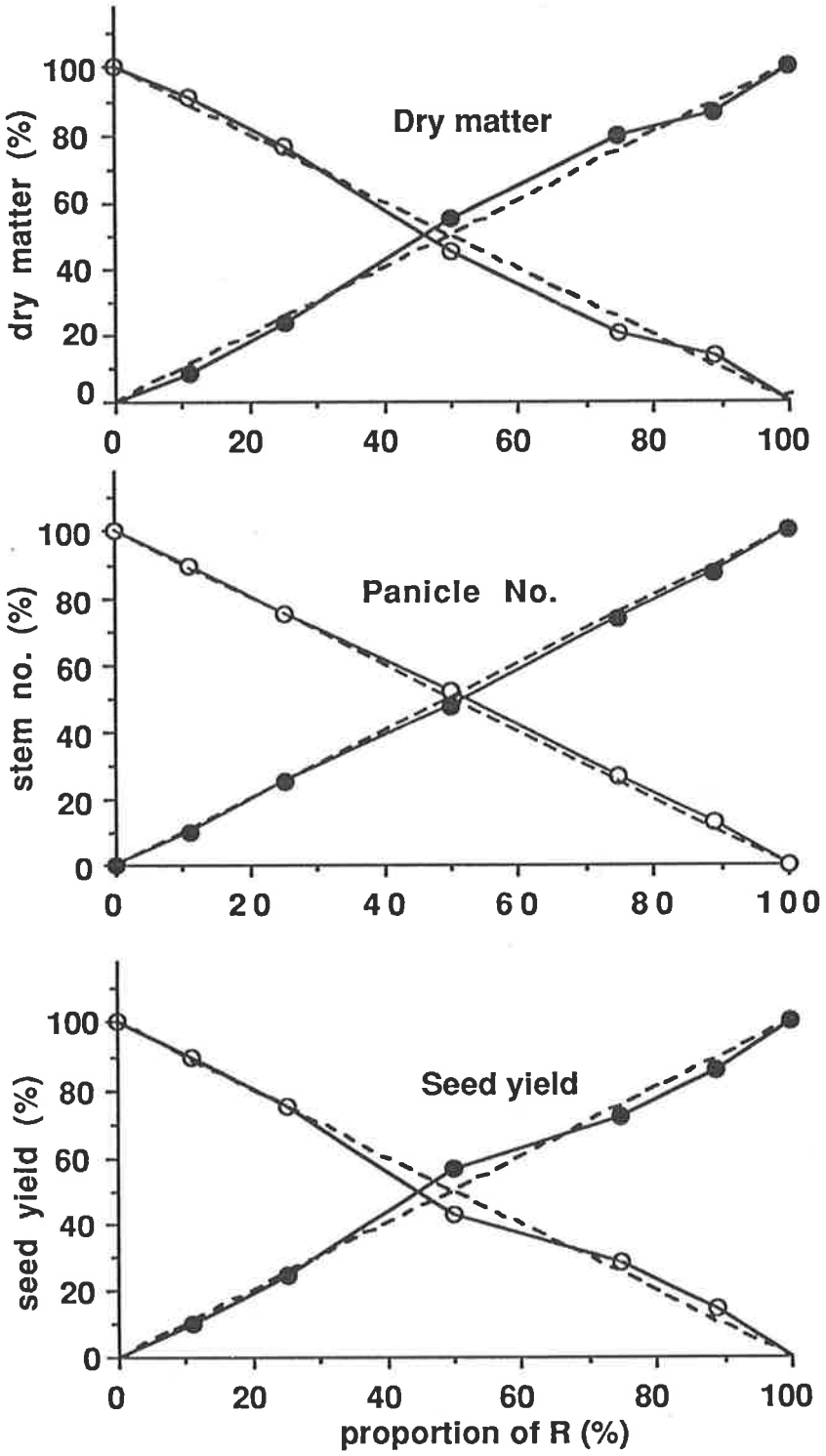


Figure 5.2. The proportion of total dry weight, number of flowering stems and seed yield of resistant, SAS1, (●) and susceptible, SAS2, (O) biotypes of *A. sterilis* ssp. *ludoviciana* grown together in various proportions at a constant plant density of 400 plants m^{-2}. Plants were harvested 126 days after transplanting.

5.4. Discussion

The results of these experiments show that, in the absence of herbicide, the resistant wild oat, SAS1, is at least equally fit with the susceptible biotype, SAS2. It must be remembered that these two populations were collected in similar environments at sites less than 100 meters apart and should not, therefore, vary widely in terms of ecotypic response to the environment.

Considering number of days from planting to emergence of the first inflorescence, there were no differences between R and S biotypes in non-competition or competition experiments (Tables 5.2 and 5.4). The same was true comparing plants derived from 1st and 2nd seeds of R and S biotypes (Table 5.2). This demonstrates that there is no difference in time of flowering between R and S biotypes which may be exploited for management of the R population.

When separating R and S seeds into bigger (1st) and smaller (2nd) seeds of the spikelets of R (R_1, R_2) and S (S_1, S_2) biotypes, the R_1 produced significantly more dry weight, more panicles and more seed yield per plant than R_2, S_1 and S_2. R_2, on the other hand, produced the least amount in all these parameters. If the germinating first seeds are successfully controlled the first year after seed set and no new seed is added to the seedbank, the more dormant 2nd or 3rd seeds will provide the majority of the resistant germinations in the next season. These plants may be less competitive and more likely to be out competed by susceptible wild oats present in the seed bank. Plants derived from second seeds of susceptible

biotype produced more dry matter, panicle and seed yield than plants derived from second seeds of the resistant biotype in non-competition situation although the differences were not statistically significant (Table 5.2). This could suggest that plants derived from S_2 seeds can compete better than plants derived from the R_2 seeds. Further work is required to determine whether a small but consistent difference exists. If so, the resistant seedlings may be less competitive in the second and third years after seed set. It should also be noted, however, that differences in biology can occur in populations of wild oat even over distances of only 100 m. Further work is, therefore, required to determine whether any putative difference in fitness between the R_2 versus S_2 is directly related to resistance.

Pooled data for 1st and second seeds indicates that there is no significant difference between resistant and susceptible biotypes for dry weight, seed yield, stem number or number of days necessary to the emergence of the first inflorescent. Although there is no significant difference, the mean values for all these parameters are greater for the resistant biotype (Table 5.2). Thus, it can be concluded that under free competition of R and S biotypes, in the absence of herbicide, the R biotype is at least as fit as the S biotype.

5.5. Conclusion

Resistant wild oat, SAS1, is equally fit with the susceptible biotype for all parameters measured. Neither the R nor the S biotype was consistently more productive in competitive or non-competitive situations. Plants derived from the first (bigger and less dormant) seeds of the spikelet

were more productive and plants derived from the second (smaller and usually more dormant) seeds were less productive than similar plants for both biotypes. This suggests that maximum emphasis should be placed on controlling resistant wild oat in the first year after seed set. In subsequent years, plants derived from less competitive 2nd seeds, face more even competition against plants derived from the 2nd seeds of the susceptible biotype.

This study is the first ever examination of the competitive ability of an ACCase resistant plant population. It is clear that this particular resistant population is not less competitive than a susceptible population growing only 100 metres away. Therefore, a population can develop resistance to a wide range of AOPP herbicides without suffering a competitive disadvantage. It will be interesting to compare this result to those that will be obtained when other workers conduct competition studies with ACCase resistant populations. No such studies have yet been published, although a similar study with a resistant population of *Lolium rigidum* has also shown no competitive disadvantage associated with resistance to ACCase herbicide (J. M. Matthews and S. B. Powles, unpublished).

These results with an AOPP resistant *A. sterilis* ssp. *ludoviciana* population which shows no competitive disadvantage are in stark contrast to the many studies with triazine resistant weed biotypes which have clearly reduced competitive ability (reviewed by Holt and Thill, 1994). While it is apparent that the particular mutation that endows resistance to triazine herbicides (Gronwald, 1994) confers reduced competitive ability (Holt and Thill, 1994) it is equally clear from the study reported here that

this need not be the case for an AOPP resistant population. This is not surprising since there is no reason to believe that all resistance mechanisms will be associated with reduced competitive ability.

CHAPTER 6

Seedbank life of *Avena sterilis* L., ssp. *ludoviciana* biotype SAS1

6.1. Introduction

The final goal of any farmer who has faced the problem of herbicide resistance is to eradicate the resistant population from the farm. Seedbank studies of weeds are tools for proposing models capable of defining the life of a given species in the seedbank. The results of seedbank studies can be useful in the prediction of future weed infestations and in developing strategies for their long term control (Fernandez-Quintanilla, 1988; Lopez Grandos and Garcia Torres, 1993). Of particular interest are any differences between R and S biotypes which might be exploited for control of the R biotype. Seedbank dynamics of R and S biotypes of *Senecio vulgaris,* however, have been shown to be more affected by weed management practices than by differences in germination characteristics between biotypes (Watson *et al.*, 1987).

The initial density of weed seeds in the soil will have a profound effect on the subsequent difficulty encountered in controlling a weed population. In farms with a carefully planned and implemented weed control program, the number of weed seeds in the soil can be quite low. Conversely, weed seed numbers can increase dramatically in the soil when weed control is poor. Maximum broomrape (*Orobanche crenata* Forsk.) seed production for a population of 53 broomrape plants m^{-2} was approximately 4 million seeds m^{-2} (Lopez Garnadas and Garcia Torres,

1993). Thus, poor control due to the development of herbicide resistance may be expected to lead to very high soil seed populations.

Seed longevity in the soil is particularly influenced by the depth of burial (Miller and Nalewaja, 1990). Seed burial will inhibit germination for species where light is required to stimulate germination, such as *Lactuca sativa* (Borthwick, et al., 1954). However, for *Avena sterilis* light actually inhibits germination (Yenish et al., 1992). Through the use of tillage, seeds of *Avena sterilis* are buried in the soil and hence are more likely to germinate (see Yenish et al., 1992).

Other factors affecting seedbank depletion are soil moisture, microbial activity, temperature and relative humidity. In an experiment on the role of soil microorganisms in the life of the seeds of *A. fatua*, Kiewnick (1963, 1964) found that at 20 ^{o}C and 100% relative humidity, the presence of soil microorganisms reduced viability of wild oat seeds by 23 percent in 3 months and 35 percent in 6 months, respectively. He also reported that by inducing secondary dormancy, through increasing relative humidity above 50 percent, the seeds became more susceptible to attack by soil microorganisms.

The literature suggests some general trends in seedbank persistence. Some authorities suggest that grass seeds are generally less persistent in the soil than dicot weed species (Wilson and Lawson, 1992). Another point to be considered is that seedbanks comprise seeds of different ages and sources. For example, in *Xanthium spp.* and *Avena sterilis*, the majority of the first seeds in each spikelet (bigger seeds) germinate in the

first year after seed set (see section 5.4). If these germinants are successfully eradicated in the first year and not allowed to add any new seed to the seedbank, then, the following year's germination will be from 2nd or 3rd seeds of the spikelets. Plants derived from 2nd and 3rd seeds are generally less productive (see sections 5.3.2 and 5.5).

Powles et al.(1992) found that viable seeds of both resistant and susceptible *Hordeum glaucum* seed was exhausted after three years prevention of seed set in the field. However, in similar experiments with *Arctotheca calendula*, viable resistant and susceptible seeds were found in the seedbank even after 6 years of prevention of seed production (E.S. Morgan, E.S. Tucker, and S.B. Powles, unpublished, 1993). Similarly, Alcocer-Ruthling et al. (1992) found that there was no difference in seed viability between sulfonylurea resistant and susceptible *Lactuca serriola* within 3 years when buried at either 7.5 and 15 cm. These results show that eradication of a resistant population can only be possible (if at all) where the seedbank life of the species is short.

Holm et al (1977) reported that seeds of *A. fatua* were found to survive from 4 to 7 years in the soil. Cartledge (1973) reporting on wild oats in Queensland, Australia, recommends " when a pasture phase is used to reduce the wild oats population, the area should remain in pasture for 3 or preferably 4 years. However, on areas that are summer cropped and winter fallowed, the period out of winter crops does not need to be so long because winter cultivations speed up germination of wild oats seed. Two or preferably 3 years of winter crop is generally effective if wild oat plants are not allowed to seed". Martin and Felton (1993) reported from

northern New South Wales, Australia that " cultivated fallow using tines increased wild oat density and reduced grain yield compared with a no tillage fallow". After 4 years they found that the seed reservoir was smaller under a no tillage fallow regime. The relative longevity of resistant and susceptible *Avena* seeds has not been reported.

Control of resistant weed populations may be possible using integrated weed management programs which include burning, cultivation, crop rotation including a pasture phase and the use of herbicides to which the resistant biotype remains susceptible. This will help to deplete the resistant weed seeds from the seed bank.

An experiment was conducted to determine the seedbank dynamics of resistant wild oats population SAS1. This field experiment was performed in the clover paddock near Bordertown, South Australia where the resistant population was first collected (see Chapter 4). The initial distribution of wild oat was surveyed prior to the commencement of these experiments. The initial population of wild oat in the seedbank was measured in mid autumn 1990 after which seed set was prevented. The effect of different cultural practices on the persistence of SAS1 in the seedbank was also examined. The decline of the wild oat population in the seedbank was measured over subsequent years.

6.2. Materials and methods

6.2.1. The experimental site

The experiment was conducted in the actual field near Bordertown, South Australia where resistant population SAS1 was collected in 1989 (section 4.1.1). Subterranean clover was grown in the field during the 1989 season. The experimental area, within the paddock was chosen as the main centre of contamination and had a dense infestation of almost 100% resistant wild oat, SAS1. The soil consists of a 3-7 cm thick, brown, silty, clay loam. Mean annual rainfall in the area is about 600 mm, occurring mainly from April to October (see sections 3.1 and 4.1 for further details of the site). The major weed species occurring in the field are listed in Table 6.1 in descending order of importance for cereal cropping in the area.

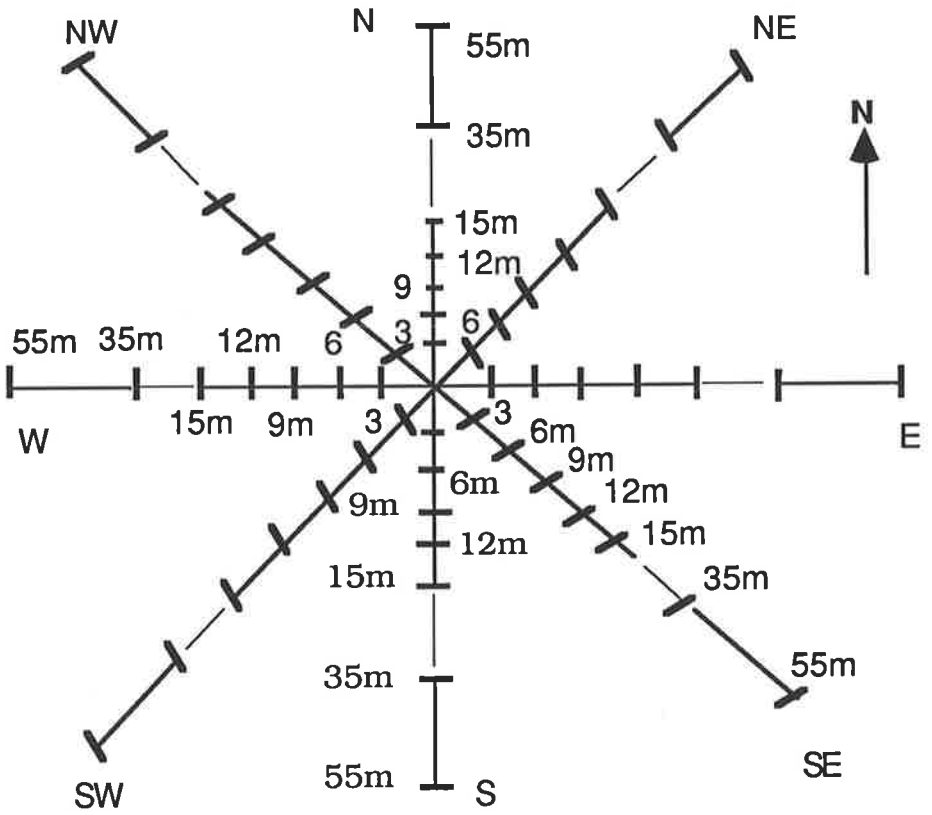
Table 6.1. Weed flora of the experimental farm, in descending order of importance for winter cereal cropping.

scientific name	family
Avena sterilis ssp. *ludoviciana*	poaceae
Lolium rigidum	poaceae
Trifolium subterraneum	fabaceae
Vicia sp.	fabaceae
Juncus sp.	juncaceae
Epilobium sp.	onograceae
Avena fatua	poaceae
Sonchus sp.	asteraceae
Rumex sp.	polygonaceae
Onopordeum sp.	asteraceae

6.2.2. Survey of distribution of resistant wild oat in the experimental field

The seedbank study was initiated by determining the wild oat seeds in the soil in different directions and at different distances from the centre of contamination (Figure 6.1). Soil samples were taken at 5 points up to 55 m from the centre of contamination along 8 transects. Wild oat seeds were extracted from these soil samples (Figure 6.1) (see section 6.2.5.1). Due to the existence of some wild oat patches at greater distances in the NW, N, NE, and N directions, further seeds were collected from standing plants from 55 m to 122 m from the centre of contamination on 15/11/90 in these directions. The seeds from these two samplings were germinated and seedlings were tested with two rates of fluazifop (0.318 and 0.414 kg ha^{-1}) to find the degree and extent of AOPP resistance in various parts of the paddock.

Figure 6.1. Map of soil sampling to determine initial variation in wild oat seed density and susceptibility. Soil samples were taken at five points along each of eight transects originating at the original centre of resistant wild oat contamination (not to scale). The prevailing wind direction during flowering is from the North West.



6.2.3. Persistence of wild oat in the seedbank under different cultural conditions

The survey of wild oat in the field revealed a dense population of fluazifop resistant wild oat in the experimental area (sections 6.2.2 and 6.3.1). Experiments were devised to determine some cultural practices a farmer could employ to most rapidly deplete wild oat seed numbers in this field. Wild oat seed set occurred in the 1989 growing season and in the 1990 growing season there was a dense wild oat infestation. It was anticipated that the very high density of resistant wild oat present in 1990 would add a huge number of new seeds to the seedbank and this would make control extremely difficult so it was determined that, irrespective of subsequent control practices, the area should be cut for hay in the 1990 season to prevent seed set. Therefore, wild oats over the entire experimental site were cut late in the season to prevent seed set in 1990. The effects of various cultural practices on the persistence of wild oat seeds in the growing seasons of 1991, 1992 and 1993 were then determined.

Four treatments were used in these experiments:

1) control undisturbed sites,

2) one spray of diclofop (0.75 kg ha^{-1}), the highest recommended rate, at the beginning of the growing season,

3) three glyphosate sprays at 0.712 kg ha^{-1} at, the beginning, the middle and near the end of the growing season to eradicate any germinated wild oat seedlings and totally prevent any seed set,

4) three cultivations at the same times mentioned above and the total prevention of any seed set (3).

The experiment was conducted using 2 X 15 m plots for each treatment in a complete randomised block design with four replications and results were analysed by analysis of variance. The means were compared using Duncan's Multiple Range test method ($P \leq 0.05$).

Each of the different treatments were designed to provide information on specific factors which may affect the persistence of resistant wild oat in the seedbank. In the undisturbed control plots the persistence of resistant wild oat in the absence of herbicide treatment and cultivation could be observed. Factors affecting wild oat persistence in this situation would be : a) lack of promotion of germination because of lack of seed burial by cultivation, b) competition between wild oat and other weeds present in the field, especially subterranean clover and annual ryegrass, c) effect of stubble mulch on the decay of wild oat seeds on the soil surface, d) allelopathic effects of any weed species on wild oat seedbank status. The effect of continuing to use the same herbicide after resistance has been discovered was examined in diclofop treated plots. The effect of using non-selective herbicides to control wild oats in the absence of tillage was examined in glyphosate treated plots. Finally, the effect of cultivation was investigated. The cultivation treatment contrasts with the other three treatments where no cultivation was used.

Cultivation was conducted using a 7 HP hand operated tiller with a rotary hoe attached. The calendar dates for the various treatments each year, depended on weather conditions and are presented in Table 6.2.

Herbicides were applied using a hand-held boom sprayer at a speed of 1 m s^{-1}. The output of the sprayer was 127 l ha^{-1} at a pressure of 250 KPa. Because of prolonged rainfall late in the 1992 growing season, there was some summer emergence of wild oats after the end of the normal growing season and the last plot treatments for the season. The late germinations were uprooted by hand on 3/2/1993 (Table 6.2).

In May 1993 the stubble of the control and diclofop treated plots were burnt for safety reasons effectively terminating these treatments. However, in 1993 measurements of seedbank reserves in control and diclofop treated plots were made to determine the number of seeds which had remained buried in the soil throughout the period of the experiment following cultivation in 1990. The cultivated and glyphosate treated plots were not burnt in May 1993 since complete weed control prevented the build up of stubble in these treatments. Decline of the seedbank for cultivated and glyphosate treated plots could, therefore, be followed through to the 1993 growing season.

Table 6.2 Time of treatments in field seedbank experiment

Date		Treatment			
		Undisturbed (control)	Diclofop (kg ha^{-1})	Glyphosate (kg ha^{-1})	Cultivated
1990	20/6/90	Entire experimental site cultivated			
	23/10/90	Wild oat cut before seed set over entire site			
1991	31/7/91		0.75	0.712	cultivated
	26/8/91			0.712	cultivated
	12/10/91			0.712	cultivated
1992	3/7/92		0.75	0.712	cultivated
	25/8/92			0.712	cultivated
	30/10/92			0.712	cultivated
1993	3/2/93	Late germinations from 1992 removed by hand weeding			
	28/5/93	Stubble burned		No stubble to be burnt	
	10/6/93		0.75	0.712	cultivated
	29/7/93			0.712	cultivated
	1/10/93			0.712	cultivated

6.2.4. Assessments of seedling emergence per unit area.

Early each growing season, prior to the first operation, (sprays, cultivation and soil sampling) the number of emerged seedlings m^{-2} were counted using a 20 X 50 cm metal quadrat. Five random counts were made for every plot. In the first year of the experiment (1991) the number of

seedlings following each treatment was also measured in a similar manner. Assessment of subsequent emergence later in the season was not possible in control and diclofop treated plots in 1992 due to the high densities of plants from earlier emergence.

6.2.5. Determination of soil seedbank

6.2.5.1. Soil sampling

Soil samples were collected more than once per year. The first sampling was made at the beginning of each season prior to early treatments and the second sampling near the end of the growing season when no new germinations were expected for that year. Samples were collected with a 10.5 cm diameter core sampler to a depth of 9.5 cm. Four randomly selected core samples were taken from each plot at each sampling. The number of seeds included both seeds on the soil surface and seeds incorporated into the soil.

6.2.5.2. Seed extraction from soil samples

Seeds were separated from soil using a set of 2 detachable metal sieves having pore sizes of 2.8 and 0.5 mm respectively. One soil sample was emptied into each of the four 10 L buckets. Water was added to cover the soil and the soil stirred by hand until thoroughly mixed. The slurry was emptied into the 2.8 mm sieve, which was fitted to the top of the 0.5 mm sieve, and washed. Wild oat spikelets were separated from stubble, where present, and the number of single seeds counted. The tiny particles on the

0.5 mm sieve were checked for any possible wild oat seeds passing the 2.8 mm sieve. Seeds of wild oat from the separate core samples were counted and the mean number of seed m^{-2} $plot^{-1}$ was computed.

6.3 Results

6.3.1. Initial seedbank assessment

Wild oat seed number m^{-2} around the centre of contamination was measured as described in section 6.2.2. The average density of seeds over the whole surveyed area on 5/4/90 was 4060 seeds m^{-2}. The seeds were then germinated and tested for fluazifop resistance. Results in Table 6.3 show that after some years of selection pressure on the farm there were still susceptible seeds in the seedbank. There were more susceptible seeds at distances remote from the centre of contamination, especially in the eastern and north eastern parts (directions N, NE and E) (see Figure 6.1 and Table 6.3). However, a large number of susceptibles also occurred at the greatest distances in the western direction. This indicates that there is considerable heterogeneity for herbicide resistance status throughout the paddock.

The existence of further patches of herbicide resistant wild oat to the north of the main centre of contamination was suggested by the pattern of herbicide failure in 1989. Testing of plants grown from seeds collected from standing wild oat plants on 15/11/90 confirmed that almost pure resistant wild oat populations existed at distances up to 120 m in the NW direction (Table 6.4). However, the existence of substantial numbers of

susceptibles in the NE and E directions again emphasises the patchy distribution of this phenomenon.

Table 6.3. Percentage of seedlings from various points in the resistant wild oat infested field which were controlled by application of 0.414 kg ha^{-1} fluazifop. Seedlings were germinated from soil cores collected on 5/4/90 testing from 4 to 20 seedlings for each sample. Average seed density over the entire area was 4060 seed m^{-2}.

direction→ distance m ↓	S	SW	W	NW	N	NE	E	SE	average
3	0	0	0	0	0	25	0	0	3
6	0	20	0	0	0	0	0	0	3
9	0	0	0	10	0	0	0	0	1
12	0	0	0	0	0	10	0	0	1
15	0	0	0	10	0	0	0	0	1
35	0	0	33	0	0	75	0	0	14
55	33	0	100	0	60	66	0	0	32
average	4.7	2.9	19.0	2.9	8.6	25.1	0	0	8

Table 6.4. Percent of seedlings grown from seeds collected from standing plants at various points in the field on 15/11/90 which were killed by application of 0.414 kg of fluazifop ha^{-1}. The number of seedlings tested were at least 20 per sample.

direction* → distance m ↓	NW	N	NE	E	average
45	0	0			
60			20	33	
115		0	20		
120	0	6.6			
average					10.0

* see section 6.2.2 and Figure 6.1.

6.3.2. Seedling emergence per unit area

The changes in the number of seedlings emerging per unit area during the 1991 growing season in response to the four treatments (section 6.2.3) are presented in Table 6.5. The final column in Table 6.5 shows the mean total emergence for the 1991 season. Numbers for glyphosate treated and cultivated plots are summations since, following each counting, all plants were destroyed by the next treatment. For control and diclofop plots the highest number, which here is the first counting, was taken as the total number since competition and other factors reduced numbers later in the season.

Table 6.5. Number of emerged seedlings m^{-2} and its change according to treatments during the 1991 growing season.

treatment	31/7/91	26/8/91	12/10/91	emergence/ year**
control	120.5 a*	86.5 a	93.5 a	120.5
diclofop	104.0 a	79.0 a	93.5 a	104.0
glyphosate	115.0 a	6.5 b	4.0 b	125.5
cultivation	146.5 a	88.0 a	92.5 a	324.0

*in each column values with different letters are significantly different at P=0.05%. Means were compared using Duncan's Multiple Range test method.

**totals for control and diclofop treated plots equal the highest density recorded (31/7/91). Totals for glyphosate and cultivation treatments equal sum of all countings.

Emergence early in the season was similar for all plots (31/7/91 Table 6.5). However, three distinct patterns can be seen for results later in the season. In control and diclofop plots there was a slight decrease in the total number of seedlings due to competition as existing seedlings continued to grow throughout the season. There were no large differences in seedling densities between control and diclofop treated plots at any sampling time confirming that diclofop has little effect on this resistant population. Most seedlings emerging in these plots after the first flush of emergence would have very restricted growth due to competition from established plants. A second pattern of emergence is seen for glyphosate treated plots where, after the first flush of emergence to 31/7/91, there was little late emergence(26/8/91 and 12/10/91, Table 6.5). This led to a

total emergence in glyphosate treated plots which was similar to that for control and diclofop treated plots (Table 6.5). A third pattern of seedling emergence was evident for cultivated plots. Cultivation had a positive effect on emergence of new seedlings. The highest total emergence during 1991 was recorded in plots with cultivation (Table 6.5). It would be expected that the stimulation of germination in the cultivated plots combined with prevention of seed set would exhaust the seedbank more rapidly than for the other treatments.

The decline in seedling numbers for the treatments in the years 1991 to 1993 are represented in Table 6.6.

Table 6.6. Total number of seedlings emerged m^{-2} in the first flush for each season from 1991 to 1993.

treatment	31/7/91	30/10/92	10/6/93
control	120.5 a*	175.5 c	
diclofop	104.0 a	177.5 bc	
glyphosate	115.0 a	133.5 c	0.4 a
cultivation	146.0 a	297.5 a	1.5 a

*in each column values with different letters are significantly different at P= 0.05. Means were compared using Duncan's Multiple Range test method.

As expected the seedling emergence in control and diclofop treated plots did not decrease between 1991 and 1992 due to input of seed in each season (Table 6.6). Even after stubble burning in May 1993, which would

have destroyed most of the seeds on the soil surface, emergence was 79.38 seedlings m^{-2} for control plots and 71.88 m^{-2} for diclofop treated plots. This indicates that as well as the build up of seed on the soil surface there were a large number of viable seeds persisting in the soil of these plots from the time of last cultivation during 1990. It should be noted that stubble burning may have increased germination in these plots due to the removal of the thick stubble mulch. The highest total number for seedling emergence was recorded in plots receiving three cultivations per year in 1991 (Table 6.6). In 1992 the cultivated plots also had the highest number of seedling emergence. By 10/6/93, however, the high number of seedling emergence ceased in the cultivated plots due to depletion of the seedbank (Table 6.6; see section 6.3.3).

The number of seedlings counted during 1991-1993 in glyphosate and cultivated plots indicate that the highest emergence, in the absence of seed set, occurred in plots receiving cultivation. The total emergence in the first flush for cultivated treatments is 442.5 plants m^{-2} while in glyphosate treated plots it was 242.9 plants m^{-2}. The increased emergence in cultivated plots after the first flush (see Table 6.5) would have further increased this difference. It would, therefore, be expected that the seedbank in cultivated plots would have been the most depleted (Figure 6.2). Seed set was also prevented in glyphosate treated plots but as the total emergence over three seasons was less it would be expected that more viable seed remains in these plots than in cultivated plots.

6.3.3. Effects of cultural practices on seedbank dynamics of resistant wild oat

The entire experimental site was cut to prevent seed set in 1990. This pre-treatment reduced seed densities in the soil seedbank from an average of 4060 seeds m^{-2} in 1990 to an average of 1160 seeds m^{-2} at the commencement of the experiment in 1991. This result indicates that a dramatic reduction in seed numbers may be achieved by preventing seed production upon first discovering a high density of herbicide resistant wild oat in the field. The dynamics of resistant wild oat population SAS1 under various cultural conditions, were subsequently determined during the period 1991 to 1993.

Number of seeds m^{-2} at various sampling times from 1990-1993 are presented in Table 6.7 and is further illustrated in Figure 6.2.

Table 6.7. Density of wild oat seed (seeds m^{-2}) in the soil seedbank under different management practices from 1991 to 1993.

treatment	28/4/91	14/4/92	30/10/92	10/6/93	1/10/93
control	1157.1 a	2494.0 a	-----	160.1* a	-----
diclofop	1168.7 a	2448.0 a	-----	312.0* a	-----
glyphosate	1197.7 a	609.0 b	558.2 a	210.0 a	116.0 a
cultivation	1128.1 a	493.0 b	391.5 a	116.0 a	44.0 a

*seed densities in 1993 for control and diclofop treated plots are underestimated due to destruction of soil surface seeds by stubble burning at the end of 1992 growing season. These values are representatives of seed number in seedbank under the soil surface only.

Following treatment of the experimental plots in 1991 the trend in seed number for the various treatments differed widely. The pattern for control and diclofop treated plots was similar while glyphosate treated and cultivated plots exhibited a completely different pattern (see 14/4/92, Table 6.7 and Figure 6.2). In 1992 there was an increase in the seedbank for control and diclofop treatments. This enrichment occurred through seed set during 1991. The stubble was not disturbed and was allowed to stand during summer. On 3/7/92 in control and diclofop treatments there were so many 20-30 cm high clover plants as to prevent successful establishment of wild oat. This would have been expected to reduce the import of seed into the seedbank in 1992 but would also have reduced seed losses due to germination. It was impossible to test these effects in later seasons, however, as the stubble of these plants was burned in April 1993. Stubble burning is mainly responsible for the dramatic drop in seed numbers of control and diclofop treated plots from 2494± 201 to 2448 ±306 in 1992 to 160.1 ±50.92 and 312 ± 168 in 1993 respectively. This loss was mainly due to the destruction of seeds on the soil surface. Seed numbers remaining in the soil seedbank after stubble burning were similar to those in glyphosate treated plots (10/6/93, Table 6.4) since neither diclofop or glyphosate treated plots nor control plots received the cultivation necessary to incorporate large numbers of seed after 1990. This suggests that in the absence of stubble burning a very high burden of buried and soil surface seed would persist in the absence of weed control or if attempted control using diclofop were continued.

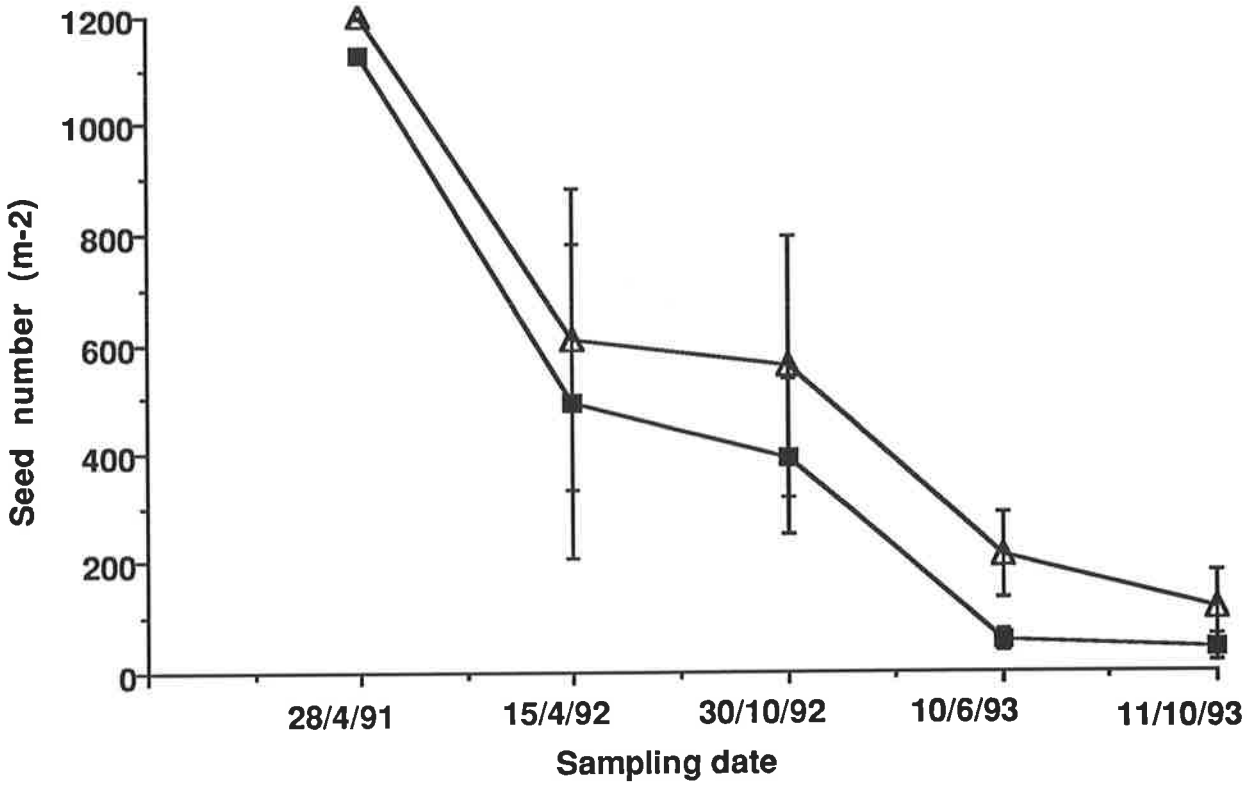


Figure 6.2. Seed number m^{-2} of *Avena sterilis* ssp. *ludoviciana*, biotype SAS1, emerging from the seedbank over four years in plots treated with 3 cultivations per year (■), or without cultivation but with 3 glyphosate applications per year (Δ). Vertical bars represent the standard errors. No fresh seed production occurred on these plots.

When no fresh seed production was allowed (glyphosate and cultivated treatments), a steady decline in seed numbers was observed (Figure 6.2). These results clearly indicate that to eradicate wild oat from the seedbank effective prevention of seed set is essential for a number of years. Total prevention of seed set by either non-selective herbicide usage or repeated cultivation gave similar reductions in seed numbers over a period of 3 seasons (Table 6.7; Figure 6.2).

6.4. Discussion

6.4.1. Susceptible seeds in seedbank

As evident from Tables 6.3 and 6.4 after at least 8 AOPP herbicide treatments between 1981-1989 (Table 3.1), there were still an average of 7.9% susceptible seeds at the centre of the herbicide resistant wild oat t infestation and at least 16.5% susceptible seed in the seedbank at a distances of 55 to 120 m from the centre of contamination in certain directions (Tables 6.3 and 6.4). This indicates a relatively long seedbank life of susceptible wild oats in the soil (Cartledge, 1973; Shilling *et al.*, 1985). Alternatively, it is possible that the persistent susceptible plants, even at the centre of contamination, represent a late germinating herbicide susceptible cohort which has escaped herbicide treatment over 8 years.

6.4.2. Trends in seedbank under different cultural conditions

The large reduction in soil seedbank from 4060 seeds m^{-2} in 1990 to between 1128.1 and 1197.7 seeds m^{-2} in 1991 obtained by prevention of seed set by cutting in 1990 agrees with literature reports (Martin, 1992; Martin and Felton, 1990; Worsham, 1991; Miller and Nalewaja, 1990). In the absence of any weed control measures, the number of wild oat seeds in control plots increased dramatically from 1991 to 1992 (Table 6.7, Figure 6.2). This large wild oat seedbank would have been expected to create a major weed control problem for the farmer in subsequent

seasons. However, there were several factors acting against wild oat even in the absence of weed control measures.

Firstly, the lack of cultivation meant that seed remained on the soil surface vulnerable to predation by ants and to decay which would be hastened by an increase in mulch. The lack of weed control also allowed the build up of *Trifolium subterraneum* and *Lolium rigidum* numbers which provided heavy competition for wild oat early and late in the season, respectively. However, stubble burning in 1992 removed the stubble and seed on the soil surface. This prevented the study of wild oat seedbank dynamics in these treatments in the third and subsequent years.

In diclofop treatments similar trends in seedbank to those observed for untreated plots were evident (Table 6.6 and Figure 6.2). However, because diclofop controlled ryegrass and susceptible wild oats in these plots there was less inter and intra-specific competition with resistant wild oat. The final number of seeds on 10/6/93 was slightly more in the diclofop treated plots than that of control treatment (Table 6.7), although the difference is not statistically different. Further experiments would be needed to determine whether a small but consistent difference exists.

The cultivation treatments destroyed all established seedlings and seeds in the seedbank were brought nearer the surface where higher light, oxygen and moisture levels would be expected, promoting germination. It should be noted that although burial of fresh seed of *Avena* enhances its chance of germination (Borthwick *et al.*, 1954) after a period of deep burial germination can be stimulated by bringing seed closer to the

surface (Yenish *et al.*, 1992). Newly established seedlings were again controlled by the subsequent cultivations in the middle of the season and another group of new seeds were stimulated to germinate. With the third cultivation near the end of the growing season the last emerged seedlings were destroyed, totally preventing seed set. As a result of these intense cultivation treatments the soil seedbank population exhibited a 96% reduction from 1991-1993 from 1128 to 44 seeds m^{-2} (Table 6.7). This 96 % reduction in wild oat seedbank confirms the suggestions of Cartledge (1973) and Martin and Felton (1993). However, in the case of herbicide resistant wild oat even control of 96% of a population may be insufficient. The farmer may have to consider strategies aimed at totally eliminating the population. These will require continued, intensive wild oat control into at least the 4th or 5th season following the detection of resistance.

Although not statistically significant, the seedbank for cultivated treatments was consistently less than that for glyphosate treatments after 1991. This is consistent with the idea that cultivation, by increasing germination, more rapidly depleted the seedbank. Further experiments would be required to determine whether a small but consistent difference does exist.

Thus it is clear that a program of cultivation which includes late cultivation to control late germinations is extremely effective in reducing wild oat numbers. However, the cost of such a strategy and the damage to soil structure make such a strategy impractical. Glyphosate and cultivation treatments showed similar reductions in wild oat seeds from

the seedbank. Thus, glyphosate treatment may be used to reduce wild oat seed numbers without the negative affects of cultivation.

The use of diclofop did not reduce wild oat seed numbers compared to untreated control and in fact may have reduced competition by ryegrass (Tables 6.5, 6.6 and 6.7). It is likely that attempts to rely on diclofop for weed control or a total lack of weed control measures would have resulted in a very high density of wild oat seed persisting on the soil surface and in the soil seedbank for many seasons.

The results of these experiments combined with those in Chapter 5 clearly indicate that it is necessary to have a seed set prevention program in the first year of the rotation in order to deplete the soil of 1st seeds of the spikelets of resistant plants (see Chapter 5). Intensive seed set prevention will need to continue into the 4th and 5th year in order to nearly eradicate the resistant wild oat. Clearly, such a determined effort to reduce resistant wild oat numbers will have considerable cost both in inputs and lost cropping. Careful planning of herbicide use in subsequent cropping rotations will be needed. Inspection of paddocks after herbicide applications should also be used to allow spot control of any escapes to prevent the potential for a repeated build up of a large population of resistant wild oat.

Chapter 7

Inheritance of resistance

7.1. Introduction

When a case of resistance in a weed biotype is detected, investigation of the biological characteristics of this resistance can be valuable. One such biological characteristic is the mode of inheritance of resistance. Knowledge of the mode of inheritance will help in predicting the occurrence of herbicide resistance and the consequences of resistance for field management of resistant populations (Darmency, 1994). The breeding system employed will have a major influence upon the likelihood of transmission of genetic information between individuals. Points to be considered are :

a) In cross-pollinated plants the trait may spread more easily from a foci in the field. The speed and the radius of spread depends upon the degree and ease of pollination, the radius over which effective pollination is possible, and the efficiency of agents mediating pollination such as gravity, wind, or insects.

b) In self-pollinated plants the spread of resistance may be more restricted owing to restricted pollen flow.

c) If the trait is recessive it will have less chance of expression than if it is dominant because only homozygotes will express resistance.

d) If a trait is controlled by a number of recessive or incompletely dominant genes, then it will be necessary for an individual to be homozygous for recessive traits or to possess a number of resistance genes before it can express a high level of resistance. The likelihood of resistance

will be inversely proportional to the number of genes required. For obligate cross-pollinating species it will be unlikely for individuals carrying rare recessive genes to cross with other plants carrying the same allele due to the abundance of susceptible pollen.

e) In the case of maternal inheritance of a resistance trait, a resistant field population will build up very rapidly from a very low frequency of the resistant genotype as resistant individuals can not be rendered susceptible by receiving susceptible pollen, even in an outcrossing species (Scott and Putwain, 1981).

Genes for herbicide resistance may or may not exist in natural weed populations prior to herbicide exposure (Darmency and Gasquez, 1990). If a gene causing herbicide resistance does not already exist in a treated population it is possible that a new mutation may appear because of existing factors in the ecosystem at the time of herbicide use (see 2.2.2.1. and 2.2.2.4.). Usually, resistant plants only become apparent after repeated herbicide use (Table 7.1).

Gressel (1979) stated that the frequency of plants resistant to any given herbicide in a wild population is unpredictable and depends on the number and dominance characteristics of genes for resistance and the ploidy of the weed genome. The frequency of resistant plants in a natural population may be between $1X10^{-6}$ and $1X10^{-10}$ for nuclear inherited resistance (Gressel, 1979), and $1X10^{-10}$ to $1X10^{-20}$ for plastid genome-inherited triazine resistance (Gressel, 1991). Even at such low frequencies, as a result of the selection pressure by a single herbicide or a number of

chemically similar herbicides for a number of years, resistant individuals will be selected and will eventually dominate.

Thus, there are a number of factors affecting the rate of onset of herbicide resistance which can vary greatly between plant species and for different herbicides for a single species. This would lead us to expect a wide variation in the time period of herbicide treatment leading to herbicide resistance. The literature confirms that there is a great deal of variation in the period of herbicide use leading to resistance (Table 7.1).

Table 7.1. The approximate period of herbicide use prior to development of herbicide resistance.

plant species	herbicide	time (years)	reference
Lolium rigidum	AOPP*	3-4	Heap and Knight, 1982
Lactuca serriola	sulfonylurea	6	Mallory-Smith *et al.*, 1990
Kochia scoparia	sulfonylurea	3-7	Primiani *et al.*, 1990
various species	triazines	5-10	Gressel *et al.*, 1982
Erigeron philadelphicus	paraquat	8-11	Itoh, 1985
Hordeum glaucum	paraquat	24	Powles, 1986
Arctotheca calendula	paraquat	24	Powles *et al.*, 1989
Hordeum leporinum	paraquat	12-24	Purba, 1993

*AOPP=aryloxyphenoxypropionate

The mode of inheritance of herbicide resistance has been studied for some herbicide resistant weeds. Most cases of triazine resistance were found to be maternally inherited, while nuclear gene control of herbicide resistance was reported for other cases of triazine resistance and resistance to most other herbicides (Table 7.2). The gene responsible for resistance may be expressed as a dominant, semi dominant or recessive character depending on the function of the modified gene. In a small number of cases the number and dominance characteristics of genes causing herbicide resistance have been reported (Table 7.3). Control of herbicide resistance by a single dominant or semidominant gene is the most commonly reported mode of inheritance. However, cases of herbicide resistance controlled by more than one gene or inherited as a recessive character have also been reported (Table 7.3).

Table 7.2. Inheritance of herbicide resistance in different plant species.

plant species	herbicide	mode of inheritance	reference
Brassica campestris	atrazine	maternal	Souza Machado *et al.*, 1978
Senecio vulgaris	triazines	maternal	Scott and Putwain, 1981
Setaria viridis	triazines	maternal	Darmency and Pernes 1985
Hordeum glaucum	paraquat	nuclear	Islam and Powles, 1988
Hordeum leporinum	paraquat	nuclear	Purba *et al.*, 1993
Arctotheca calendula	paraquat	nuclear	Purba *et al.*, 1993
Abutilon theophrasti	triazine	nuclear	Anderson and Gronwald, 1987
Avena sativa	diclofop	nuclear	Warkentin *et al.*, 1988
Lactuca serriola	sulfonylurea	nuclear	Mallory-Smith *et al.*, 1990
Lolium multiflorum	diclofop	nuclear	Betts *et al.*, 1992
Conyza bonariensis	paraquat	nuclear	Shaaltiel *et al.*, 1988
Erigeron philadelphicus	paraquat	nuclear	Itoh and Miyahara, 1984
Erigeron canadensis	paraquat	nuclear	Yamasue *et al.*, 1992
Lolium perenne	paraquat	nuclear	Faulkner, 1974
Ceratopteris richardii	paraquat	nuclear	Hickok and Schwarz, 1986

Table 7.3. Dominance relationships and number of genes controlling herbicide resistance (dominant = D, semi-dominant = I, recessive = R, Q = quantitative inheritance).

plant species	herbicide	gene No.	dominance	reference
Hordeum glaucum	paraquat	1	I	Islam and Powles, 1988
Hordeum leporinum	paraquat	1	I	Purba *et al.*, 1993
Arctotheca calendula	paraquat	1	I	Purba *et al.*, 1993
Abutilon theophrasti	triazine	1	I	Anderson and Gronwald, 1987
Lactuca serriola	sulfonylurea	1	I	Mallory-Smith *et al.*, 1990
Lolium multiflorum	diclofop	1	I	Betts *et al.*, 1992
Conyza bonariensis	paraquat	1	I	Shaaltiel *et al.*, 1988
Erigeron philadelphicus	paraquat	1	I	Itoh and Miyahara, 1984
Erigeron canadensis	paraquat	1	I or D	Yamasue *et al.*, 1992
Lolium perenne	paraquat	several	Q	Faulkner, 1974
Ceratopteris richardii	paraquat	1	R	Hickok and Schwarz, 1986
Avena sativa	diclofop	2	R	Warkentin *et al.*, 1988

The objectives of the present study were: (1) to investigate the genetic control of AOPP resistance in *Avena sterilis* ssp. *ludoviciana*, (2) to determine whether resistance could be transferred to cultivated oat breeding lines.

7.2. Materials and methods

7.2.1. Plant materials

Seeds of resistant *Avena sterilis* ssp. *ludoviciana* biotype SAS1, were collected from a clover field at Bordertown, South Australia in 1989 (see Table 3.1 for field history). Susceptible cultivated oats, *A. sativa* cv Echidna (classed as susceptible to AOPP herbicides) and cv Mortlock (classed as moderately susceptible to AOPP herbicides) were supplied by the South Australian Department of Agriculture.

7.2.2. Germination of seeds and herbicide application

Seeds were dehulled, pricked and placed on 0.6% agar gel with 0.1% KNO_3. Germination on agar allows easy transfer of seedlings to soil with minimum root damage. They were kept in a cold room (2-4^{o}C) for 12 days and then transferred to the germinator for 5-7 days. Germinated seedlings were transferred to soil and placed either in the glasshouse or in the field. Plants were treated with herbicide when most plants were at the two leaf stage (see Chapter 3).

7.2.3. Method of crossing

In spring 1990, reciprocal crosses were made in glasshouse conditions. For crossing, the bracts (lemma and palea) of flowers on the female parent plant were cut 1/3 from tip, flowers were emasculated and contained with

several male spikelets in a bag in order for pollen to shed from the upper male florets to fertilise the female flowers below.

7.2.4. Breeding strategy

F1 seeds were produced by reciprocal crosses between the resistant parent, SAS1, and susceptible cultivated oats cv Echidna and cv Mortlock. The initial crosses and the treatment of F2 progeny with fluazifop in 1992 were conducted in collaboration with Dr. A. Barr from the South Australian Department of Agriculture.

In the summer of 1990-1991, F2 seeds were obtained using some unsprayed F_1 plants from spring 1990. In 1991, some of the F_1 and F_2 and the parental SAS1, Mortlock, Echidna and SAS2 susceptible were tested with two herbicides. These populations were tested with fluazifop-butyl at 0.550 kg ai ha^{-1} and fenoxaprop-ethyl at 0.3 and 0.6 kg ai ha^{-1} in glasshouse conditions. At the end of these experiments, seeds were harvested from surviving treated plants and control plants. Seeds were harvested from single plants so that the pedigree of individual families could be recorded. F_3 seeds were produced from unsprayed F_2 controls from the 1991 winter studies (see Figure 7.1).

To allow comparison of seed lots under identical environmental conditions, a series of experiments were conducted in 1992 using F_1, F_2 and F_3 seed lots grown in pots during winter 1992. F_1 seeds were the original seeds produced in spring 1990. F_2 seeds came from four sources. Some F_2 seeds

were those produced in summer of 1990-1991 from untreated plants. Other F_2 seeds were taken from untreated controls of 1991 winter F_1 tests. Two other F_2 seed lots were produced from F_1 plants treated with herbicide during the 1991 winter F_1 experiments. These seeds were collected from the F_1 plants which survived 0.3 and 0.6 kg ha^{-1} fenoxaprop treatments. Thus, the following populations were tested using plants grown in pots in the field during the winter of 1992:

-F_1 seeds from crosses made in September-November 1990

-F_2 seeds from unsprayed F_1s in December1990-March 1991

-F_2 seeds from unsprayed F_1s in July-November 1991

-F_2 seeds from F_1s tested with 0.3 kg ha^{-1} fenoxaprop in July-November 1991

-F_2 seeds from F_1s tested with 0.6 kg ha^{-1} fenoxaprop in July-November 1991

-F_3 seeds produced from unsprayed F_2s in July-November 1991

The following populations were also used as controls in all experiments:

-cultivated oats, Mortlock (susceptible parent)

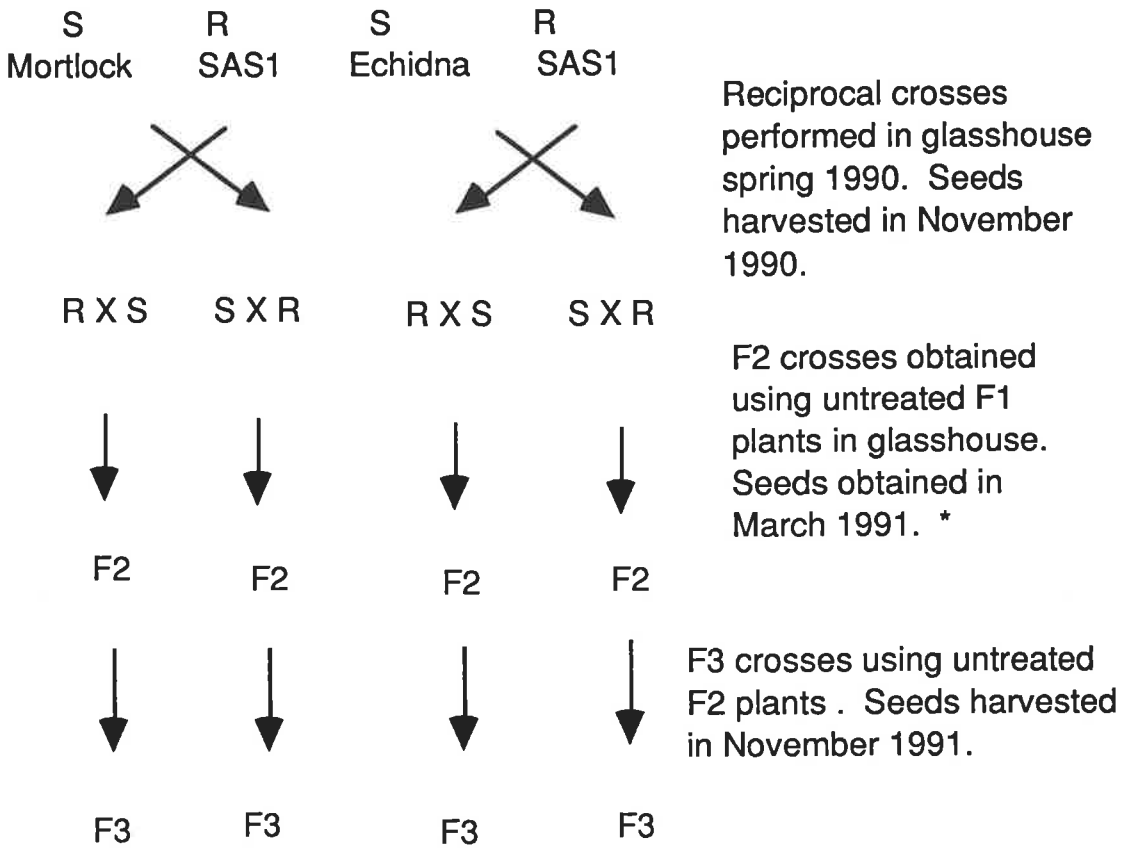
-cultivated oats, Echidna (susceptible parent)

-wild oats SAS1, (resistant parent)

-wild oats SAS2, (susceptible control not used as parent).

Each population was treated with a number of AOPP and CHD herbicides at several rates as described in section 7.3. Where possible, equal numbers of seedlings of each population were tested at each herbicide rate.

Figure 7.1. Origin of seed samples used in breeding experiments.



* Following testing of F1 and F2 seeds in winter 1991 plants were allowed to grow and produce three further seed lots in November 1991:

(a) F2 progeny of untreated F1 controls
(b) F2 progeny of F1 plants surviving 0.3 kg fenoxaprop ha-1
(c) F2 progeny of F1 plants surviving 0.6 kg fenoxaprop ha-1

7.2.5. Testing of progeny

For both glasshouse and field pot trials, assessment of herbicide response were made from 14 days after treatment. Surviving plants were classed as resistant if their response was similar to that of the treated resistant parent SAS1. Treated resistant parent plants or progeny generally exhibited little or no injury or growth reduction in response to herbicide. A second class of live plants were clearly discernible. These plants showed clear symptoms of herbicide injury including leaf tip necrosis, leaf death and severe growth reduction. Such plants were classed as intermediate. A third class of plants was clearly evident. These plants exhibited herbicide response similar to the susceptible parents (*A. sativa* cv Mortlock or Echidna) and were killed by herbicide treatment at the recommended rate and often by herbicide application at lower rates.

The susceptible wild oat population, SAS2, was not used in crossing experiments but was included in progeny tests as a control. SAS2 plants were mostly killed at the lowest rate of herbicides used in each experiment. For clarity of presentation only data for biotypes actually used in crosses is presented in most cases.

7.3. Results

7.3.1. Testing of F_1 and F_2 progeny during winter 1991 in the glasshouse

Plants grown from F_1 seeds and F_2 seeds produced in the glasshouse in summer 1990-1991 were tested in winter 1991 using 0.3 or 0.6 kg ai ha^{-1}

fenoxaprop or 0.55 kg ai ha^{-1} fluazifop. Experiments were conducted in glasshouse conditions with warmer temperatures than outdoors grown plants. Some plants were maintained through to seed production to produce seed for screening in 1992.

The results of glasshouse testing of F_1 and F_2 progenies in winter 1991 are presented in Table 7.4.

Table 7.4. Response to graminicide of parent populations, F_1 and F_2 plants of reciprocal crosses of Mortlock and the R* biotype.

herbicide (rate)	population	response R#	I	S	X^2 (1:2:1)	P (2 d.f.)	X^2 (3:1)	P (1 d.f.)
fenoxaprop 0.3 kg ha^{-1}	Mortlock	0	0	50				
	R parent	50	0	0				
	F_1 Mortlock/R	0	1	1				
	F_1 R/Mortlock	0	3	0				
	F_1 pooled	0	4	1				
	F_2 Mortlock/R	14	24	12	0.24	0.75<P<0.90	0.03	0.75<P<0.90
	F_2 R/Mortlock	16	24	10	1.52	0.25<P<0.50	0.07	0.75<P<0.90
	F_2 pooled	30	48	22	1.44	0.25<P<0.50	0.48	0.75<P<0.90
fenoxaprop 0.6 kg ha-1	Mortlock	0	0	50				
	R parent	50	0	0				
	F_1 Mortlock/R	0	3	0				
	F_1 R/Mortlock	0	2	1				
	F_1 pooled	0	5	1				
	F_2 Mortlock/R	11	24	15	0.72	0.50<P<0.70	0.24	0.50<P<0.70
	F_2 R/Mortlock	15	27	8	2.76	0.20<P<0.30	6.75	0.001<P<0.01
	F_2 pooled	26	51	23	0.21	0.70<P<0.90	0.21	0.50<P<0.70
fluazifop 0.55 kg ha^{-1}	Mortlock	0	0	50				
	R parent	50	0	0				
	F_1				not	determined		
	F_2 Mortlock/R	34	43	36	6.52	0.01<P<0.05	2.83	0.05<P<0.10
	F_2 R/Mortlock				not	determined		

* R plants were from the resistant *A. sterilis* population (SAS1)

R,I and S stand for resistant, intermediate and susceptible response, respectively (see section 7.2.5).

Table 7.5. Response to graminicides of parents, F_1 and F_2 plants of reciprocal crosses of Echidna and the R* parent biotype.

herbicide (rate)	population	response R**	I	S	X^2 (1:2:1)	P (2 d.f.)	X^2 (3:1)	P (1 d.f.)
fenoxaprop	Echidna	0	0	50				
0.3 kg ha^{-1}	R parent	50	0	0				
	F_1 Echidna/R	0	5	1				
	F_1 R/Echidna	0	2	1				
	F_1 pooled	0	7	2				
	F_2 Echidna/R#	10	18	13	1.04	0.50<P<0.70	0.98	0.30<P<0.40
fenoxaprop	Echidna	0	0	50				
0.6 kg ha^{-1}	R parent	50	0	0				
	F_1 Echidna/R	0	0	2				
	F_1 R/Echidna	0	0	5				
	F_1 pooled	0	0	7				
	F_2 Echidna/R	10	15	14	2.89	0.20<P<0.30	2.47	0.10<P<0.20
fluazifop	Echidna	0	0	50				
0.55 kg ha^{-1}	R parent	50	0	0				
	F_2 Echidna/R	33	90	37	2.7	0.20<P<0.30	0.3	0.50<P<0.70

*R parents were from the resistant *A. sterilis* population (SAS1)

**R,I and S stand for resistant, intermediate and susceptible, respectively (see section 7.2.5).

#F_1 plants of SAS1/Echidna did not produce any viable seeds prior to this experiment.

The results for crosses between resistant SAS1 and Mortlock (Table 7.4) are similar to the results for crosses between SAS1 and Echidna (Table 7.5) and suggest that resistance is controlled by a single gene.

Firstly the results for F_1s of crosses between resistant SAS1 and susceptible cultivars Mortlock and Echidna both exhibit an intermediate response between that of the resistant and susceptible parents (Tables 7.4 and 7.5). This indicates that herbicide resistance can be passed from *A. sterilis* to *A. sativa*. The uniform intermediate response of F_1 plants from reciprocal crosses indicates that the character is controlled by the nuclear genome and suggest that it may be inherited as a semidominant character.

In the F_2 populations for both SAS1 by Mortlock and SAS1 by Echidna crosses, 3 phenotypes could be clearly distinguished. These were, resistant, intermediate and susceptible, as described in section 7.2.5. The most appropriate ratio of the three phenotypes for the data was 1:2:1, resistant : intermediate : susceptible for all herbicide treatments (Tables 7.4 and 7.5). Thus, the data from both crosses and for all three herbicide treatments suggest that AOPP resistance in population SAS1 is under the control of a single, semidominant nuclear gene.

7.3.2. Testing of F_1, F_2 and F_3 populations in 1992 using potted plants grown in the field

Progeny of all populations were further tested using plants cultured in pots in the field as described in Section 7.2. As plants were grown outdoors during the normal winter growing season, the cool wet conditions were closer to those experienced under agronomic conditions than the warmer glasshouse conditions of experiments in 1991 (section 7.3.1).

7.3.2.1. Characterisation of pot grown F_1 progeny in the field

Twenty two F_1 plants from the four groups of the reciprocal crosses (R/Mortlock, Mortlock/R, R/Echidna and Echidna/R) and 20 plants of parental R, Mortlock and Echidna controls and susceptible wild oat control SAS2 were tested with 0.083 kg ha^{-1} of fluazifop. The herbicide was applied in two sequential treatments of 0.019 kg ha^{-1} followed by a 0.064 kg ha^{-1} 15 days later. Herbicide was applied using a laboratory spray cabinet as described earlier (see section 3.6.1).

Eight days after the final spraying (DAS), the resistant SAS1 seedlings were alive with no symptoms of herbicide damage. All F_1 seedlings were highly affected, yellow in colour and water soaked in appearance. All susceptible SAS2 plants were dead. Echidna control plants were similar to F_1 plants but Mortlock control plants were completely necrotic and appeared near death. Forty seven DAS, Echidna plants died while a few F_1 plants were completely necrotic but many had already started to recover. Sixty one DAS, some F_1 plants which appeared completely necrotic at 47 DAS started

to regrow from the meristem. Thus, the response of the F_1s for all reciprocal crosses were intermediate between those of the resistant parent, SAS1, and the susceptible parents, Mortlock and Echidna. This confirms the results obtained in previous glasshouse studies (7.3.1) and provides a clear criteria for identifying putative heterozygotes in F_2 and F_3 populations in pot field studies (sections 7.3.2.2 and 7.3.2.3).

7.3.2.2. Testing F_2 plants derived from unsprayed F_1 plants

F_2 seeds were collected from untreated control F_1 plants from the 1991 glasshouse studies (section 7.3.1). In 1992 trials of pot grown plants in the field, 924 plants from these F_2 seeds were used. Two AOPP and two CHD herbicides were used at the following rates:

herbicide	rate, kg ai ha^{-1}
diclofop	0.563, 0.938, 1.875
fluazifop	0.032, 0.064, 0.128, 0,318, 0.636

The response to fluazifop of F_2 plants derived from unsprayed F_1 plants of the previous year are shown in Table 7.6 and 7.7.

Table 7.6. Response of parents and F_2 plants (derived from non-sprayed F_1 parents) to fluazifop at 0.636 kg ha^{-1},16 days after spray in field conditions.

genotype or population	response R*	I	S	X^2 (1:2:1)	P (2 d.f.)	response R+I : S	X^2 (3:1)	P (1 d.f.)
Mortlock	1		7					
Echidna	0		8					
R parent**	7		1					
F2***	33	68	34	0.058	0.70<P<0.90	101 : 34	0.002	0.95<P<0.99

*R, I and S stand for resistant, intermediate and susceptible, respectively.

**R parent = SAS1

***F_2 data are pooled for reciprocal crosses and for Mortlock X SAS1 and Echidna X SAS1 crosses.

Table 7.7. Response of parents and F_2 plants (derived from non-sprayed F_1 plants) to different rates of fluazifop 23 days after spray grown in pots under field conditions.

genotype or population	response R*	I	S	X^2 (1:2:1)	P (2 d.f.)	response R+I : S		X^2 (3:1)	P (1 d.f.)
Mortlock									
0.064 kg ha^{-1}	0		12						
0.128 kg ha-1	0		8						
Echidna									
0.064 kg ha^{-1}	0		12						
0.128 kg ha^{-1}	0		8						
R# parent									
0.064 kg ha^{-1}	8		0						
0.128 kg ha^{-1}	19		1						
F_2**									
0.064 kg ha^{-1}	57	108	45	1.54	0.30<P<0.50	165	45	1.428	0.20<P<0.30
0.128 kg ha^{-1}	29	64	18	4.78	0.05<P<0.10	93	14	8.56	0.01<P<0.05

#R parent = SAS1

*R,I and S stand for resistant, intermediate and susceptible response, respectively.

**F2 data are pooled for reciprocal crosses and for Mortlock X SAS1 and Echidna X SAS1 crosses.

The data in Tables 7.6 and 7.7 for segregation of resistant, intermediate and susceptible phenotypes in the F_2 progeny again best fit the ratio of 1:2:1. This result for F2s produced from glasshouse plants in summer 1991

confirms the previous result obtained from F_2s produced in the glasshouse in summer 1990-1991 and tested in the glasshouse in winter 1991 (Tables 7.4 and 7.5) but in conditions closer to those experienced by wild oat plants in the field (section 7.3.1).

7.3.2.3. 1992 testing of F_2 plants derived from sprayed F_1 plants from 1991

F_2 seeds from F_1 plants surviving herbicide treatment in 1991 were tested to trace the inheritance of herbicide resistance from SAS1. The response of F_2 plants derived from sprayed F_1s was compared to the response of F_2 plants derived from unsprayed F_1s to determine whether the small number of mortalities in F_1 experiments (Tables 7.4 and 7.5) indicate a significant number of susceptibles contaminating the F_1 population or are simply due to variation in the genetic background of each plant. From 0.3 and 0.6 kg ha^{-1} fenoxaprop sprayed F_1 seedlings of 1991, 644 and 118 seeds, respectively, were collected for use in this experiment. The progeny of reciprocal crossed were pooled. Parents, progeny and the susceptible SAS2 wild oat were treated as described in section 7.2.

Two herbicides were tested at two rates each:
diclofop at 0.28 and 3.75 kg ha^{-1}
fluazifop at 0.035 and 2.12 kg ha^{-1}

The lower rates were those at which all susceptible wild oat plants had died in previous experiments (see Chapter 4). The low rate of each herbicide allowed the intermediate and resistant progeny to be

distinguished from each other and from the susceptible progeny (as discussed in section 7.2.5). The higher rates were selected to kill intermediate plants and select resistant ones. Measurements were made 6, 16, 22, 45 and 93 days after treatment. Some of the results of these experiments are presented in Table 7.8.

Table 7.8. Response to diclofop applied at 3.75 kg ha^{-1} of pot grown plants in the field, of parents, and F_2 plants derived from F_1 plants which survived treatment with 0.3 and 0.6 kg ha^{-1} of fenoxaprop.

genotype or population	response R*	I	S	X^2 (1:2:1)	P (2 d.f.)	R+I : S	X^2 (3:1)	P (1 d.f.)
Mortlock	0	0	5					
Echidna	0	0	5					
R** parent	2	0	0					
F_2 from 0.3 kg ha^{-1} fenoxaprop survivors #	3	4	3	0.4	0.70<P<0.90	7	3	0.13	0.70<P<0.90
F_2 from 0.6 kg ha^{-1} fenoxaprop survivors #	6	19	8	0.94	0.50<P<0.70	25	8	0.01	0.90<P<0.95

*R, I and S stand for resistant, intermediate and susceptible response, respectively (as described in section 7.2.5)

**R parents were from resistant wild oat population, SAS1.

.#results for F_2 from Fi survivors of 0.3 kg ha^{-1} fenoxaprop were determined 9 DAS and results for F_2 from 0.6 kg ha^{-1} of fenoxaprop were determined 16 DAS.

The results in Table 7.8 indicate that 3.75 kg ai ha^{-1} diclofop kills the susceptible plants but does not injure the resistant. The data for F_2 plants fit the ratio of 1 resistant : 2 intermediate : 1 susceptible. The pooled data also fit the 3 alive : 1 dead ratio. This was true for both F_2 derived from F_1 survivals of 0.3 kg ha^{-1} and F_2 derived from survivors of 0.6 kg ai. ha^{-1} fenoxaprop.

The fact that F_2 progeny from plants surviving 0.3 and 0.6 kg ha^{-1} fenoxaprop in 1991 fit a ratio of 1:2:1, R:I:S suggests that they are genetically similar to the F_2 progeny produced from unsprayed plants (Tables 7.4 and 7.5 and 7.6). This result is in agreement with the hypothesis that the F_1 are genetically uniform with respect to AOPP resistance.

7.3.2.4. Testing of F_3 progeny winter 1992

From control pots of 1991 F_2 experiments in the glasshouse, F_3 seeds were harvested. Seed was harvested from individual plants separately. The F_2 and F_3 families were derived from individual plants of the following crosses:

-Echidna X SAS1

-Mortlock X SAS1

-SAS1 X Mortlock

F_3 progeny from each F_2 plant were treated with two concentrations of fluazifop (0.062 and 0.318 kg ai ha^{-1}) to deduce the genotype of the F_2 plants. For every family 14 seeds were planted where possible. F_3 seeds

were germinated, planted and sprayed in the same way as described in sections 3.4, 3.5.2 and 3.6.1, respectively. Data from assessments of each family 22 DAS are presented in Table 7.9.

Table 7.9. Response to fluazifop, at 0.318 kg ai. ha^{-1}, of individual families derived from parent populations and F_3 families from crosses of R and S *Avena spp.*. The results of experiments using 0.062 kg ai. ha^{-1} fluazifop were similar (data not shown).

population	response*			X^2	P	X^2#	P
	R	I	S	(1:2:1)	(2 d.f.)	(3:1)	(1 d.f.)
Mortlock	0	0	14				
Echidna	0	0	13				
R parent**	20	0	0				
Mortlock/R	13	19	15	1.89	0.25<P<0.50	1.20	0.25>P>0.50
R/Mortlock	15	18	6	4.38	0.05<P<0.10	1.92	0.10<P<0.25
Echidna/R	8	27	10	1.19	0.50<P<0.75	0.18	0.50<P<0.75
sum of all F_3s	36	64	31	0.45	0.75<P<0.90	0.12	0.50<P<0.75

*numbers of families exhibiting uniform resistant response (R), segregating for resistance (I) or exhibiting uniform susceptible response (S).

#ratio for R+I vs S

**R stand for resistant (SAS1)wild oat.

Homozygous resistant and homozygous susceptible F_2 plants should produce only resistant and susceptible plants, respectively. Only the heterozygous F_2 plants would be expected to show segregation in the F3.

The number of F_3 families showing uniform resistant response, segregating for resistance and showing uniform susceptible response were in the ratio of 1:2:1 (Table 7.9). The segregation of these three groups suggests that resistance to AOPP herbicides in resistant wild oat, SAS1, is controlled by a single semidominant gene.

7.3.3. Discussion

The results for crosses of resistant wild oat SAS1 with susceptible cultivated oats demonstrate that resistance to AOPPs can be transferred from wild oats to cultivated oats Mortlock and Echidna. However, it should be noted that the relative infrequency of outcrossing for this species would reduce the likelihood of this occurring in the field. The successful transfer of resistance from one species to another, has previously been reported. Nuclear inherited triazine resistant may be transferred from *Brassica napus* L. to *B. oleracea* L. (Ayotte *et al.*, 1987). Chiang *et al.*, (1977) also transferred resistance to *Plasmodiophora brassica* from *Brassica napus* to cabbage (*B. oleracea* var. Capitata). Also sulfonylurea resistance from *Lactuca serriola* to *L. sativa* (Mallory-Smith, *et al.*, 1990). These findings highlight the potential for gene transfer between crop and weed species.

The results provide strong evidence that AOPP resistance in SAS1 is under the control of a single, semidominant, nuclear encoded gene. Glasshouse tests using fenoxaprop and fluazifop (Tables 7.4 and 7.5) as well as tests of pot cultivated plants in the field using fluazifop (section 7.3.2.1) all indicate that the response of the F_1 progeny is intermediate between that of the susceptible and resistant parent for crosses with both Mortlock and

Echidna. Reciprocal F_1 crosses exhibited similar herbicide response indicating that the trait is under nuclear control (Tables 7.4 and 7.5). Tests of four different groups of F_2 progeny using pots in the field and in the glasshouse indicate that the ratio of resistant, intermediate and susceptible individuals best fit the 1:2:1 ratio which is expected for a single semidominant gene. segregation of F_2 progeny from F_1 plants surviving 0.3 or 0.6 kg ha^{-1} fenoxaprop also fit this model. This tends to confirm the notion that the F_1s are genetically uniform for the herbicide resistance trait (section 7.3.2.3). The number of F_3 families produced from the F_2 segregating for herbicide resistance is also in agreement with the single, semidominant nuclear gene hypothesis.

The results described in this chapter represent a considerable body of data which all suggest that AOPP herbicide resistance in *A sterilis* biotype SAS1 is controlled by a single, semidominant nuclear encoded gene. Thus, control of AOPP resistance in *A. sterilis* biotype SAS1 is similar to many previously reported cases of herbicide resistance controlled by a single semidominant nuclear encoded gene. Control by a single semidominant gene has been reported for paraquat resistance in *Hordeum* species and *Arctotheca calendula*. Sulfonylurea resistance in *Lactuca serriola* is also controlled by a single semidominant gene (Table 7.3). Resistance to AOPP herbicides controlled by a single semidominant gene has been previously reported in *Lolium multiflorum* (Betts *et al.*, 1992). Single nuclear gene control of triazine resistance has also been reported for *Abutilon theophrasti* (Anderson and Gronwald, 1987) although triazine resistance is more commonly controlled by a chloroplastic gene (Table 7.2)

CHAPTER 8

Other populations

8.1. Introduction

AOPP and CHD herbicides have been widely used for wild oat control throughout the cereal belt of Australia since the introduction of the AOPP herbicide diclofop-methyl in 1982. Following the development of resistance in wild oat population SAS1 in South Australia (Chapter 4) and SAF1 in Western Australia (Boutsalis, 1989; Piper, 1990), the widespread use of these herbicides over such a vast area would suggest that resistance would also have developed in other wild oat populations. Such a trend would be consistent with previously reported cases of resistance in Australia for *Lolium rigidum* (Heap, 1988) and for *Hordeum glaucum* (Tucker, 1989) and in cases of resistance in *Avena* reported from North America (Heap *et al., 1993*, Seefeldt *et al.*, 1993). After the discovery of resistant biotype SAS1 in 1989, wild oat seed samples were collected from around Australia (see 8.2.1). The populations were tested for resistance to the AOPP and CHD herbicides which are most widely used for wild oat control in Australia. These populations are separated by many hundreds of kilometres and any resistance has probably developed independently, as a result of local treatments with herbicides. If resistance to both AOPP and CHD herbicides in wild oat is increasing, it is also possible that the spectrum of resistance may expand to other herbicides groups such as dinitroanilines, thiocarbamates, triazines, bipyridiliums. If newly

discovered resistant biotypes may have identical patterns of resistance this would suggest that the mechanism of resistance is similar between biotypes (Heap, 1988). "It might also suggest that exposure to any one of the herbicides to which the populations were resistant would be selecting for resistance to all the other herbicides" (Heap, 1988). Conversely, differing spectra of herbicide resistance would suggest a multitude of mechanisms as has previously been reported for *L. rigidum* (reviewed in Powles and Matthews, 1992).

One of the populations tested in these experiments is of particular interest because of its relationship with population SAS1. Biotype SAS154 was collected from the same paddock as SAS1 but after the farm was sprayed with tralkoxydim in 1990. Dose response experiments had indicated that SAS1 is highly resistant to AOPP herbicides after exposure to diclofop and fluazifop. Population SAS1 was not resistant to CHD herbicides at recommended rates for wild oat control but exhibited more survivors than susceptible biotype SAS2 at lower rates (Chapter 4). Population SAS154 was tested to determine whether exposure to tralkoxydim in 1990 would lead to CHD resistance.

This chapter reports experiments to examine 59 wild oat populations collected throughout Australia. Resistance status of these populations was compared to the known resistant population SAS1 and a known susceptible SAS2 to determine the extent and spectrum of resistance to herbicides.

8.2. Materials and methods

8.2.1. Plant material

Seeds were collected during 1990, 1991 and 1992 from farms throughout Australia where farmers suspected herbicide resistance. Further samples were sent from different states of Australia, by individuals or Departments of Agriculture, representatives of the chemical companies and seed cleaners. Different seed samples contained *A. sterilis* ssp. *ludoviciana*, *A. fatua* or *A. barbata*. Some of these seeds were almost pure for species but most of them were mixtures of *A. sterilis* ssp. *ludoviciana* and *A. fatua* and one sample contained all three species (biotype NAF5). Seed samples thought to be herbicide susceptible as well as suspected resistant samples were submitted. Some of these samples were accompanied by records of the herbicide history of the paddock while many were not. Some suspected resistant samples were accompanied by information for only the herbicide which did not control the population in the previous year. The available herbicide history for the populations is presented in Appendix 4.

8.2.2. Seedling establishment and spray

Seeds were germinated on agar media and sown in potting soils as described in Chapter 3. Populations were treated with AOPP herbicides and/or CHD herbicides depending on the herbicide history of the paddock. Plants were grown outdoors in pots using 5 plants per pot and

treated in the laboratory spray cabinet as described in Chapter 3. The experimental designs were complete randomised design or completely randomised block design with 3-5 replications per treatment. Plant survival was recorded at appropriate intervals after treatment and usually recording was extended up to flowering.

All populations were screened for resistance to field rates of AOPP and CHD herbicides in preliminary experiments. Populations exhibiting resistance were further tested in wide range dose response experiments. In preliminary screening experiments biotypes were tested with at least two AOPP herbicides and one CHD herbicide. Treatment rates were usually 0.5, 1, 1.5, or 2 times the recommended rate of each herbicide for wild oat control. The AOPP herbicides diclofop-methyl and fluazifop-butyl and the CHD herbicide tralkoxydim were most commonly used. Some biotypes were also tested with the AOPP herbicide fenoxaprop and the CHD herbicide sethoxydim, particularly where the herbicide history reported failure of these herbicides. On the basis of these experiments populations were classified as resistant or susceptible. Biotypes exhibiting at least 20% survival at the lowest recommended rate for wild oat control for any of the herbicides were considered as resistant biotypes. Those biotypes which exhibited resistance to any of the herbicides used in preliminary screening were tested in wide dose range evaluation experiments where sufficient seed was available. The data from wide dose range experiments was used to calculate a resistance index (RI) for each biotype (Appendices 7 - 10).

8.3. Results

8.3.1. Preliminary evaluation of new biotypes

The response of populations in preliminary experiments varied greatly. As might be anticipated, populations with a history of exposure to AOPP and CHD herbicides were more likely to be resistant but there were a number of exceptions. Some susceptible populations with a considerable recorded history of AOPP and CHD exposure were identified while resistant populations with little or no previous exposure has been recorded.

On the basis of preliminary experiments, 22 out of the 59 populations were classified as susceptible (Table 8.1; Appendix 5; Appendix 6). The recorded history of exposure to AOPP and CHD herbicides for these populations are given in Table 8.2. Only 7 of the susceptible populations were known to have had no previous history of AOPP or CHD exposure although it is possible that the two populations which lacked herbicide history may also have had no exposure to this group of herbicides. At least 12 of the 22 susceptible populations are known to have been previously exposed to AOPP and CHD herbicides. Populations SAS90 and SAF30 had been exposed to 5 applications of AOPP and CHD herbicides without developing resistance (Table 8.2).

Most resistant populations had some history of exposure to AOPP or CHD herbicides. Out of the 59 biotypes tested, 37 had more than 20% survival at the lowest recommended rate of at least one of the

herbicides tested (Table 8.1; Appendix 5; Appendix 6). Of the 37 resistant biotypes, 34 showed resistance to diclofop, 5 to fluazifop, 15 to fenoxaprop, 1 to sethoxydim and 5 to tralkoxydim (Table 8.1). Some biotypes with a long history of herbicide use were found to be resistant even though the farmers did not suspect resistance. When resistance was confirmed in biotypes with herbicide history the degree of resistance was usually related to the amount of herbicides used in the paddock. In contrast, biotypes VAF3, VAS1 and WAF11 were found to be resistant despite a complete lack of recorded exposure (Table 8.1).

Most resistant biotypes were resistant to the herbicide to which they had maximum exposure, although resistance may also appear against other members of the two chemical groups. To properly determine whether a biotype is resistant the dose responses to a variety of herbicides are required. Wide range dose response experiments using several herbicides were possible for 13 of the resistant populations where sufficient seed existed.

There were 24 resistant populations for which wide range dose response experiments could not be conducted. However, some of these biotypes are worthy of comment. For example *A. barbata* biotypes SAB3 and SAB4, collected from roadside sites, not only showed resistance to diclofop but exhibited a high percentage of survival against up to 0.244 kg ha^{-1} tralkoxydim which is 2.44 times the lowest recommended rate for wild oat control (Table 8.1, Appendix 6). As these biotypes were collected from the road side and probably had little or no history of selective herbicide exposure, the reason for their apparent high level of

tralkoxydim resistance is not known. These are the only biotypes known to have high levels of resistance to tralkoxydim in South Australia. Further studies of these biotypes are required.

8.3.2. Wide herbicide dose range experiment

Based on preliminary experiments 13 wild oat biotypes, classified resistant to at least one herbicide, were examined using a wider dose range of AOPP and CHD herbicides. Biotypes SAS2 and SAF34 were included as susceptible controls. The results of these experiments are shown in Table 8.3 and Appendices 7-10.

Out of 13 resistant biotypes tested in wide herbicide range experiments twelve biotypes exhibited more than 20% survival at the lowest recommended rate of diclofop. Five of the diclofop resistant populations exhibited >85% survival at the rate of 3 kg ha^{-1} diclofop which is 5.3 times the lowest recommended rate (Table 8.3, Appendix 7). The five highly resistant biotypes had all been exposed to diclofop (Table 8.3). However, biotypes NAF8 and NAF9 had only had one exposure to the herbicide and as few as two exposures to AOPP and CHD herbicides (Table 8.3).

Eight biotypes showed more than 20% of survival at 0.106 kg ha^{-1} fluazifop (Table 8.3, Appendix 8). Only biotype SAS154 exhibited a very high level of resistance to sethoxydim with 100% survival at 16 times the recommended rate (Table 8.3, Appendix 8). Most resistant biotypes had been exposed to fluazifop (Table 8.3).

Five biotypes exhibited resistance to sethoxydim although none of the thirteen biotypes had a recorded history of sethoxydim exposure. Biotype WAF14 exhibited a high degree of resistance with 100% survival at 0.280 kg ha^{-1} of sethoxydim which is 3 times the minimum recommended application rate for wild oat control. This is the first report of high level of sethoxydim resistance in wild oat in Australia.

Biotype WAF14 was the only biotype to exhibit tralkoxydim resistance (Table 8.3, Appendix 9). WAF14 had been previously exposed to tralkoxydim. Although four other tested populations had a recorded history of tralkoxydim exposure none had more than 2 treatments.

Population SAS154 which was derived from population SAS1 but had been exposed to two further tralkoxydim treatments did not exhibit resistance to either of the CHD herbicides tested (Table 8.3).

The pattern of resistance to AOPP and CHD herbicides for these thirteen biotypes varied greatly and did not consistently correlate with the number of exposures to these herbicides. For example, biotype NAS4 had the highest total number of previous AOPP and CHD exposures with 11 exposures to 6 different compounds but was highly resistant to diclofop only. In contrast, biotype NAF7 with only 3 recorded AOPP and CHD exposures was resistant to all four herbicides tested.

8.4. Discussion

The results of preliminary screening of 59 wild oat populations from around Australia confirmed that resistance to AOPP and CHD herbicides has developed in populations throughout the area of the Australian cereal belt. This finding is in agreement with reports of resistance for *Lolium* and *Hordeum* in Australia (Heap, 1986; Tucker, 1989; Purba, 1993) and for *Avena* in North America (Heap *et al.*, 1993).

It was generally true that populations with higher numbers of exposures to AOPP and CHD herbicides were more likely to be resistant. However, several biotypes with up to 4 exposures to these groups of herbicides were found to be susceptible (Table 8.2). Further experiments are required to determine whether biotypes which failed to develop resistance after many herbicide treatments have the potential to develop resistance after further exposure to these herbicides and, if so, how many exposures are required. In contrast, were populations VAF3, VAS1 and WAF11 which exhibited resistance even though they have probably not been exposed to selective herbicides (Tables 8.1, 8.2). The reasons for these differences are yet to be elucidated.

Wide range dose response studies revealed a wide variation in response to any given herbicide and a wide variation in the range of herbicides for which resistance is expressed in the 13 resistant populations investigated. This suggests that the resistance mechanisms vary between biotypes. Resistance mechanisms may include mutant forms of the target enzyme (ACCase) which exhibit varying levels of reduced

sensitivity for the analogues of AOPP and CHD herbicides. This has been reported for resistant wild oat in Australia (Piper, 1990) and North America (Heap, *et al.*, 1993, Seefeldt *et al.*, 1993). A second group of potential mechanisms could be changes in catabolising enzyme systems. Metabolism based sulfonylurea and imidazolinone herbicide resistance in *Lolium rigidum*, for example, does not extend to certain analogues because they are not rapidly metabolised. Increased metabolism of AOPP herbicides is suspected as a mechanism of resistance in wild oats in Australia (C. Maneechote, personal communication). It is also possible that mechanisms of resistance to AOPP and CHD herbicides which have not been previously identified may be found in these resistant populations.

It is clear from these studies that AOPP and CHD resistance in wild oat is widespread in the Australian wheat belt. Past experience suggest that the incidence of resistance will continue to increase. The differences in the level and spectrum of AOPP and CHD herbicide resistance in wild oat biotypes suggests that no universal mechanism prevails.

Table 8.1 Resistance status of wild oat populations tested in preliminary herbicide response experiments using AOPP and CHD herbicides and history of exposure to the herbicide (number of applications). Mortality data for preliminary screening experiments are given in Appendices 2 and 3.
* R = resistant (more than 20% survival at minimum recommended dose for wild oat control), S = susceptible.
** recorded number of exposures to the herbicide
#nr indicates that no records of herbicide exposure are available
For system of biotype coding see section 3..2.

# #	diclofop		fluazifop		fenoxaprop		tralkoxydim		sethoxydim	
Biotype	status	history	status	history	status	history	status	history	status	history
NAS1	R*	5**	R	1	R	1	-	2	-	-
NAS4	R	5	S	1	R	1	-	2	-	-
NAS5	R	-	-	-	S	1	-	-	-	-
NAS6	R	-	-	-	R	2	-	-	-	-
NAS7	R	-	S	-	R	-	-	-	-	-
NAS8	R	-	-	-	S	-	R	1	-	-
NAF3	S	3	S	2	-	-	S	-	R	-
NAF4	R	4	S	2	-	-	-	-	-	-
NAF5	R	-	-	-	S	-	-	-	-	-
NAF6	R	n r #	R	n r	R	n r	-	n r	-	n r
NAF7	R	2	-	1	R	-	R	-	-	-
NAF8	R	1	-	1	R	-	S	-	-	-
NAF9	R	1	-	1	R	-	S	1	-	-
NAF10	R	3	-	1	R	1	S	1	-	-
QAS1	S	n r	-	n r	S	n r	-	n r	-	n r
SAS45	S	n r	S	n r	S	n r	S	n r	S	n r
SAS46	S	3	S		S		S		S	
SAS47	S	-	S	-	S	-	S	1	S	-
SAS63	S	-	S	-	S	-	S	2	S	-
SAS64	R	-	-	-	-	-	-	-	-	-
SAS90	S	1	S	2	S	-	S	1	S	-
SAS91	R	n r	-	n r	S	n r	-	n r	-	n r
SAS92	R	n r	-	n r	R	n r	-	n r	-	n r
SAS93	R	n r	-	n r	S	n r	-	n r	-	n r
SAS147	R	n r	-	n r	S	n r	S	n r	-	n r
SAS148	S	2	S	1	S	-	-	-	-	-
SAS149	S	1	-	-	S	-	S	-	-	-
SAS150	S	2	-	-	S	-	S	-	-	-
SAS151	S	-	-	-	S	-	S	-	-	-
SAS152	S	-	-	-	S	-	S	-	-	-
SAS156	S	-	-	-	-	-	-	-	-	-

Table 8.1. continued

	diclofop		fluazifop		fenoxaprop		tralkoxydim		sethoxydim	
Biotype	status	history	status	history	status	history	status	history	status	history
SAF14	R	3	R	3	R	-	S	1	R	-
SAF15	S	-	S	-	S	-	S	-	S	-
SAF21	S	-	S	1	S	-	S	-	S	-
SAF23	S	n r	-	n r	R	n r	S	n r	-	n r
SAF25	R	-	-	1	S	-	S	1	-	-
SAF26	R	-	-	1	R	-	S	-	-	-
SAF27	R	3	-	-	R	-	-	-	-	-
SAF28	S	1	-	-	S	-	-	-	-	-
SAF29	S	-	-	-	S	-	S	-	-	-
SAF30	S	2	-	-	S	-	S	-	-	-
SAF31	S	1	-	2	S	1	-	-	-	-
SAF32	R	3	-	1	S	-	S	1	-	-
SAF33	R	1	-	-	S	-	S	1	-	-
SAF34	S	-	-	-	-	-	S	-	S	-
SAF35	S	-	-	-	-	-	S	1	-	-
SAF36	R	n r	S	n r	S	n r	S	n r	S	n r
SAB1	S	-	-	-	S	-	S	-	-	-
SAB2	S	-	-	-	S	-	S	-	-	-
SAB3	R	-	-	-	S	-	R	-	-	-
SAB4	R	-	-	-	S	-	R	-	-	-
VAS1	S	-	-	-	R	-	S	-	-	-
VAF1	R	2	-	2	S	-	R	-	-	-
VAF2	R	3	-	2	S	-	S	-	-	-
VAF3	R	-	-	-	R	-	R	-	-	-
WAF11	R	-	-	-	S	-	-	-	-	-
WAF12	R	2	-	-	S	-	-	-	-	-
WAF13	R	1	S	-	S	-	-	-	-	-
WAF14	R	3	R	1	R	-	-	1	-	-

Table 8.2 History of reported exposure to AOPP and CHD herbicides for susceptible biotypes identified in preliminary experiments (number of exposures)(see Appendix 4). Total recorded number of exopsures to AOPP and CHD herbicides is shown in last column.

biotype	diclofop	fluazifop	fenoxa-prop	tralkoxy-dim	sethoxy-dim	other	Total
QAS1	nr**	nr	nr	nr	nr	nr	nr
SAS45	nr	nr	nr	nr	nr	nr	nr
SAS46	3						3
SAS47	__	__	__	1	__		1
SAS63	__	__	__	2	__		2
SAS90	1	2	__	1	__	1	5
SAS148	2	1	__	__	__		3
SAS149	1	__	__	__	__		1
SAS150	2	__	__	__	__		2
SAS151	__	__	__	__	__		0
SAS152	__	__	__	__	__		0
SAS156	__	__	__	__	__		0
SAF15	__	__	__	__	__		0
SAF21	__	1	__	__	__		1
SAF28	1	__	__	__	__	1	2
SAF29	__	__	__	__	__		0
SAF30	2	__	__	__	__		2
SAF31	1	2	1	__	__	1	5
SAF34	__	__	__	__	__		0
SAF35	__	__	__	1	__		1
SAB1	__	__	__	__	__		0
SAB2	__	__	__	__	__		0

**no records available

Table 8.3 Resistance status of wild oat populations tested in wide dose range herbicide response experiments using AOPP and CHD herbicides and history of exposure to the herbicide (number of applications). Mortality data for wide dose range experiments are given in Appendices 7 to 10. Total history (number of applications) of all AOPP and CHD herbicides are recorded in Appendix 4.

##	diclofop		fluazifop		sethoxydim		tralkoxydim		Total
Biotype	status	history	status	history	status	history	status	history	history
NAS4	RR#	5	S	1	S	-	S	2	11
NAS6	R	-	S	-	S	-	S	-	3
NAS8	R	-	S	-	R	-	S	1	1
NAF3	S	3	S	2	R	-	S	-	5
NAF6	R	2	R		R	-	S		
NAF7	RR	2	R	1	R	-	S	-	3
NAF8	RR	1	R	1	S	-	S	-	2
NAF9	RR	1	R	1	S	-	S	1	3
SAF14	RR	3	R	3	S	-	S	1	6
SAF27	R	3	R	-	S	-	S	-	4
SAF36	R		S		S	-	S		
WAF14	R	3	R	1	RR	-	R	1	5
SAS154	RR	3	RR	3	S	-	S	2	8
SAS2	S	-	S	-	S	-	S	-	
SAF34	S	-	S	-	S	-	S	-	

For system of biotype coding see section 3.2.

RR = highly resistant (>85% survival to 5.3 times the lowest recommnded rate of diclofop, 100% survival at 16 times the lowest recommended rate of fluazifop or 100% survial at 23 times the lowest recommended rate of sethoxydim).

R = resistant (more than 20% survival at minimum recommended dose of wild oat control),

S = susceptible

* recorded number of exposures to the herbicide

CHAPTER 9

General conclusion and recommendations

9.1. Conclusions

The results of this study represent as extensive examination of herbicide resistance in *Avena* species in Australia. The first instance of an *Avena* population resistant to very high levels of AOPP herbicides (SAS1) is reported. Prior to these studies only one case of herbicide resistance in *Avena* had been reported and this biotype exhibited resistance to only moderate levels of AOPP herbicides (Piper, 1990; Boutsalis *et al.*, 1990). Since initiation of this study, highly resistant biotypes of *Avena* have been discovered throughout the cereal belt of Western Australia (Chapter 8; Gurjeet Gill, unpublished), Northwestern Canada (Heap *et al.*, 1993) and North America (Seefeldt, *et al.*,1993).

The highly resistant biotype SAS1 has been characterised in detail for its response to herbicides, mode of inheritance of resistance, its relative fitness and its persistence in the seedbank. Studies of other *Avena* populations confirm that herbicide resistance in *Avena* populations is widespread throughout the wheat belt of Australia.

Herbicide response experiments with biotype SAS1 revealed it to be highly resistant to all AOPP herbicides tested including diclofop, fluazifop, fenoxaprop, quizalofop, haloxyfop, paraquizafop and quinfurop. New AOPP herbicides quinfurop and paraquizafop were

more effective on SAS1 than older ones like diclofop, fluazifop, haloxyfop and fenoxaprop. Quizalofop had the lowest LD_{50} among all AOPPs to which SAS1 showed to be resistant. The reason for this difference in response to AOPP analogues is yet to be determined. Cyclohexanediones controlled SAS1 at the recommended rates but at lower rates of these herbicides SAS1 exhibited less mortality than the susceptible biotype SAS2 (see Chapter 4).

As no resistance was detected in SAS1 to cyclohexanedione herbicides the farmer applied tralkoxydim for two years after AOPP herbicides had been applied. After 2 years of selection pressure by tralkoxydim a slight increase appeared in R_1 of the population. This suggests that when resistance has occurred in a biotype to a herbicide which targets a specific target enzyme, there is a greater possibility for resistance to develop to other herbicides targeting that enzyme.

Pot and field competition experiments using resistant wild oat, SAS1, provide the first reports of the relative fitness of herbicide resistant wild oat. The results of these studies reveal that there are no significant differences in productivity or competitive ability between SAS1 and the susceptible biotype SAS2 in terms of time to first flowering, stem number, aboveground dry weight and seed yield (Chapter 5). Productivity experiments confirmed that the first seeds of spikelets of biotype SAS1 are more fit than second seeds as are those of the susceptible biotype. As there is no discernible fitness differential it is, therefore, suggested that maximum emphasis should be placed on controlling resistant wild oat in the first year after seed set where most

of the nondormant first seeds germinate (Chapter 5). Further studies of the competition between resistant wild oat and crops such as various wheat cultivars, barley, broadbean and canola will also be required to allow better strategies for management of wild oat to be devised.

Seedbank studies revealed that after the farmer reported the incidence of resistance in 1989 there were still 7.9% susceptible seeds in the central patch of contamination (Table 6.3). These susceptible seeds however are unlikely to be useful in management of resistance due to the lack of any superiority in productivity or competitive ability relative to the resistant biotype (see chapter 5).

Cultural practices after the detection of resistance were found to have profoundly different effects on the persistence of wild oat, SAS1, in the seedbank. Three glyphosate treatments or three cultivations per season were used to remove emerged seedlings. Cultivated treatments promoted germination from the seedbank which were destroyed by further cultivation. However, glyphosate had a similar effect in reducing the soil seedbank with the difference that glyphosate application is faster, cheaper and involves no soil structure damage. A phase involving medic or clover could be used in an Integrated Weed Management (IWM) program to control resistant wild oat. Cutting the crop for forage, or heavy grazing followed by a spray of a non selective herbicide late in the season will have the effect of preventing seed set and thereby depleting the soil seedbank.

Breeding experiments reported in Chapter 7 establish that herbicide resistance in SAS1 is controlled by a single partially dominant nuclear encoded gene and can be transferred from resistant wild oat to cultivated oats. Recent, as yet unpublished experiments show that resistance in SAS1 is due to a herbicide resistant form of target enzyme ACCase (C. Maneechote, unpublished). The evidence in Chapter 7 suggests that the single gene is most likely that encoding the ACCase protein.

Fifty nine other populations than SAS1 and SAS2 were studied from wheat belt regions of Australia. Many of these biotypes (37) were found to be resistant to at least one AOPP or CHD herbicide. Diclofop resistance usually appears after 3-5 years of the herbicide application either in rotation or continuously (Table 7.1, Table 8.3). The rate at which resistance appears is related to chemical group even in different weed species. For example resistance to diclofop-methyl in *Lolium rigidum* occurred within 3-4 years (Table 7.1). Similarly it appeared within 3-4 years of diclofop use in *A. sterilis* and *A. fatua* (Tables 3.1 and 8.3).

9.2. Recommendations

The herbicide history of farms having herbicide resistant wild oats suggest that the resistant populations are often present well before they are reported by the farmer. This appears to have been so in the case of population SAS1 (see Table 3.1). It is essential that farmers be aware of the potential for herbicide resistance to develop in wild oats so that they may detect the problem before a large seedbank of highly resistant

plants can develop. The results of these studies in combination with those from North America (Heap *et al.*, 1993) clearly indicate that the number of instances of herbicide resistance in wild oats in Australia can be expected to increase in coming years.

Secondly, farmers need to be made aware of how to deal with herbicide resistant populations of wild oat when they develop. In the past, farmers may have reacted to a case of herbicide failure by repeating the application using a higher rate. This strategy may lead to the selection of plants resistant to higher levels of application, compounding the problem. Farmers need to be made aware of which chemicals can be used to control AOPP or CHD resistant wild oat populations. Recent surveys indicate that many farmers are not aware of the mode of action of various herbicides. Therefore, awareness of this must be increased.

It should be stressed to farmers that paddocks should be checked after AOPP or CHD herbicide applications for small patches of escapees. Escapees may be controlled by spot applications of non-selective herbicides avoiding the potential for large populations of resistant wild oat to develop.

The advent of herbicide resistance in wild oats adds a new factor to be considered when designing management strategies. Both new and old agronomic techniques must be integrated to provide maximum yield but also reducing the likelihood of resistance developing in wild oats. Techniques such as variations in the use of herbicides, crops to be used and the pattern of rotation will have to be considered by each farmer in

the light of this new potential problem. Farmers are already used to dealing with the potential problem presented by fire, for example. The potential problem of herbicide resistance in wild oat is simply one more factor to be considered.

It is recommended that farmers, government authorities and advisers consider taking the following actions:

-Train farm advisers in methods of combating herbicide resistance.

-Employ active resistance specialists in Departments of Agriculture

-Increase scientific contact between farm advisers and research centres which may mainly be concentrated in universities.

-Present informative programs in the news media especially targeted for farmers.

-During the course of these studies it has become apparent that adequate controls to prevent the spread of herbicide resistant wild oat seed do not exist. Therefore it is suggested that seeds which are going to be sold as cropping seeds (even if 2 or 3 year old seeds) should be tested for the presence of herbicide resistant wild oat before they may be approved for sale.

9.3. Further studies

If final seedbank life of wild oats could be determined through a long term study for each region, eradicatory field management programs may be devised. Meanwhile, the crops which are most competative with *Avena* species should be determined.

Some biotypes are resistant to one or few AOPP herbicides whereas others resist both AOPP and CHD herbicides. Laboratory experiments are necessary to determine the mechanism(s) of resistance to herbicides to allow for herbicide rotation programs to be devised.

Rotation of both crops and herbicides are necessary. An economic crop rotation which could prevent wild oat seed set would not only help economic production but would also help scientists to study long term farm management for wild oat seedbank depletion. Similarly, a herbicide rotation program combined with cropping rotation may indicate whether herbicides will contain resistance or will lead to new and more complicated case of resistance.

Finally, and most importantly, agronomic studies are necessary to find the best IWM systems combining all of the management tools available.

Appendix 1: The composition of the soil (University of California mix) used in pot experiments

University of California mix (U.C.):

2/3 m^3 of washed coarse sand is sterilised at 100°C for 1/2 hour in a sterilising mixer. One bale of peatmoss (1/6 m^3 which expands to 1/3 m^3) is added and mixed for 10 s. The combined temperature drops to about 75 °C.

After about 10 min (more cooling) the following fertilisers were added and mixed with the sand and peat for 20 s. pH was about 6.5.

Fertilisers

Calcium hydroxide	700 g.
Calcium carbonate	480 g.
Nitrophoska (15-4-12)	600 g.

Nitrophoska analysis

15%	**N**	Total nitrogen. 5% NH4 ammonium form. 4% NO3 nitrate form. 1% NH2 amide form. 5% IBDU.
3.9%	**P**	Total Phosphorus. 2.7% Citrate soluble. 1.2% Water soluble.
12.4%	**K**	Potassium sulphate.
1.25%	**Mg**	Magnesium carbonate.
3.4%	**Ca**	Dicalcium phosphate.
5.3%	**S**	Sulphates.
0.3%	**Fe**	Iron oxide.
0.0002%	**Cu**	Copper oxides.
0.01%	**Zn**	Zinc oxide.
0.01%	**B**	Calcium borate.
0.0003%	**Mo**	Molybdenum oxide.

Appendix 2: The composition of the recycled and John Innes soil used in pot experiments

1) Recycle soil:

1/2 m^3 of finished with and composed experimental soils (U. C.; J. I.; + R. S.) and plants therein (composed for over 2 y) in steam sterilised at 100 °C for 45 min and cooled after which 1/10 m^3 of Eurotorf peatmoss and the following nutrients are mixed in.

Blood meal	500 g.	Agricultural lime	200 g.
Potassium sulphate	200 g .	Super phosphate	100 g.

The mix is sieved through a 1 cm. grid size sieve. The pH was about 6.5.

2) John Innes soil:

5 parts by volume of coarse sand is mixed with 5 parts by volume of a medium loam and steam sterilised at 100 °C for 45 min and cooled quickly after 4 parts by volume of Eurotorf peatmoss and the following nutrients are mixed in with each 1/2 m^3 of mix.

Blood meal	600 g	Superphosphate	550 g
Potassium sulphate	300 g	Agricultural lime	200 g

The mix is sieved through a 1cm. grid sized sieve. The pH was about 6.5.

Appendix 3. Amount of rainfall (mm) during different years of experiments at the Waite Institute.

Month	1990	1991	1992	1993
January	22.4	15.4	1.4	31.4
February	13.6	0.0	14.0	40.0
March	1.2	5.0	89.2	18.8
April	17.0	42.6	44.4	1.4
May	18.4	12.8	95.2	49.4
June	99.2	141.8	84.8	44.8
July	110.0	103.0	72.4	74.8
August	98.8	88.0	162.6	59.8
September	50.0	90.0	141.4	75.2
October	45.6	76.0	90.2	77.6
November	16.4	35.2	94.4	23.0
December	50.4	5.6	88.2	
Total	543	615.4	978.2	

Appendix 4. Reported history of selective herbicide control for biotypes of wild oats discussed in Chapter 8. Total number of years for which herbicide history was reported are presented in the last column. Abbreviations used are as follows : dicl = diclofop; flua =fluazifop; feno = fenoxaprop = quiz = quizalofop; halo = haloxyfop; flam = flamprop; seth = sethoxydim; tral = tralkoxydim;sima = simazine; trif = trifluralin; tria = triallate.

*nt indidcates that the total number of years of selective herbicide control was not reported.

#nr indicates that there was no record (herbicide history is not known).

biotype	dicl	flua	feno	quiz	halo	flam	seth	tral	sima	trif	tria	year
NAS1	5	1	1	-	1	1	-	2	-	2	-	10
NAS4	5	1	1	-	1	1	-	2	-	2	-	10
NAS5	-	-	1	-	-	-	-	-	-	-	-	nt*
NAS6	-	-	2	-	-	1	-	-	-	-	-	nt
NAS7	-	-	1	-	-	-	-	-	-	-	-	nt
NAS8	-	-	-	-	-	-	-	1	-	-	-	nt
NAF3	3	2	-	-	-	-	-	-	2	-	-	nt
NAF4	4	2	-	-	-	-	-	-	2	-	-	5
NAF5	-	-	-	-	-	-	-	-	-	-	-	0
NAF6												nr#
NAF7	2	1	-	-	-	-	-	-	-	-	-	3
NAF8	1	1	-	-	-	-	-	-	-	-	-	3
NAF9	1	1	-	-	-	-	-	1	-	-	-	3
NAF10	3	1	1	-	-	1	-	1	-	1	-	5
QAS1												nr
SAS 45												nr

Appendix 4. continued

biotype	dicl	flua	feno	quiz	halo	flam	seth	tral	sima	trif	tria	year
SAS46	3											nt
SAS47	-	-	-	-	-	-	-	1	-	-	-	1
SAS63	-	-	-	-	-	-	-	2	-	-	-	1
SAS64	-	-	-	-	-	-	-	-	-	-	-	0
SAS90	1	2	-	1	-	-	-	1	-	-	-	0
SAS91												nr
SAS92												nr
SAS93												nr
SAS147												nr
SAS148	2	1	-	-	-	-	-	-	-	3	-	5
SAS149	1	-	-	-	-	-	-	-	-	2	-	5
SAS150	2	-	-	-	-	-	-	-	-	2	-	5
SAS151	-	-	-	-	-	-	-	-	-	-	-	0
SAS152	-	-	-	-	-	-	-	-	-	-	-	0
SAS156	-	-	-	-	-	-	-	-	-	-	-	0
SAF14	3	3	-	-	-	-	-	1	1	-	-	6
SAF15												0
SAF21	-	1	-	-	-	-	-	-	-	-	-	1
SAF23												nr
SAF25	-	1	-	2	-	-	-	1	-	3	1	8
SAF26	-	1	-	-	-	-	-	-	-	-	-	1
SAF27	3	-	-	1	-	-	-	-	-	-	-	5
SAF28	1	-	-	-	1	-	-	-	-	-	-	nt
SAF29	-	-	-	-	-	-	-	-	-	2	-	2
SAF30	2	-	-	-	-	-	-	-	-	6	-	8
SAF31	1	2	1	1	-	-	-	-	2	-	-	7

Appendix 4. continued

biotype	dicl	flua	feno	quiz	halo	flam	seth	tral	sima	trif	tria	year
SAF32	3	1	-	1	-	-	-	1	-	-	-	7
SAF33	1	-	-	-	-	-	-	1	1	-	-	3
SAF34	-	-	-	-	-	-	-	-	-	-	-	0
SAF35	-	-	-	-	-	-	-	1	-	-	-	1
SAF36												
SAB1												0
SAB2												0
SAB3												0
SAB4												0
VAS1	-	-	-	-	-	-	-	-	-	-	-	0
VAF1	2	2	-	-	-	-	-	-	-	-	-	4
VAF2	3	2	-	-	-	-	-	-	-	-	-	5
VAF3	-	-	-	-	-	-	-	-	-	-	-	0
WAF11	-	-	-	-	-	-	-	-	-	-	-	0
WAF12	2	-	-	-	-	-	-	-	-	-	1	4
WAF13	1	-	-	-	1	-	-	-	-	-	-	4
WAF14	3	1	-	-	-	-	-	1	3	-	-	10

Appendix 5. Percentage survival of different biotypes of wild oats against aryloxyphenoxypropionate herbicides in preliminary experiments.

biotype	diclofop kg ha^{-1}					fluazifop kg ha^{-1}				fenoxaprop kg ha^{-1}			
	0.188	0.375	0.469	0.750	1.125	0.085	0.106	0.159	0.212	0.060	0.090	0.120	0.180
NAS 1	100	---	100	100	---	100	---	---	---	100	---	---	100
NAS 4	100	100	100	100	---	100	---	---	---	100	---	---	100
NAS 5	---	---	35	10	10	---	---	---	---	---	0	---	0
NAS 6	---	---	26	36	35	---	---	---	---	---	55	---	28
NAS 7	100	60	35	8	---	0	---	---	---	14	---	---	20
NAS 8	---	---	85	85	90	---	---	---	---	---	---	5	0
NAF 3	15	15	7	---	---	0	---	---	---	---	---	---	---
NAF 4	35	25	20	---	---	0	---	---	---	---	---	---	---
NAF 5	---	---	22	15	0	---	---	---	---	---	0	---	0
NAF 6	100	100	100	100	---	100	---	---	---	100	---	---	100
NAF 7	---	---	100	100	100	---	---	---	---	100	---	---	100
NAF 8	---	---	100	100	100	---	---	---	---	100	---	---	100
NAF 9	---	---	100	100	100	---	---	---	---	100	---	---	100
NAF 10	---	---	---	100	---	---	---	---	---	---	---	50	---
QAS 1	---	---	14	6	7	---	---	---	---	---	0	0	0

Appendix 5. continued

biotype	diclofop kg ha^{-1}					fluazifop kg ha^{-1}				fenoxaprop kg ha^{-1}			
	0.188	0.375	0.469	0.750	1.125	0.085	0.106	0.159	0.212	0.060	0.090	0.120	0.180
SAS 45	___	___	0	___	___	___	___	0	0	___	___	0	0
SAS 46	___	___	0	___	___	___	___	0	0	___	___	0	0
SAS 47	___	___	0	___	___	___	___	0	0	___	___	0	0
SAS 63	___	___	0	___4	___	___	___	0	0	___	___	0	0
SAS 64	35	15	20	4	3	___	___	___	___	___	___	___	___
SAS 90	___	___	0	0	___	___	___	0	0	___	___	0	0
SAS 91	___	___	35	20	4	___	___	___	___	___	0	___	0
SAS 92	___	___	58	55	25	___	___	___	___	___	25	___	12
SAS 93	___	___	20	38	12	___	___	___	___	___	0	___	0
SAS 147	___	___	50	0	0	___	___	___	___	___	___	0	0
SAS 148	50	0	0	0	___	0	___	___	___	0	___	___	0
SAS 149	___	___	10	0	0	___	___	___	___	___	___	0	0
SAS 150	___	___	0	0	0	___	___	___	___	___	___	0	0
SAS 151	___	___	0	0	0	___	___	___	___	___	___	0	0
SAS 152	___	___	0	0	0	___	___	___	___	___	___	0	0
SAS 156	___	5	0	0	___	___	___	___	___	___	___	___	___
SAF 14	___	___	100	___	___	___	___	0	0	___	___	95	90
SAF 15	___	___	0	___	___	___	___	0	0	___	___	0	0
SAF 21	___	___	0	___	___	___	___	0	0	___	___	0	0
SAF 23	___	___	0	0	0	___	___	___	___	___	___	20	10
SAF 25	___	___	20	30	0	___	___	___	___	___	___	0	0
SAF 26	___	___	36	54	16	___	___	___	___	___	___	40	0

Appendix 5. continued

biotype	diclofop kg ha^{-1}					fluazifop kg ha^{-1}				fenoxaprop kg ha^{-1}			
	0.188	0.375	0.469	0.750	1.125	0.085	0.106	0.159	0.212	0.060	0.090	0.120	0.180
SAF 27	100	100	100	100	100	100	___	___	___	___	___		100
SAF 28	___	100	0	0	0	___	___	___	___	___	___	100	0
SAF 29	___	___	0	0	0	___	___	___	___	___	___	0	0
SAF 30	___	___	5	5	0	___	___	___	___	___	___	0	0
SAF 31	___	___	0	0	0	___	___	___	___	___	___	0	0
SAF 32	___	___	30	30	0	___	___	___	___	___	___	0	0
SAF 33	___	___	53	27	0	___	___	___	___	___	___	0	0
SAF 34	___	___	0	0	0	___	___	___	___	___	___	0	0
SAF 35	___	0	0	0	0	___	___	___	___	___	___	0	___
SAF 36	___	0	90	___	___	___	___	___	___	___	___	___	___
		___							0	___	___	___	0
SAB 1	___		15	10	0							___	
SAB 2	___	___	5	0	0	___	___	___	___	___	___		0
SAB 3	___	___	33	13	7	___	___	___	___	___	___	0	0
SAB 4	___	___	47	53	13	___	___	___	___	___	___	0	0
		___										0	0
VAS 1	___	___	13	0	0							0	
						___	___	___	___	___	___	50	10
VAF 1	___	___	87	67	67								
VAF 2	___	___	20	20	10	___	___	___	___	___	___	0	0
VAF 3	___	___	60	80	0	___	___	___	___	___	___	0	0
		___				___	___	___	___	___	___	20	0
WAF 11	___	___	80		33	16							
WAF 12	___	___	65		50	40	___	___	___	___	___	___	13
WAF 13	90	25	10		0	___	0	___	___	___	___	___	0
WAF 14	100	100	100		100	___	100	___	___	___	0	___	___
											100	___	___

Appendix 6. Survival (%) of different wild oat biotypes treated with sethoxydim and tralkoxydim on different biotypes in preliminary experiments. The lowest recommended rates for wild oat control, in this table, were considered as 0.093 and 0.095 kg ha^{-1} for sethoxydim and tralkoxydim, respectivily.

biotype	sethoxydim kg ha^{-1}				tralkoxydim kg ha^{-1}		
	0.047	0.093	0.140	0.187	0.075	0.095	0.115
NAS 1	---	---	---	---	---	---	---
NAS 4	---	---	---	---	---	---	---
NAS 5	---	---	---	---	---	---	---
NAS 6	---	---	---	---	---	---	---
NAS 7	---	---	---	---	---	---	---
NAS 8	---	---	---	---	85	30	10
NAF 3	95	30	5	0	0	0	0
NAF 4	---	---	---	---	---	---	---
NAF 5	---	---	---	---	---	---	---
NAF 6	---	---	---	---	---	---	---
NAF 7	---	---	---	---	30	---	0
NAF 8	---	---	---	---	10	---	0
NAF 9	---	---	---	---	40	---	0
NAF 10	---	---	---	---	---	---	0
QAS 1	---	---	---	---	---	---	---
SAS 45	---	---	0	0	---	---	0
SAS 46	---	---	0	0	---	---	0
SAS 47	---	---	0	0	---	---	0
SAS 63	---	---	0	0	---	---	0
SAS 64	---	---	---	---	---	---	---
SAS 90	---	---	0	0	---	---	0
SAS 91	---	---	---	---	---	---	---
SAS 92	---	---	---	---	---	---	---
SAS 93	---	---	---	---	---	---	---
SAS 147	---	---	---	---	0	---	0
SAS 148	---	---	---	---	---	---	---
SAS 149	---	---	---	---	0	---	0
SAS 150	---	---	---	---	0	---	0
SAS 151	---	---	---	---	0	---	0
SAS 152	---	---	---	---	0	---	0
SAS 156	---	---	---	---	---	---	---
SAF 14	---	---	0	0	---	---	0
SAF 15	---	---	0	0	---	---	0
SAF 21	---	---	0	0	---	---	0
SAF 23	---	---	---	---	10	0	0
SAF 25	---	---	---	---	10	---	0

Appendix 6. continued

biotype	sethoxydim kg ha^{-1}				tralkoxydim kg ha^{-1}		
	0.047	0.093	0.140	0.187	0.075	0.095	0.115
SAF 26	---	---	---	---	50	10	10
SAF 27	---	---	---	---	---	---	---
SAF 28	---	---	---	---	---	---	---
SAF 29	---	---	---	---	0	---	0
SAF 30	---	---	---	---	0	---	0
SAF 31	---	---	---	---	---	---	---
SAF 32	---	---	---	---	0	---	0
SAF 33	---	---	---	---	30	10	10
SAF 34	0	0	0	0	20	0	0
SAF 35	---	---	---	---	0	0	0
SAF 36	---	---	---	0	---	---	0
SAB 1	---	---	---	---	0	---	0
SAB 2	---	---	---	---	0	---	0
SAB 3	---	---	---	---	80	93	27
SAB 4	---	---	---	---	27	20	20
VAS 1	---	---	---	---	40	0	0
VAF 1	---	---	---	---	40	20	0
VAF 2	---	---	---	---	20	10	0
VAF 3	---	---	---	---	20	20	20
WAF 11	---	---	---	---	---	---	---
WAF 12	---	---	---	---	---	---	---
WAF 13	---	---	---	---	---	---	---
WAF 14	---	---	---	---	---	---	---

Appendix 7. Survival (%) and diclofop resistance status of selected biotypes in broad dose range experiments using diclofop-methyl at different rates (kg ha^{-1}). The minimum recommended rate for wild oat control is 0.562 kg ha^{-1} diclofop. Diclofop history (applications) of each biotype is presented in the last column.

biotype	0.188	0.375	0.750	1.125	1.500	2.250	3.000	3.750	4.500	6.000	status	history
NAS 4	100	100	100	100	100	___	100	___	___	100	RR#	5
NAS 6	90	90	45	75	75	___	25	___	___	15	R	0
NAS 8	100	80	60	__	36	3	6	10	___	___	R	0
NAF 3	15	15	7	___	7	0	0	0	___	___	S	3
NAF 6	92	60	80	48	32	___	___	__	___	___	R	2
NAF 7	100	100	100	___	100	100	85	92	___	___	RR	2
NAF 8	100	93	93	___	93	93	93	93	___	___	RR	1
NAF 9	85	93	100	___	100	90	100	100	___	___	RR	1
SAF 14	100	100	100	___	100	___	100	___	100	___	RR	3
SAF 27	90	80	75	___	70	65	0	0	___	___	R	3
SAF 36	100	100	72	___	40	0	0	0	___	___	R	nr**
WAF 14	100	95	97	___	50	16	10	5	___	___	R	3
SAS 154	100	100	100	___	100	95	100	100	___	100	RR	3
SAS 2	18	0	0	___	0	0	0	0	___	___	S	0
SAS 34	___	0	0	___	0	0	0	0	___	___	S	0

#RR = >85% survival at 3 kg ai ha^{-1} (5.3 times the lowest recommended rate for wild oat control)
R = >20% survival at the lowest recommended rate for wild oat control but <85% survival at 3 kg ai ha^{-1}
S = <20% survival at the lowest recommended rate for wild oat control
* nr means that the herbicide history was not reported for the biotype

Appendix 8. Survival (%) of selected resistant biotypes in broad dose range experiments using fluazifop-butyl at different rates (kg ha^{-1}). The minimum recommended dose for wild oat control is 0.106 kg ha^{-1} fluazifop*. Fluazifop history (applications) of biotypes are presented in the last column.

biotype	0.013	0.026	0.053	0.106	0.159	0.212	0.318	0.424	0.636	0.848	1.272	1.696	status	history
NAS 4	___	___	___	___	0	0	___	0	___	0	___	0	S	1
NAS 6	___	___	___	___	0	0	___	0	___	0	___	0	S	0
NAS8	___	85	5	5	___	___	0	0	0	___	0	0	S	0
NAF 3	___	0	0	0	___	0	___	0	0	___	0	___	S	2
NAF 6	___	___	64	50	20	7	___	0	___	___	___	___	S	nr**
NAF 7	___	100	100	94	___	___	75	25	5	___	0	0	R	1
NAF 8	___	100	100	80	___	80	___	0	0	___	___	___	R	1
NAF 9	___	100	85	95	___	90	___	45	0	___	___	___	R	1
SAF 14	85	___	90	100	100	___	55	80	___	30	___	___	R	3
SAF 27	___	100	100	96	7	___	0	0	0	___	0	0	R	0
SAF 36	___	90	20	0	___	___	0	0	0	___	0	0	S	nr
WAF 14	___	100	100	100	___	___	55	40	7	___	3	0	R	1
SAS 154	100	100	100	100	100	100	100	100	100	100	100	100	RR	3
SAS 2	___	0	5	0	___	___	0	0	0	___	0	0	S	0
SAF 34	___	20	0	0	___	0	___	___	___	___	___	___	S	0

#RR = 100% survival at 1.696 kg ai ha^{-1} (16 times the lowest recommended rate for wild oat control)

R = >20% survival at the lowest recommended rate for wild oat control but <100% survival at 1.696 kg ai ha^{-1}

S = <20% survival at the lowest recommended rate for wild oat control

** nr = not reported

Appendix 9. Survival (%) of selected resistant biotypes in broad dose range experiments using sethoxydim at different rates (kg ha^{-1}). The minimum recommended dose for wild oat control is 0.093 kg ha^{-1} sethoxydim. Sethoxydim history (applications) of biotypes are presented in last column.

biotype	0.012	0.028	0.047	0.056	0.080	0.093	0.140	0.187	0.224	0.280	0.374	status	history
NAS 4	___	___	___	___	0	___	0	0	0	0	___	S	0
NAS 6	___	___	___	___	0	___	0	0	0	0	___	S	0
NAS8	___	100	___	100	63	21	0	0	___	0	___	S	0
NAF 3	___	95	___	95	80	30	10	0	0	___	___	S	0
NAF 6	___	___	___	___	70	___	40	20	5	0	___	S	0
NAF 7	___	100	___	100	69	54	10	3	___	0	___	S	0
NAF 8	___	80	___	95	90	40	10	___	___	___	___	S	0
NAF 9	___	55	___	75	85	15	10	___	___	___	___	S	0
SAF 14	95	___	80	___	___	75	20	0	___	0	0	S	0
SAF 27	___	100	___	50	45	12	5	0	___	0	___	S	0
SAF 36	___	100	___	80	55	15	15	0	___	0	___	S	0
WAF 14	___	100	___	100	100	100	100	100	___	100	___	R	0
SAS 154	___	80	___	55	20	10	5	0	___	___	___	S	0
SAS 2	50	___	46	___	___	6	0	0	___	0	0	S	0
SAF 34	___	10	0	0	0	___	___	___	___	___	___	S	0

#RR = 100% survival at 0.280 kg ai ha^{-1} (23 times the lowest recommended rate for wild oat control)

R = >20% survival at the lowest recommended rate for wild oat control but <100% survival at 1.696 kg ai ha^{-1}

S = <20% survival at the lowest recommended rate for wild oat control

** nr = not reported

Appendix 10. Survival (%) of selected resistant biotypes in broad dose range experiments using tralkoxydim at different rates (kg ha^{-1}). The minimum recommended dose for wild oat control is 0.100 kg ai ha^{-1} tralkoxydim. Tralkoxydim history (applications) of biotypes is presented in the last column.

biotype	0.013	0.050	0.075	0.090	0.110	0.140	0.170	0.2	0.3	status	history.
NAS 4	---	---	0	0	0	0	0	---	---	S	2
NAS 6	---	---	20	15	0	5	2	---	---	S	0
NAS8	---	100	25	10	0	0	0	0	0	S	1
NAF 3	---	10	0	0	0	0	0	---	---	S	0
NAF 6	---	---	60	60	40	22	8	---	---	S	0
NAF 7	---	80	35	20	5	0	5	0	0	S	0
NAF 8	---	50	7	0	0	0	0	---	---	S	0
NAF 9	---	55	45	7	7	0	0	---	---	S	1
SAF 14	100	60	0	---	0	0	---	0	0	S	1
SAF 27	---	20	6	0	0	0	0	0	0	S	0
SAF 36	---	100	60	25	10	5	5	0	0	S	nr**
WAF 14	---	100	100	65	55	23	30	10	5	R	1
SAS 154	---	15	25	15	5	5	0	---	---	S	2
SAS 2	---	10	6	0	0	0	0	0	0	S	0
SAF 34	---	7	7	0	0	0	0	0	0	S	0

#R = >20% survival at the lowest recommended rate for wild oat control.
S = <20% survival at the lowest recommended rate for wild oat control
** nr = not reported

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