DISTRIBUTION AND SEVERITY OF HERBICIDE RESISTANCE IN THE REPUBLIC OF SOUTH AFRICA

By

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INTRODUCTION

Weeds constitute a serious and continuing limitation to crop production in all agricultural systems (Devine, Duke & Fedtke, 1993). It is a well-known fact that effective weed control is of utmost importance in order to produce wheat (*Triticum aestivum* L.) economically. Weeds such as wild oats (*Avena fatua* L.) reduce yield quantity and seed quality of wheat (Prize Jones, 1976). Friezen (1961) confirmed that the protein content of wheat increases when weed control is effective. Chancellor & Peters (1972) found that a yield reduction of 30% could be expected when the plant density of *A. fatua* exceeds 40 plants per m^2 during wheat production. The effect of ryegrass (*Lolium* spp.) on wheat grain yield is directly proportional to the square root of its density. Levick (1969) concluded that the most important result of ryegrass competition was a reduction in the number of grain-bearing ears of wheat. According to Lemerle, Verbeek & Coombes (1995) *Lolium* spp. are a very competitive weed during wheat production and yield losses can be as much as 75% depending on various factors. In addition, Lumb & McPherson (1964) and Reeves & Tuohey (1972) have reported increased wheat grain yields resulting from ryegrass control measures. Little seeded canary grass (*Phalaris minor* Retz.) is a common and troublesome grassweed in winter cereals (Afentouli & Eleftherohorinos, 1999). The most likely reasons for its prevalence are its similar growth period to winter cereals, early seed dispersal, and ability of seeds to remain dormant in the soil for several years. Several studies indicate 30 to 60% wheat yield reduction from *P. minor* competition related to species, plant density, timing of emergence, wheat density as well as environmental factors (Afentouli & Eleftherohorinos, 1996; Cudney & Hill, 1979). Taking the above-mentioned effects of different weed species on crop production into mind one must come to the conclusion that weeds need to be controlled by one way or another.

According to Gressel (1991) chemical weed control is a practice invented by man. Our predecessors were not conscious of weeds in the modern sense, but as the human population multiplied, the pressure for producing crops with higher yields became the order of the day. The result of such pressure led to the optimization of cropping practices, including chemical weed control. Herbicide usage is far cheaper than cultivation, uses less fuel and usually does less damage to the crop roots and soil structure. According to Cobb (1992) the post-emergent alaninopropionates were introduced between 1969 and 1972 for the control of wild oats, and the introduction

of the aryloxyphenoxypropionates and the cyclohexanediones in the mid 1970s has enabled the control of certain annual grasses during cereal production.

Although the future of chemical weed control seems bright, a new element namely herbicide resistance, has been thrust into the agricultural sector. Resistance must be defined for uniform use worldwide, and can be described as the inherited ability of a plant to survive a herbicide treatment, which under normal spraying conditions would effectively kill the plant (Anonymous, 1997 & Gressel, 1991). The recent advent of herbicide resistant weeds increasing in significant numbers and broad distributions, has compelled mankind to remember that such genetic and ecological adaptability is almost universally present in all classes of living organisms. Even though the appearance of resistance to pesticides in previously susceptible insects and diseases has become quite common, the industry had assumed that in the case of weeds it would be different (LeBaron & Gressel, 1982). In reality the appearance of herbicide resistance had been long predicted and should have been expected. Interspecific resistance or selectivity of plant species to survive herbicide treatments has always existed, and is the very reason for the phenomenal success and value of modern weed control chemicals in crop production. According to LeBaron (1991) screening methods for new herbicides are designed for selecting new chemicals that will kill weeds to which crops will survive. Therefor one can assume that natural resistance against herbicides does occur in each and every plant.

The recognition of herbicide resistance in weeds is relatively recent, despite the widespread use of selective herbicides for over 40 years (Moss & Rubin, 1993, & Purba, Preston & Powles, 1995). Herbicide resistance tends to occur when a similar mode of action is used repeatedly, or when herbicides are very effective in killing the weed. The latter implies that the herbicide imposes a greater selection pressure on weeds and allows only the truly resistant plants to pass on their genes to the next generation, resulting in the survival of the specie if a herbicide with a similar mode of action is used the following season. Once herbicide resistance is established, the elimination thereof is hardly possible due to genetic inheritance and should therefore be taken in a very serious light (Martin, Kelly & Gill, 1993).

The main aim of this study is to provide the agricultural industry with a complete summary regarding herbicide resistance in the Republic of South Africa. Such a summary will empower the industry to target resistance with a thorough knowledge regarding herbicide resistance as well as to develop effective strategies to prevent

and/or to retard the development and spreading of herbicide resistance in South Africa. To be effective in this regard, weeds and herbicides should be better understood than present, and certain practices to manage herbicide resistance should be implemented to control the situation (Gressel, 1991).

Herbicide resistance is a relatively new aspect in South Africa and certain actions should be taken to ensure effective weed control in the long-term future of this country and should involve steps such as:

- Classification of herbicides according to their mode of action in order to be efficient in herbicide rotation.
- Testing of suspected resistant weed seed to be able to advise the producer to ensure efficient weed control.
- To complete a national survey to monitor the development and distribution of the situation.
- To monitor weed control strategies throughout the country to ensure that herbicides are applied correctly to obtain optimum efficacy.
- To advise the producer regarding effective management strategies to prevent further development of herbicide resistant weeds.

Herbicides are important for effective weed management during crop production and one can assume that they will still be as important in the future. However, to ensure the efficacy of herbicides in the future, one must obtain thorough knowledge of these products to ensure their continuous efficacy. To conclude, the future of herbicides depends greatly on how they are used at present.

CHAPTER 1

HERBICIDE RESISTANCE IN PERSPECTIVE

1.1 Plant responses

The repeated use of herbicides with similar modes of action on the same site over a period of years has resulted in weed biotypes that are resistant to such herbicides. Since the discovery of herbicide-resistant weeds (Ryan, 1970) the phenomenon has become well known in scientific and agricultural communities around the world (Anderson, 1997). There is no evidence that any herbicide resistant weed biotype has occurred through mutations caused by a particular herbicide, nor is there evidence to show whether or not these resistant biotypes were already present prior to herbicide treatment at the site where they were detected. Herbicide-resistant weed biotypes are presumed to arise through naturally occurring mutations, from small pre-existing populations of the specie. Therefore, resistance becomes apparent when selection eliminates the susceptible biotypes, leaving the resistant biotypes. Therefor, one can conclude that relying on chemical weed control rather than utilizing a combination of weed control practices brought resistance of weed biotypes in croplands forward.

Herbicides are major technological tools that are used successfully throughout the world. An adverse consequence of their use has been the emergence of herbicide resistance among certain weed species. The differential response of plants to herbicides has generally been attributed to differences in morphological and physiological factors between and within species that affect the accumulation of the herbicide in toxic amounts at their site of action. The failure of a herbicide to provide anticipated control is often first attributed to misapplication or unsuitable environmental conditions. When these factors are successfully eliminated, herbicide resistance is often the reason for poor weed control. There are three types of plant responses to applied herbicides and they can be characterized as susceptibility, tolerance and resistance.

1.2 Susceptibility

Plant susceptibility can be identified as a positive response to an applied herbicide. The degree of response is a measure of a plant's susceptibility to the applied herbicide, under the conditions involved. The degree of susceptibility may range from an imperceptible response, to a marked growth abnormality or to total death of the plant. Susceptibility is influenced by factors such as:

- stage of plant growth when treated
- herbicide concentration absorbed by plant
- inherent toxicity of applied herbicide and
- environmental factors such as light, moisture and temperature.

Plants are most susceptible when rapid growth is taking place and when rapid growth has ended and food reserves are temporarily depleted or exhausted. Plants growing in subdued light are generally more susceptible to foliar-applied herbicides than those growing in high light intensity, apparently due to the formation of a thinner, more permeable cuticle. Also, water-stressed plants are less susceptible to foliar-applied herbicides than those not water stressed, due to a less expanded and therefore less permeable cuticle (Anderson, 1997).

In general, plants are less susceptible to herbicides applied when temperatures are below or above those favoring growth. Annual plants are most susceptible to herbicides in their seedling stage, while perennial plants are most susceptible to herbicides in their flowering stage.

1.3 Tolerance

Tolerance and resistance both describe a condition whereby a plant withstands a herbicide treatment, but their use often results in confusion. Until the occurrence of what is now termed "herbicide resistance," the term tolerance was often used to designate crop response to a herbicide. According to Anderson (1997) a tolerant crop was a crop that was not significantly injured by a herbicide applied at a recommended rate. A workable definition of tolerance is the ability of a crop plant to withstand a predetermined dosage of a herbicide, which may be overcome by higher dosages. Thus, tolerance is rate dependent. And it is largely attributed to differential factors such as absorption, translocation and / or detoxification. All these factors can be morphological or physiological.

As stated by Anderson (1997) it would appear desirable, in view of recent developments, to restrict the use of the term tolerance to crop plants only.

1.4 Resistance

Many definitions describing herbicide resistance are noted throughout the world in scientific papers, journals, books ect. However, a workable definition of resistance is the ability of a biotype to survive treatment with a given herbicide to which the weed

specie is normally susceptible (Anderson, 1997). There appear to be three types of resistance, namely (1) herbicide resistance, (2) cross-resistance and (3) multiple resistance.

1.4.1 Herbicide resistance

This type of resistance refers to a weed biotype that is resistant to only one specified herbicide, as in the case of resistance to amitrole or glyphosate. However, the term may also be used in a general sense to denote the phenomenon of herbicide resistance in weed biotypes (Anderson, 1997).

1.4.2 Cross-resistance

To add a second phenomenon, cross-resistance also occurs and it is well-documented (Burnet, Loveys, Holtum & Powles, 1993; Burnet, Hildebrand, Holtum & Powles, 1991 & Burnet, Barr & Powles, 1994a).

Cross-resistance may be defined as resistance to two or more herbicides from different chemical groups resulting from the presence of a single resistance mechanism (Gressel, 1988). As stated by Burnet, Hart, Holtum & Powles (1994b), cross-resistance is a phenomenon whereby weed biotypes become resistant to distinct classes of herbicides as a consequence of selection by a chemically unrelated herbicide. This phenomenon has been observed in many different instances, with the best example being that of cross-resistance to chlorsulfuron in a biotype of *Lolium rigidum* (Gaud.) exposed mainly to diclofop and trifluralin (Heap & Knight, 1986; Heap & Knight, 1990). According to Preston, Tardif & Powles (1996), cross-resistance frequently occurs to herbicide groups to which the population has never been exposed. Currently populations of *L. rigidum* demonstrate resistance across 16 different herbicide chemical classes with 11 different modes of action.

As cited by Kudsk, Mathaissen & Cotterman (1995) sulfonylurea resistance has also been reported as a result of metabolic cross-resistance. Biotypes of rigid ryegrass (*L. rigidum*) and blackgrass (*Alopecurus Myosuroides* Huds.), selected by the continuous use of diclofop-methyl and chlorotoluron/isoproturon, respectively, have been found to be resistant to a number of chemically unrelated herbicides, including sulfonylurea herbicides. According to Burnet *et al.*, (1994b) cross-resistance is of considerable practical importance because it may render alternative herbicides ineffective. With the advent of widespread herbicide resistance in *Lolium* spp. more attention is being given to developing alternative herbicides to be used with different

crops to vary herbicide regimes. The success of such development would depend on the absence of cross-resistance to new herbicides.

One of the implications of cross-resistance in rigid ryegrass is that the selection pressure of a previous herbicide can also alter susceptibility of a biotype to an unrelated herbicide (Burnet *et al.*, 1994b). According to Preston *et al.*, (1996) *L. rigidum* is an obligate out-crossing specie, and any survivors of herbicide application must cross with another survivor to produce offspring. This out-crossing means that all mechanisms of herbicide resistance present will tend to be mixed and distributed within the progeny of these individuals.

1.4.3 Multiple-resistance

Over the past two decades, herbicide usage exposed huge weed populations to strong selection pressures for herbicide resistant traits (Tardif & Powles, 1994). According to Christopher *et al.*, (1991) and Holt, Holtum & Powles (1993) herbicide resistant populations within more than 100 weed species have been documented. In most cases resistance developed following exposure to one chemical, or chemicals which are similar in structure and mode of action. They were not resistant to chemicals of a different structure, which have different modes of action. At present, there is a relatively new phenomenon, called multiple resistance and the most dramatic commercially significant example has been documented by Heap & Knight (1990) and Powles & Mathews (1992).

Multiple resistance occurs when resistance to several herbicides results from two or more distinct resistance mechanisms affecting different target sites in the same plant (Tardif & Powles, 1994). The term "multiple resistance" describes a situation where a weed population has been subject to selection by a range of herbicides to which it eventually becomes resistant. Multiple resistance refers therefore to the accumulation of resistance to particular herbicides within a population as a consequence of selection pressure by those herbicides (Powles & Mathews, 1992). LeBaron (1991) pointed out the danger if weeds develop cross- and multiple resistance during crop production. Not only is herbicide resistance appearing after fewer repeated annual applications of some of these newer herbicides, but also there seem to be more species that have potential to resistance.

According to Tardif, Preston & Powles (1997), in order to understand multiple resistance it is important to know that two mechanisms, viz. target site- and non-

target site modifications can be responsible for the development of multiple resistance. Non target site mechanisms can be subdivided into mechanisms of resistance not involving modification of the herbicide get-site and include enhanced metabolism, reduced retention or absorption reduced translocation, and differential sequestration.

As cited by Holt *et al.*, (1993) both multiple- and cross-resistance can occur in a single population and to identify the correct phenomenon, accurate records of herbicide exposure are required. According to LeBaron (1991) the evolution of populations resistant to other herbicides, and especially biotypes with multiple resistance to different classes of herbicides, poses a great threat. The agricultural practices most conclusive to the occurrence of the cross- and multiple-resistance are those that rely exclusively on one or a few herbicides for a high level of weed control (Burnet *et al.*, 1994b). Morrison & Bourgeois's (1995) answer to this problem is to establish glyphosate resistant crops and to kill the weeds with glyphosate treatments. If resistance develop against glyphosate, however, the problem would start all over again.

1.5 GENE-FACTORS CONTROLLING THE RATE OF EVOLUTION

The factors controlling the rates of evolution vary and can be a function of the genetic systems controlling resistance. Resistance to herbicides can develop in two ways, viz. target site resistance and creeping resistance (Gressel, 1991).

1.5.1 Target-site resistance

Usually, target-site resistance develops under a regime of high herbicide dosage rates, confers a high degree of resistance and is usually controlled by a single or relatively few genes and has up to date received by far the greater attention by the herbicide industry (Gressel, 1991). According to Devine (1997), the enzyme acetyl-coenzyme A carboxylase (ACCase) is the target site of two major groups of synthetic herbicides, the aryloxyphenoxypropionates and cyclohexanediones. As cited by Devine, Duke & Fedtke (1993) the target site is usually the site to which the herbicide binds, or with which it interferes in some manner, resulting in death of the plant.

It's important to keep in mind that by using chemicals new genes are not produced, although chemical enrichment may occur. The use of herbicides can however increase resistance in a population (Reinhardt, 1997). Single-gene resistance was the first to appear and is also the simplest to understand. Although genetic

modification occurs in the target sites of the key enzymes, the herbicide is still able to function. The mutant enzyme often acts with reduced efficacy, leading to reduced fitness of the mutant when it competes with the wild biotype. In the case of paraquat, metabolic resistance seems to be offered by a single dominant major gene (Gressel, 1993). There are two ways of lowering selection pressure and delaying resistance. The first is to reduce the rate of herbicide application and secondly, a less persistent herbicide from the same group may be used, so that the more susceptible individuals are able to survive. Furthermore, the rate of herbicide enrichment can be reduced when the resistant individual is less fit than the wild type. The resistant and susceptible weed are able to compete during the season when applying the herbicide. Herbicide rotation is a major resistance-preventing strategy where competitive fitness is low (Gressel & Segal, 1990).

Another resistance mitigating factor is the seed soil bank. If the seeds of a given specie germinate over many years, the susceptible plants in the seed bank slowing the rate of evolution will buffer resistant seeds. Resistance evolves more quickly in no-till situations than where tillage is practiced. Seeds on the soil surface usually germinate within a year or die without entering the seed bank (Gressel, 1993).

1.5.2 Creeping-resistance

Creeping-resistance develops mainly due to the use of herbicides with a similar mode of action, and in areas subjected to low dosages of a herbicide and may therefore confer a lower degree of resistance to a greater number of individuals in a population. Creeping-resistance can develop as a result of the presence of more than one resistant gene (Gressel, 1991). Low threshold levels of many herbicides can be degraded by weeds, even though the amount applied can inhibit the target enzyme while the herbicide is being degraded. Added gene doses could result from amplification of the present genes, mutations in promoter genes, or mutations in structural genes conferring changes in substrate specificity. There may be different genes that can mutate to give one more gene dose for resistance (Gressel, 1993). Continuous herbicide use will result in continued selection for resistant biotypes, thereby further increasing the levels of resistance. Both seed bank and any fitness differentials should affect the rate of creeping-resistance in the same way that target-site resistance is affected (Gressel, 1993).

1.6 Worldwide occurrence of herbicide resistance

When the first cases of evolution of newly resistant weeds appeared, the weeds were either quietly rouged without saving material for study, or ignored as being unimportant. Because of the occurrence on a single farm or two, resistance seemed unimportant, especially in a different state or country from one's own. The spread of herbicide resistance from farm to farm was due to co-evolution and usually not by the spreading of pollen or seed (Gressel, 1993).

The extent of the area affected by herbicide-resistance is poorly documented due to a lack of comprehensive survey data. The fact that resistant seed is spreading in unlimited quantities during each season, makes it more difficult to keep track of the problem. There have been relatively few reports of herbicide resistance prior to the late 1960s, and was first observed during 1968 when Ryan (1970) reported that *Scenecio vulgaris* developed resistance to both atrazine and simazine. However, since the detection of the triazine-resistant weed (*S. vulgaris*) there has been a steady increase in the number of resistant weed species throughout the world (Moss & Rubin, 1993). According to LeBaron (1991) it has been estimated that triazine-resistant weeds infest about one million ha in the United States of America and at least two million ha in other countries. A survey *of L. rigidum* infestations within cropped fields in an intensively cropped area of about 250 km^2 in South Australia in 1994 revealed that 40% of all fields surveyed contained diclofop-methyl resistant populations (Nietschke, Llewellyn, Mathews, Powles & Reeves, 1996). Jutsum and Graham (1995) stated that resistance is found in over 100 grass and broad-leaved species, occurring in more than 40 countries. LeBaron (1991) reported that by the year 1990, 113 herbicide-resistant weed biotypes had evolved in various locations worldwide. This total included 58 species (41 dicotyledonous and 19 grass weeds) resistant to 14 classes of herbicides. There are now resistant weeds present in all but 10 of the 50 States of the USA, all but two provinces of Canada, 18 countries of Europe and 10 other countries (LeBaron, 1991). According to Clay (1989) and Van Oorschot (1991) a total of 15 different modes of action is involved and occurs in 62 countries.

In Australia, wild oats (*A. fatua*) and ryegrass (*Lolium* spp.) infest millions of hectares, which are resistant to aryloxyphenoxypropionates and cyclohexanediones (Morrison & Bourgeois, 1995). As cited by Christopher, Powles, Liljegran & Holtum (1991) the first report of resistance to diclofop-methyl was found in *L. rigidum* during 1982, (Heap & Knight, 1982). Table 1 presents combined statistics of resistance found for

the first time for distinguish chemical groups of herbicides (LeBaron, 1991, LeBaron & Gressel, 1982).

Table 1: Worldwide occurrence of herbicide resistance (LeBaron, 1991; LeBaron & Gressel, 1982)

Herbicide group	Specie	Country	Year
Sulfonylureas & Imidazoliones	*Lolium rigidum*	Australia	1986
Amides	*Echinochloa crus-galli*	Greece	1986
Organoarsenic	Xanthium strumarium	USA	1984
Carbamates	*Amaranthus hybridus*	Hungary	1988
Dinitroanilines	*Eleusine indica*	USA	1973
Bipyridiliums	*Lolium perenne*	United Kingdom	1976
Hydroxybenzonitriles	*Chenopodium album*	West Germany	1988
Aryloxyalkanoic acids	*Commelia diffusa*	Hawaii	1954
Pyridinecarbolic acids	*Centaurea soltstitialis*	USA	1988
Aryloxyphenoxy propionic acids	*Lolium rigidum*	Australia	1982
Urea compounds	*Alopecurus myosuroides*	West Germany	1983
Uracils	*Amaranthus Hybridus*	Hungary	1988
Triazoles	*Poa annua*	Belgium	1986
Triazines	*Scenecio vulgaris*	USA	1968
Glyphosate	*Agropyron repens*	USA	1978

1.7 Herbicide resistance in South Africa

Cairns & Hugo (1986) reported the first confirmed case of herbicide resistance to diclofop-methyl in the Western Cape on *A. fatua*, while Smit (1993) confirmed herbicide tolerance of different biotypes of *A. fatua* (Plate 1). Up to date, only one broad-leaved weed specie, viz. smooth pigweed (*Amaranthus hybridus* L.), (Plate 2) was confirmed to be resistant to triazine treatments (Sereda, Erasmus & Coetzer, 1996). Experiments done at the ARC-Plant Protection Institute (Republic of South Africa) indicated that a herbicide rate 20 times higher than registered, still did not provide satisfactory results on the specific *A. hybridus* biotype.

Plate 1: *Avena fatua* (Grabandt, 1985)

Plate 2: *Amaranthus hybridus* (Grabandt,1985)

At a conference held during 1997 in the Western Cape, a total area of 6000 ha was speculated as being herbicide resistant to ACC-ase inhibitors. In the Western Cape three grass species are of economical importance regarding herbicide resistance, namely *L. rigidum* (Plate 3), *A. fatua* and *Phalaris minor* (Retz.), (Plate 4) all suspected to be resistant to grass weed herbicides.

Plate 3: *Lolium* spp. (Grabandt, 1985)

Plate 4: *Phalaris minor* (Grabandt, 1985)

To stress the importance of effective weed control regarding above-mentioned weed species, one could refer to literature. As cited by Lemerle, Verbeek & Coombes (1995), *Lolium* spp. is a very competitive weed during wheat production and yield losses of wheat in competition with *Lolium* spp. can be as much as 75%, depending on sowing date, cultivar and seeding rate. Afentouli & Eleftherohorinos (1996) found a reduction of up to 60% of wheat yield if *P. minor* competition occurs and Carlson & Hill (1985) stated that wheat yield declined rapidly as the density of *A. fatua* increases. At this stage only one broad-leaved weed, viz. wild radish (*Raphanus raphanistrum* L.) appears to be resistant to sulfonylureas (Plate 5). Currently, herbicide resistance in wheat production has only been observed in the winter rainfall area. The occurrence of resistance goes hand in hand with herbicide usage and crop production systems. By investigating herbicide sales during 1996, certain conclusions can be made regarding the announcement of resistance for the future. Table 2 summarizes the percentage sales of herbicides for the winter and summer rainfall areas, while Table 3 indicates the percentage of herbicides sold in the two respective main wheat producing areas in South Africa.

Plate 5: *Raphanus raphanistrum* (Grabandt, 1985)

Table 2: Herbicide sales in wheat production in South Africa (Senekal, 1996)

HERBICIDE SALES (Million Rand)					
Area (Rainfall)	**Non-selective**	**Sulfonylureas**	**Grassweed Herbicides**	**Bromoxynil & Hormone**	**Total**
Winter	1,156,476.00	11,484,989.00	22,464,523.00	7,637,493.00	42,743,481.00
Summer	23,405.00	5,665,710.00	1,848,847.00	15,762,171.00	23,300,133.00
Total	1,179,881.00	17,150,699.00	24,313,370.00	23,399,664.00	66,043,614.00

Summer – 962350 ha

Winter - 400800 ha

Table 3: Percentage herbicides used in respective areas (Senekal, 1996)

HERBICIDE CLASSES					
Area (Rainfall)	**Grass Herbicides**	**Sulfonylureas**	**Hormone & Bromoxynil**	**Non-selective**	**Total**
Summer	7.9%	24.3%	67.6%	0.1%	35.3%
Winter	52.5%	26.8%	17.8%	2.7%	64.7%

Conclusions made from tables two and three:

- A total of 64.7% of the herbicide sales occurred in the winter rainfall area. The intensity of use of herbicides is much higher than that of the summer rainfall area (35.3%) and therefore resistance should develop much faster in the winter rainfall area.
- Over fifty percent (52.5%) of the herbicides sold in the winter rainfall area are grass weed herbicides. According to the Herbicide Reaction Committee (HRAC), it is possible for resistance to develop against grass weed herbicides within 5-7 years of consecutive use.
- A total of 79.3% of the sales in the winter rainfall area belongs to grass weed herbicides and sulfonylureas. Both classes of herbicides are most likely to develop resistance if frequent use occur.
- Because of the low percentage (7.9%) of grass weed herbicides sold in the summer rainfall area, chances for developing resistance to ACCase inhibitors are less likely than those of the winter rainfall area.
- The more liberal use of hormone based herbicides & bromoxynil (67.6%) in the summer rainfall area reduces the possibility for development of resistance against sulfonylureas.

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CHAPTER II

CLASSIFICATION OF HERBICIDES

2.1 Introduction

Herbicides may be classified in several ways. For example, Vermeulen, Dreyer, Grobler & Van Zyl (1996) classified herbicides according to physiological grouping. Knowledge of the mode of action is basic to understand the principles of herbicide synthesis. According to Asthon & Crafts (1981) the term "mode of action" refers to the entire sequence of events from introduction of a herbicide into the environment to the death of plant and "mechanism of action" refers to the primary biochemical or biophysical lesion leading to death. As cited by Devine, Duke & Fedtke (1993), herbicide action might be described as the physiological and biochemical interaction of a herbicide with a plant.

Herbicide action may be thought of as comprising two phases; the first phase involves movement of the herbicide to the target site, while the second phase involves the metabolic consequences resulting from interaction at that site. Phase 1 starts with the application of the herbicide to the plant, the herbicide then enters the plant through foliar absorption or via the roots. Entry into the plant is quickly followed by a series of steps that precede the arrival of the herbicide at its site of action. The interaction of the herbicide at the target site can be viewed as the first step in phase 2 and is followed by a series of toxic consequences that result in death of the plant (Devine, *et al*,. 1993).

The primary aim of this chapter is twofold: (a) to classify the different herbicides according to their physiological characteristics, as well as to the distinguish modes of action and (b) to summarize a study on the modes of action of the two most widely applied herbicides (ACC-ase and ALS inhibitors) in wheat production.

2.2 Herbicide classification according to physiological characteristics

The first classification system in this chapter organizes herbicides into those, which are applied to foliage, and those herbicides, which are applied almost strictly to the soil (Table 1). The foliar applied groups are divided into three categories according to movement through the plant, which are:

1. Symplastic translocated (Table 2)
2. Apoplastic translocated (Table 3)

3. Those which do not move appreciably (Table 4).

According to Senekal (1996) approximately 98.2% of the herbicides used during wheat production belongs to the Aryloxyalkanoic acids, the Aryloxyphenoxy propionic acids, the Cyclohexanediones and to the Hydroxybenzonitriles. It is therefore of utmost importance to understand the mode of action of each of these chemical groups to be able to address the resistance phenomonem.

Table 1: Soil Applied herbicides (Anonymous, 1997; Retzinger & Mallory-Smith, 1997 ;Ross & Childs, 1998; Vermeulen *et al.*, 1996)

Mode of Action	Chemical Group	Active Ingredient	Characteristics
Microtuble assembly inhibition	Dinitroanilines	Pendimethalin, trifluralin, oryzalin	Root inhibitors, little or no foliar activity. Applied mostly preplant incorporated. Inhibit the steps in plant cell division, and responsible for chromosome separation. Relatively few roots. Bind to soil colloids and unlikely to leach. Losses occur through volatilization and photodegradation. Incorporation into soil is suggested.
Inhibition of lipid synthesis – not ACCase inhibition	Thiocarbamates	EPTC, pebulate	Shoot inhibitors. Soil applied. Injury appears as malformed dark green shoots. Very volatile herbicides and is preplant incorporated.
Inhibition of cell division	Chloroacetanilides	Acetachlor, alachlor, metazachlor, metolachlor, propachlor	Pre-emergence herbicides, incorporated
	Amides	Propyzamide	Shoot and root inhibitors. Preplant incorporated, pre-emergence and early post-emergence grass control.
Inhibition of cell wall (cellulose) synthesis	Amides	Isoxaben	

Table 2: Foliar applied herbicides, symplastically translocated (Anonymous, 1997; Retzinger & Mallory-Smith, 1997; Ross & Childs, 1998; Vermeulen *et al.*, 1996)

Mode of Action	Chemical Group	Active Ingredient	Characteristics
Auxin Growth Regulators	Aryloxyalkanoic acids	2,4-D; 2,4-DB; MCPA; Triclopyr; Fluroxypyr	Moving from leaves with sugar to sites of metabolic activity. Potential to kill perennial weeds. Symptoms are pigment loss, growth stoppage and malformed new growth. Most injury appears after several days.
	Benzoic acids	Dicamba; chlorthal-dimethyl	
Microtuble assembly inhibition	Pyridinecarbolic acids	Clopyralid; Picloram; Thiazopyr	
Inhibition of EPSP synthase	Unclassified	Glyphosate; Sulfosate	Usage is limited for foliar use, because chemicals are rapidly inactivated in soil. Symptoms are yellowing of new growth. It is a non-selective herbicide.
Inhibition of acetolactate synthase	Imidazoliones	Imazapyr; Imazethapyr; Imazamox	Shoot meristems cease growth, Yellow to purple symptoms appear, roots develop poorly. Symptom development requires up to three weeks
	Triazolopyrimides	Metosulam	
	Sulfonylureas	Chlorsulfuron, chlorimuron-ethyl, ethoxsulfuron, metsulfuron-methyl, nicosulfuron, primisulfuron, prosulfuron, sulfosulfuron, rimsulfuron, thifensulfuron, triasulfuron, tribenuron	
Inhibition of carotenoid biosynthesis	Triazoles	Amitrole	
	Unclassified	Clomazone	
	Pyridazinones	Norflurazon	
Inhibition of acetyl CoA carboxylase	Aryloxyalkanoic acids	Diclofop-methyl, fluazifop-P-butyl, Quiazlofop-P-butyl, fenoxaprop-P-ethyl, propaquizafop, clodinafop-propargyl, haloxyfop	Symptoms are discoloration and disintegration of meristematic tissue. Leaves turn yellow and red. Usage for selective grass control. Use early postemergence on seedling grasses.
	Cyclohexanedione	Tralkoxydim, sethoxydim, cycloxydim	

Table 3: Foliar applied herbicides, apoplastically translocated (Anonymous, 1997; Retzinger & Mallory-Smith, 1977; Ross & Childs, 1998; Vermeulen *et al.*, 1996)

Mode of Action	Chemical Group	Active Ingredient	Characteristics
Photosynthetic Inhibitors at photostem II	Triazines	Ametryn, atrazine, cyanazine, demestryn, metribuzin, prometryn, propazine, simazine, terbuthylazine, terbutryn	Symptoms develop from bottom to top of plant shoots. Chlorosis appears between leaf veins, followed by necrosis of the tissue. Chemicals have excellent soil activity. Most have foliar activity as well. Used for pre-plant incorporated, pre-emergence and limited post-emergence.
	Uracils	Bromacil, terbacil	
	Urea compounds	Linuron, diuron, ethimuron, tebuthiuron, flumeturon, methabenzthiazuron	
	Hydroxybenzonitriles	Bromoxynil, ioxynil	
	Unclassified	Pyridate, bendioxicide	
Inhibition of carotenoid biosynthesis	Pyridazinones	Chloridazon, norflurazon	

Table 4: Non-translocated herbicides (Anonymous, 1997; Retzinger & Mallory-Smith, 1997; Ross & Childs, 1998; Vermeulen *et al.*, 1996)

Mode of Action	Chemical Group	Active Ingredient	Characteristics
Photosystem-I-electron diversion	Bipyridylium	Paraquat, diquat	Result in rapid disruption of cell membranes. Herbicide penetrates into cytoplasm, cause formation of peroxides and free electrons, which destroy cell membranes. Destruction prevents translocation. Postemergence use only, can expect only shoot kill.
Inhibition of glutamine syntheses	Unclassified	Glyfosinate-ammonium	
Inhibition of protopophyrinogen oxydase	Diphenyl esters	Bifenox, fluroglycofen, fomesafen, oxyfluorfen	Have foliar and soil activity. Control broadleaf weeds. Relatively unaffected by soil texture and organic matter

2.3 Herbicide classification according to mode of action

Awareness of herbicide resistant weeds prompted Universities and agro-chemical personnel to develop guidelines to minimize the risk of selecting herbicide resistant weeds. One of these guidelines is to provide the industry with a tool to enable the farmer to rotate the herbicides used on a specific land. According to Ross & Childs (1998) the mode of action is the overall manner in which a herbicide affects a plant at the tissue or cellular level. Herbicides with the same mode of action will have the same translocation pattern and produce similar injury symptoms. To succeed, herbicides had to be classified according to their mode of action and therefore a classification system was developed as illustrated in Figure 1 (Smit, 1997).

Figure 1: Herbicide classification regarding mode of action (Smit, 1997)

2.4 Mode of action of ACCase inhibitors

Since the discovery of the first synthetic organic herbicides in the late 1940s, there has been great interest in understanding the mechanisms by which herbicides interfere with plant growth. Among the herbicides with mechanisms that are currently understood, there are about 100 herbicides that inhibit photosystem II electron transport, 37 that inhibit branched chain amino acid synthesis, 32 that are active auxins, and 28 that interfere with microtubular synthesis or function (Devine *et al.*, 1993).

Both aryloxypropanoic acid (Figure 2) and cyclohexanedione (Figure 3) herbicides are selective post-emergence graminicides and produce similar effects on grass weeds and acetyl-CoA carboxylase (ACCase) is the site of inhibition. ACCase is a complex enzyme that contains three functional sites: a biotin carboxyl carrier site, an ATP-dependent biotin carboxylase, and a carboxyltransferase (Dekker & Duke, 1995). According to Hoppe (1980) as stated by Cobb (1992) diclofop-methyl does not interfere with photosynthesis, respiration, protein synthesis, nor nucleic acid synthesis, but the inhibition of acetate incorporation into fatty acids could be demonstrated in susceptible species (Harwood 1989). The first definitive evidence that aryl-propanoic acid herbicides are potent lipid synthesis inhibitors, was the inhibition of ^{14}C-acetate incorporation into the free fatty acids. It is clear that the free fatty acids are the herbicidally active structures (Dekker & Duke, 1995). Cobb (1992) stated that plant membranes contain unique fatty acids that have crucial structural and biochemical roles and the activity of the enzyme is strongly enhanced by light (Dekker & Duke, 1995). At least 70% of the total leaf fatty acids consist of the unsaturated α-linolenic acid, which itself makes up between 40 and 80% of the lipid fraction in the chloroplast. According to Cobb (1992) trans-$\triangle_3$-hexadecanoic acid and linoleic acid are synthesized both in the chloroplast stroma and the cytoplasm (Figure 4). Essentially malonyl CoA is formed from acetyl CoA and converted to saturated palmitate by the action of soluble stromal enzyme complex, termed as fatty acid synthetase. This complex contains seven enzymes covalently bound to an acyl carrier protein (ACP), which transfers intermediates between the seven enzymes. The seven enzyme cycles are needed for the condensation of seven additional C_2 units into one palamite.

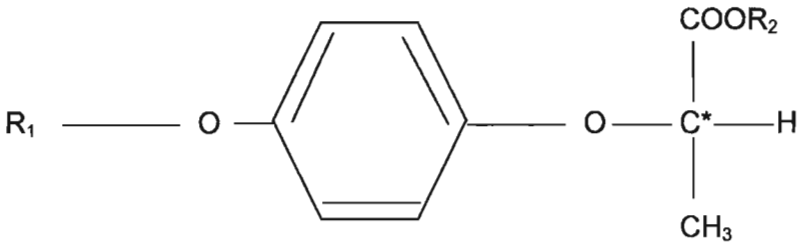

Figure 2: Chemical structure of aryloxyphenoxypropionates (Cobb, 1992).

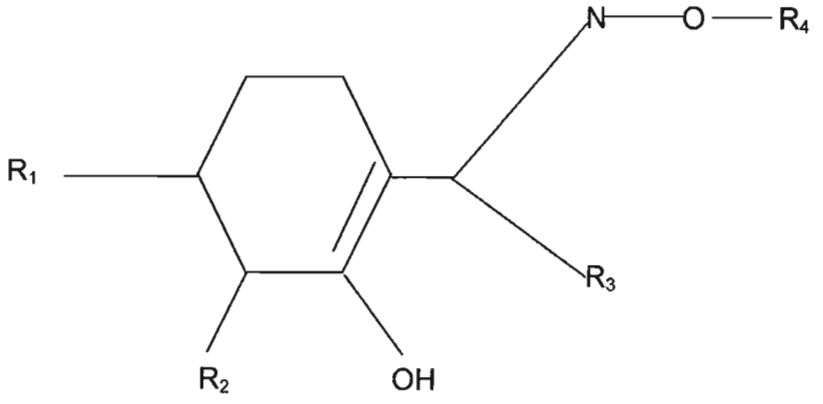

Figure 3: Chemical structure of cyclohexanediones (Cobb, 1992).

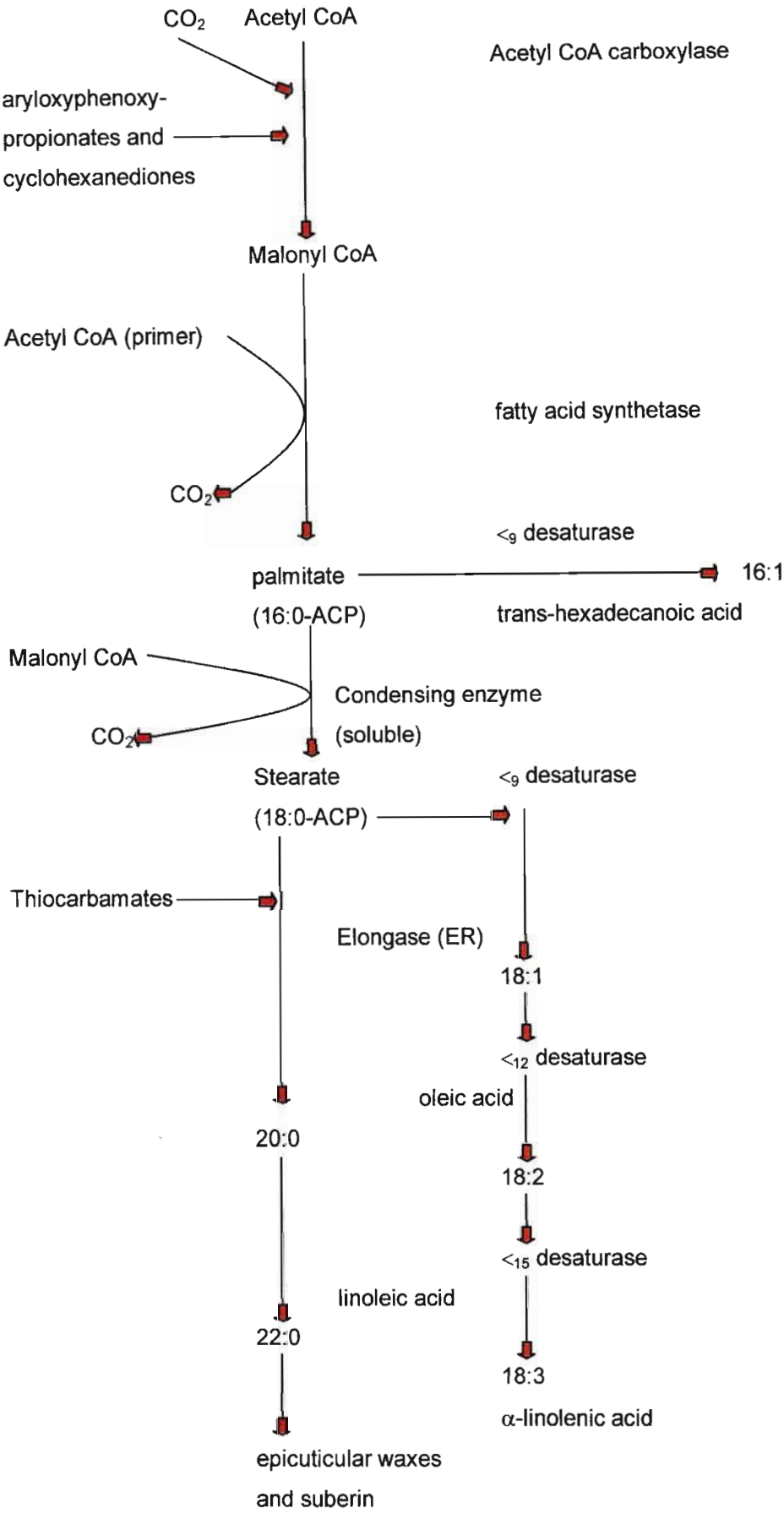

Figure 4: Fatty acid biosynthesis in plants (Cobb, 1992).

According to Harwood (1988) two metabolic routes are now possible from palamite. Firstly, a soluble condensing enzyme and elongase bound to the endoplasmic reticulum are able to add further C_2 units in the cytoplasm to yield the long-chain saturated fatty acids found in suberin and the epicuticular waxes of plant surfaces. Secondly, desaturases are present in the chloroplast to form the unsaturated fatty acids.

Acetyl CoA carboxylase (ACC-ase) is the first step for fatty acid biosynthesis and catalyses the formation malonyl CoA (Harwood 1988). ACC-ase is a high molecular weight, multifunctional protein with three distinct enzyme functions that involve biotin as an essential cofactor that functions as CO_2 carrier (Figure 5). A carboxyl group is donated from a bicarbonate anion and ATP hydrolysis is used to allow the formation of carboxybiotin intermediate by biotin carboxylase. Carboxybiotin is attached to an ε-amino group of lysine residue on the biotin carboxyl carrier protein (BCCP). Carboxybiotin then functions as a CO_2 donor in malonyl CoA formation.

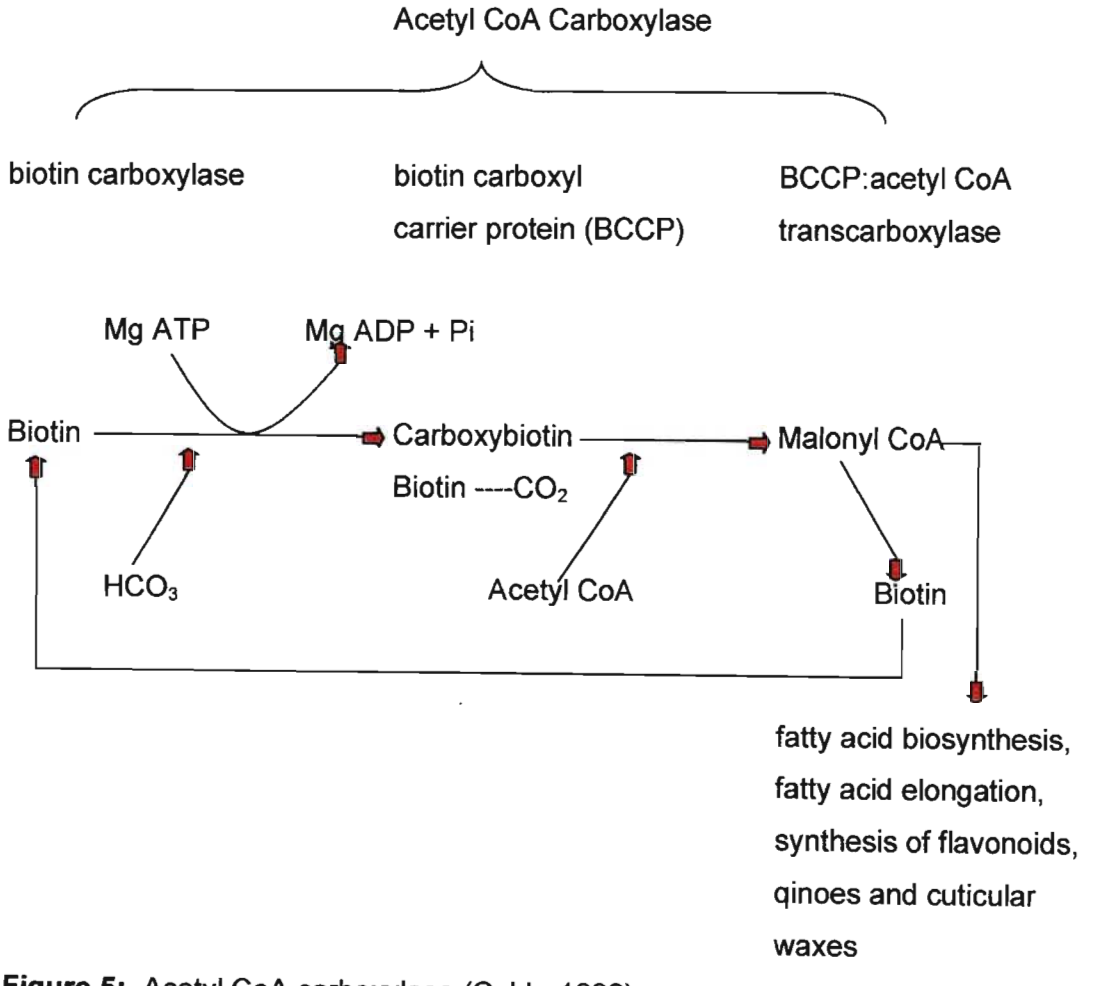

Figure 5: Acetyl CoA carboxylase (Cobb, 1992).

Rendina, Graig-Kennard, Beudion & Breen (1990) have found that the transcarboxylase is inhibited rather than the biotin carboxylase. They suggest that both aryloxyphenoxypropionates and cyclohexanediones compete for the same site on the enzyme and that the inhibition is reversible. According to Cobb (1992) ACC-ase is not the only site of graminicide action. The substituted pyridazinones have been found to inhibit lipid biosynthesis in addition to their known inhibition of carotenoid biosynthesis. Weisshaar, Retzlaff & Böger (1988) demonstrated that micromolar concentrations of the chloroacetamides may also inhibit inhibit fatty acid biosynthesis by preventing the elongation of palmitate and the desaturation of oleate in green microalge *Scenedesmus acutus*. A direct effect on membrane function has been proposed as a site of action of these herbicides (Shimabukuro & Hoffer, 1992) however, the action at this site does not appear to kill plants at herbicidal doses (DiTomaso, Brown, Stowe, Linscott & Kochian, 1991). Cobb (1992) stated that the foliar uptake of aryloxyphenoxypropionates are very rapid and de-esterification occurs in the leaf tissues. The next step is that of when phytotoxic acid accumulates at the apical meristem which becomes necrotic. Also, interference with lipid biosynthesis causes an irreversible disruption in membrane synthesis resulting in a drastically altered metabolism. These processes are encouraged by active plant growth and warm temperatures. Plant growth will also stop within two days as meristems cease to function and chlorotic symptoms will appear on younger leaves.

2.5 Mode of action of ALS inhibitors

During the 1980s three new herbicidal classes emerged that were potent, selective, broad-spectrum inhibitors of plant growth at field rates measured in grams rather than kilograms per hectare. The sulphonylureas (Figure 6), imidazoliones (Figure 7) and the triazolopyrimidine (Figure 8) are chemically different, yet they share the same mode of action, known as acetohydroxyacid synthase. All three classes of ALS inhibitors possess remarkable herbicidal properties. They are able to control a wide spectrum of troublesome grass and broadleaf weeds at very low doses. Furthermore, formulations have proved to be both foliar- and soil-active with very low mammalian toxicity (Cobb, 1992). According to Beyer, Duffy, Hay & Schlueter (1987), sulfonylurea herbicides are composed of three moieties: An aryl group, a nitrogen-containing hetrocycle portion and a sulfonylurea bridge that links the other two moieties (Figure 9).

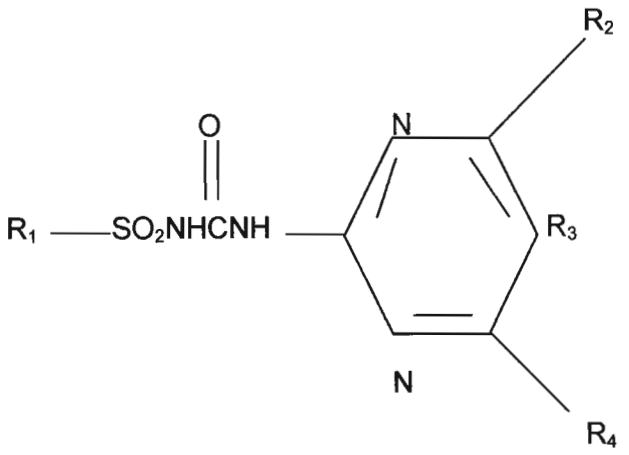

Figure 6: Structure of sulphonylureas (Cobb, 1992).

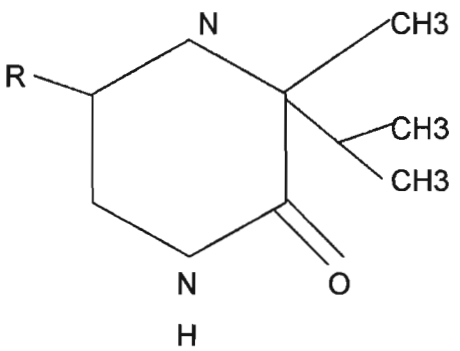

Figure 7: Structure of imidazoliones (Cobb, 1992).

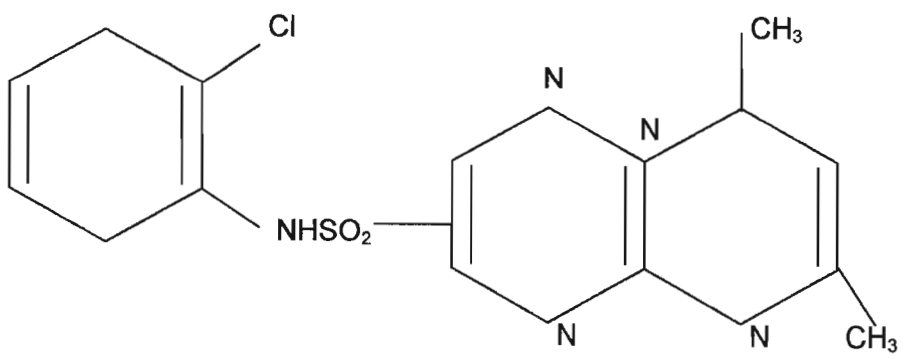

Figure 8: Structure of triazolopyrimidine (Cobb, 1992).

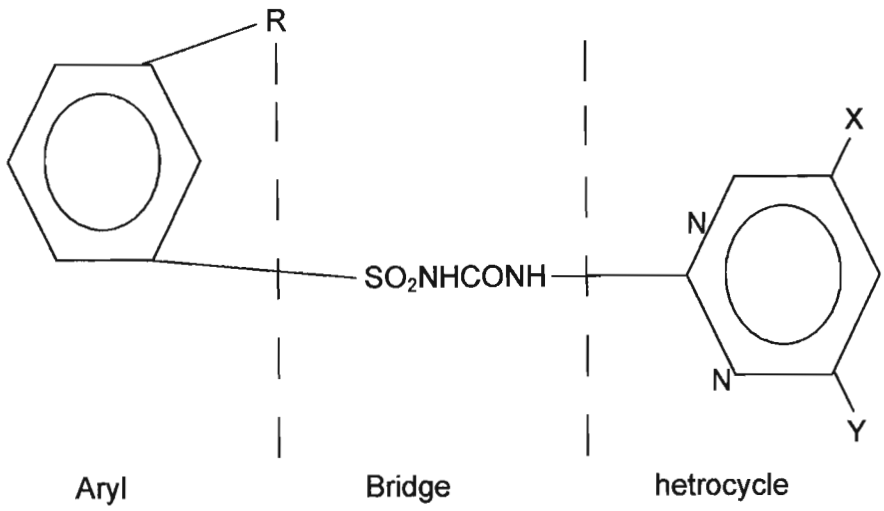

Figure 9: General structure of an ALS inhibitor (Beyer *et al.*, 1987).

When the aryl portion is a phenyl group, the highest herbicidal activity occurs when this group contains a substituent *ortho* to the bridge. Small substitution differences in each part of the molecule can give rise to large shifts in biological activity and selectivity. Beyer *et al.*, (1987) stated that sulphonylureas are potent inhibitors of plant growth. These compounds can be considered growth inhibitors that cause plant death within a period ranging from several days to more than a week. Depending on the plant specie, dose, and environmental conditions, a variety of secondary plant responses often develop. These include enhanced anthocyanin formation, loss of leaf nyctinasty, vein discoloration, necroses and chlorosis. The amino acids valine, leucine and isoleucine are products of the branched-chain amino acid pathway. Four of the enzymes of the pathway are common to synthesis of all three branched-chained amino acids. In the past decade, it had been found that sulfonylureas act on acetolactate synthase (ALS), the first enzyme in this pathway.

ALS is a nuclear-encoded, chloroplast-localized enzyme in higher plants, and also occupies a strategic location in the biosynthetic pathway of essential amino acids. Essentially, synthesis occurs in the stroma from threonine and pyruvate in a common series of reactions (Figure 10). In isoleucine synthesis threonine is first deaminated to 2-oxybutyrate by valine and isoleucine. ALS catalyses the first common step of branched-chain amino acid biosynthesis to yield acetohydroxy acids which undergo oxidation and isomerization to yield derivatives of valeric acid. Dehydration and transamination then produces isoleucine and valine. 2-Oxoisovalerate reacts with acetyl CoA to form α-isopropylmaleate which is then isomerized, reduced, and transaminated to yield leucine (Cobb, 1992).

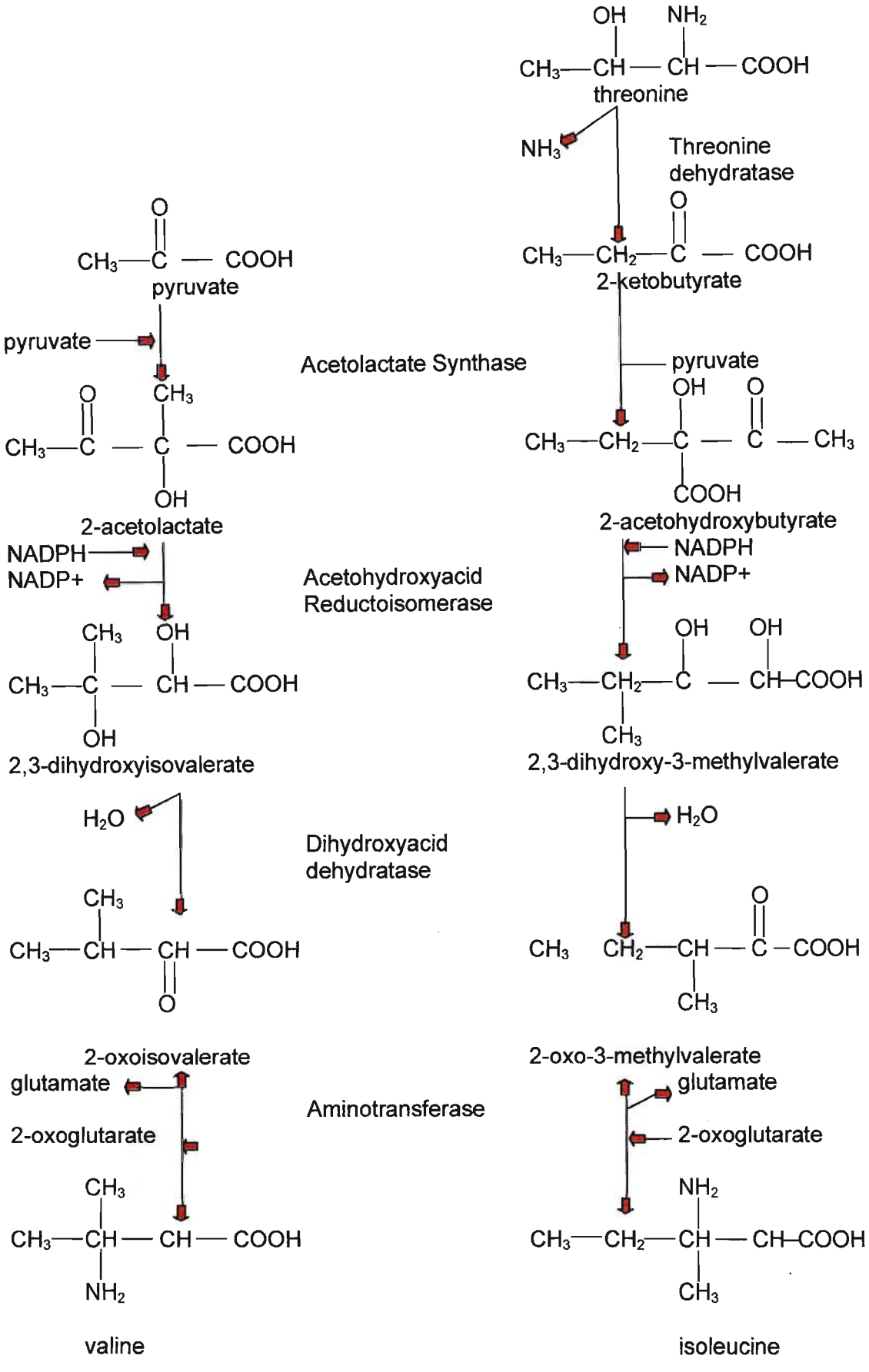

Figure 10: Biosynthesis of branched-chain amino acids (Cobb, 1992).

The precise mechanism of ALS inhibition remains uncertain. The isozyme II *from S. typhimurium* requires flavin adenine dinucleotide (FAD), thiamine pyrophosphate (TPP), and Mg^{2+} for complete activation, and the reaction proceeds in a biphasic manner. Firstly, a pyruvate molecule binds to TPP at the active site and is decarboxylated to yield an enzyme-substrate complex plus CO_2. Preliminary studies suggest that all three classes of ALS inhibitors bind at the same site. These observations suggest that ALS have some unique and unusual properties with respect to herbicide binding.

It is not known how treated plants die following ALS inhibition. An observation following treatment with ALS inhibitors is a very rapid and potent inhibition of cell division, with the result that an inhibition of elongation of young roots and leaves is evident within 3 hours after application. Studies done in the early 1980's indicated that chlorsulfuron blocked the progression of the cell cycle in dividing root cells from peas within 24 hours from G_2 to mitosis and reduced movement from G_1 to DNA synthesis. A striking feature of ALS inhibitors is their highly selective action at low dosage. Various studies have shown that extreme specie sensitivity is not due to herbicide uptake, movement, or sensitivity to ALS, but is correlated to very rapid rates of metabolism in the tolerant crop. A sulphonylurea may have a half-live of only two hours in the crop compared to two days in the weed (Cobb, 1992).

To summarize, ALS herbicides are highly potent at low rates and growth of weeds is inhibited within hours after foliar application, but physical symptoms may take days to appear. Chlorosis and necrosis will appear firstly in young meristematic regions. Young leaves appear wilted, leaf veins developed increased anthocyanin formation and leaf abscission commonly observed, both symptoms being typical responses to stress ethylene production.

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CHAPTER III

LOLIUM SPP. RESISTANCE TO ACCASE INHIBITORS IN WHEAT(*TRITICUM AESTIVUM* L.) WITHIN THE RSA: A PRELIMINARY STUDY.

3.1 Abstract

Herbicide resistance raises the possibility that the agricultural industry and crop producers might lose a valuable chemical tool that is essential for the control of weeds in wheat (*Triticum aestivum* L.) production. The recognition of herbicide resistance occurred relatively recently (1960), despite the widespread use of selective herbicides worldwide. Resistant biotypes of *Avena fatua* L. to diclofop-methyl (±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid were confirmed in the Western Cape. Recently, farmers and chemical companies reported resistance of *Lolium* spp. to several selective herbicides in small grains in the Western Cape. The main objective of the present study was to assess resistance of *Lolium* spp. against ACC-ase inhibitors used in wheat production. Seed of *Lolium* spp. which were suspected of being resistant were sown in pots in a glasshouse at a temperature regime of 15°C/20°C (night/day). During the 2-4-leaf stage, the plants were sprayed with three herbicides viz. diclofop-methyl, clodinafop-propargyl (2-propynyl (R)-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy-propionate], tralkoxydim (2-(1-[ethoxyimo)propyl]-3-hydroxy-5- (2,4,6-trimethylphenyl)cyclohex-2-enome) and imazamox, 2-[4,5-dihydro-4- methyl-4-(1-methylethyl) -5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarbolic acid at double the registered rates. Four weeks after herbicide application, percentage control was measured by comparing fresh plant mass. This study confirmed resistance of *Lolium* spp. to ACC-ase inhibitor graminicides registered for use in wheat in the Western Cape.

Keywords: ACC-ase Inhibitors, *Lolium* spp., resistance, wheat

3.2 Introduction

The recognition of herbicide resistance in weeds is relatively recent, despite the widespread use of selective herbicides for over 40 years (Moss & Rubin, 1993, Purba, Preston & Powles, 1996).

Weed resistance to herbicides concerns many sectors of the agricultural community such as farmers, advisors, researchers and the agrochemical industry. As cited by Bandeen, Stephenson & Cowett (1982) the development of herbicide resistant weeds was first observed in 1960 and Ryan (1970) reported that *Scenecio vulgaris* L. was resistant to both simazine (6-chloro-N,N'-diethyl1-1,3,5-triazine-2,4-diamine) and atrazine (6-chloro-N-ethyl-N'-(1-methylethyl-1,3,5-triazine-2,4-diamine). In most cases, observers do not usually recognize resistance if the percentage survival of treated weeds is less than 25% and when it is noticed it is often blamed on inadequate performance of the herbicide (Gill, Holmes & Kelley, 1994; Gressel, 1988). Sawicki (1987) as cited by Moss & Rubin (1993) defined resistance as a change in response to selections by toxicants that may impair control in the field. Jutsum & Graham (1995) reported that resistance is found in over 100 grass and broad-leaved species, occurring in more than 40 countries. LeBaron (1991) as cited by Rubin (1991) reported that by the year 1990, 113 herbicide-resistant weed biotypes had evolved at various locations worldwide. In Australia, wild oats *(Avena fatua* L.) and ryegrass (*Lolium* spp.), which are resistant to the aryloxyphenoxypropionates and cyclcohexanediones, infest millions of hectares (Morrison & Bourgeois, 1995). Cairns & Hugo (1986) confirmed resistant biotypes of wild oats in the Western Cape, while Smit (1993) confirmed tolerant biotypes of wild oats in the Western Cape.

Once resistance has been established, elimination thereof is impossible due to inheritance of the seed (Martin, Kelly & Gill, 1993). Because of dissemination with harvesting equipment and cross-pollination of *Lolium* spp., the spreading of resistance is unlimited (Holt & LeBaron, 1990). Anonymous (1997) reports that resistance is not built up against the active ingredient, nor the chemical group of a specific herbicide, but against the mode of action of a herbicide. In South Africa clodinafop-propargyl (2-propynyl (R)-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy-propionate], diclofop-methyl (±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid and tralkoxydim (2-(1-[ethoxyimo)propyl]-3-hydroxy-5- (2,4,6-trimethylphenyl)cyclohex-2-enome) are registered for the control of ryegrass (Vermeulen, Dreyer, Grobler & Van Zyl, 1996) in wheat. Clodinafop-propargyl *and*

diclofop-methyl belong to the aryloxphenoxypropionic acids, while tralkoxydim belongs to the cyclohexanediones. Both these two chemical groups have the same mode of action, which leads to inhibition of acetyl-CoA carboxylase (Anonymous, 1997 ; Retzinger & Mallory-Smith, 1997). Although chemically different, both groups target the same molecular site and produce similar effects on grass weeds. The ACC-ase group inhibit the plastic-localized enzyme that catalyses ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA in the lipid synthesis pathway of plants. ACC-ase is a pivotal enzyme in the plant lipid biosynthesis pathway (Dekker & Duke, 1995). A direct effect on membrane function has also been proposed as a site of action of these herbicides (Shimabukuro & Hoffer, 1992). However, this action does not appear to control plants at herbicidal doses (DiTomaso, Brown, Stowe, Linscott & Kochian, 1991).

Resistance will continue to increase if present herbicide use patterns are not altered. It cannot be overcome by the regular introduction of herbicides with new modes-of-action which are effective on resistant weeds, because the mode of action spectrum will increase and therefore resistance could develop easily towards the newly registered products. Therefore one should look at different practices such as crop rotation (Powles, Preston, Bryan & Jutsum, 1977), as an alternative control strategy. Resistance must also be assessed for herbicides which are used in other crops, such as pastures. Various pastures are the main crops used in a rotation system with wheat in the Western Cape (Smit, 1998, Small Grain Institute, Bethlehem, Republic of South Africa) and therefore it is of critical importance that imazamox, [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridine-carbolic acid], a newly registered herbicide for controlling *Lolium* spp. during the pasture phase, was included in the trials. Imazamox belongs to the imidazolinone group and does not share the same mode of action *as* diclofop-methyl, clodinafop-propargyl and tralkoxydim, but is an inhibitor of the acetolactate synthase enzyme (Anonymous,1997).

3.3 Materials and Methods

The study was conducted in a glasshouse with a day/night temperature regime of ±20°C/15°C. Seeds sampled at 10 different locations in the southern and western Cape were used in the trial (Table 1). All the seeds collected were from mature surviving plants and packed in paper bags and were transported to the Small Grain Institute (Bethlehem, Republic of South Africa), where experiments were done to

confirm resistance. Ryegrass was germinated in 2-L pots filled with sandy loam soil containing 15% clay.

Table 1: Seed collection sites.

No.	Nearest town	District
1	Hopefield	Western Cape
2	Ceres	Western Cape
3	Malmesbury	Western Cape
4, 5 & 6	Paarl	Western Cape
7	Durbanville	Western Cape
8 & 9	Caledon	Southern Cape
10	Riviersonderend	Southern Cape

At the 1-leaf stage the plants were thinned to three plants per pot to obtain plants of similar size and vigour. Seeds from susceptible biotypes were also established for control references. A water-soluble nutrient medium (6.5% N, 2.8% P, 13.0% K, 7.0% Ca, 2.2% Mg and 7.5% S) was supplied with regular irrigation during the trial.

3.3.1 Herbicide application

During the 2- to 4-leaf stages of the plants, herbicides were applied with an air-pressured pot sprayer operating at 200 kPa, fitted with two 8001 flatfan nozzles, delivering 310-L water ha^{-1}. The following herbicides were applied at double the registered rates, (1) diclofop-methyl (710 g a.i. ha^{-1}), (2) tralkoxydim, (320 g a.i. ha^{-1}), (3) clodinafop-propargyl (182.4 g a.i. ha^{-1}), (4) imazamox (96 g a.i. ha^{-1}). Although not yet registered in South Africa, the granular formulation of tralkoxydim was used in the trial. Registered adjuvants were added according to registration to obtain optimal efficacy.

3.3.2 Statistical analysis

Plant survival was recorded four weeks after herbicide application, by means of measuring fresh plant mass. The trial was a factorial experiment arranged as a randomized block design with four replications. Each pot represented a replication and there were four replications per treatment.

Data were expressed as a percentage reduction in fresh leaf mass in comparison with the treated control where 0% was total survival, 50%, suppression and 100% total mortality. Data were subjected to analysis of variance to determine the

significance of differences between means at the 5% level of probability using the Tukey test. After termination of experiments, surviving plants were rouged out by hand and left to dry before they were burned to prevent further seed depositing.

3.3.3 Results and Discussion

Significant differences were found herbicides and localities, as resistance occurred to the ACC-ase inhibitors, which includes herbicides from the aryloxyphenoxypropionates and the cyclohexanediones (Table 2).

Table 2: Control achieved by treating seedlings of *Lolium* spp. from 10 different locations with different herbicides

Herbicides	Collections of 10 Locations (No.)									
	1	2	3	4	5	6	7	8	9	10
Diclofop-methyl	R	R	R	R	R	C	R	R	R	R
Tralkoxydim	R	R	R	C	R	C	R	C	R	S
Clodinafop-Propargyl	R	R	R	R	R	R	R	C	R	R
Imazamox	C	C	C	C	C	C	C	C	C	C

R=Resistant, C= Control, S= Suppress

$LSD_T(0.05)=2.64$

CV = 8.5

Resistance to diclofop-methyl and clodinafop-propargyl was found in 90% of the localities, while resistance against tralkoxydim was found in 60% of the locations and at location no. 10, suppression of the weed was achieved. The differences in plant mass between the resistant and suppressed biotypes were large (Figure 1) and can be identified as a case of target-site resistance because the herbicide no longer binds to the enzyme. If it were a case of metabolism-based or another mechanism, the difference in percentage control between these two biotypes might be smaller. According to Devine, Duke & Fedtke (1993), differences in susceptibility between the aryloxypropionates and the cyclohexanediones might occur and could therefore be an explanation for the variation in susceptibility to ACC-ase inhibitors (Table 2). Imazamox gave excellent control of the plants tested, probably because of the different mode of action. Although imazamox is not registered for the use in wheat production, it can be applied in a crop rotation system with a pasture such as medics (*Medicago truncatula* L.).

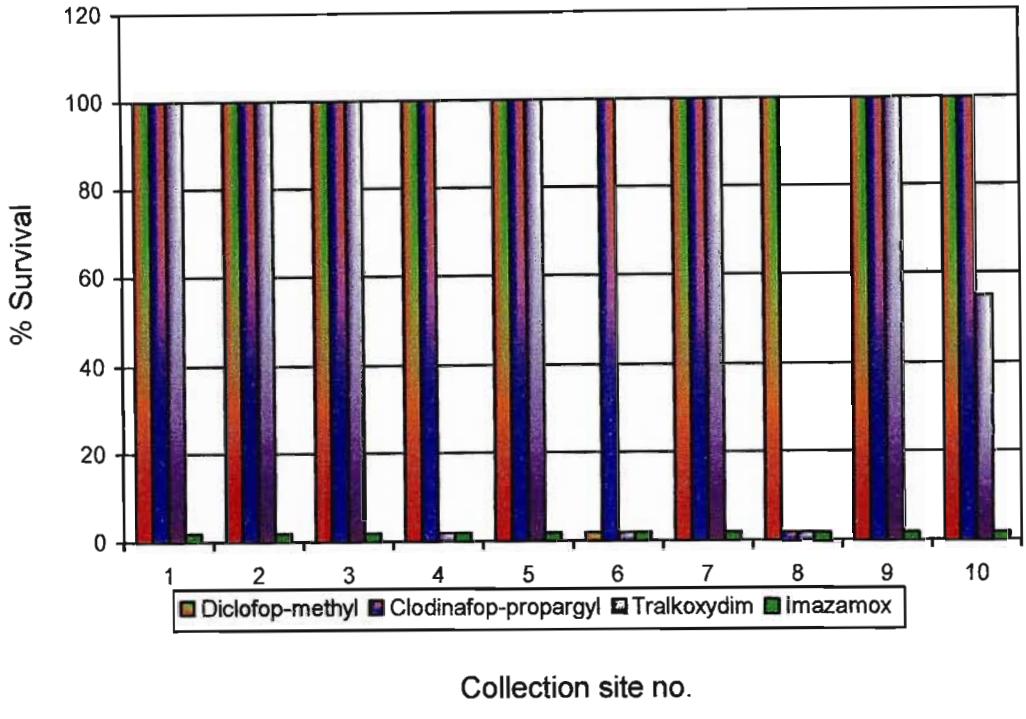

Figure 1: *Lolium* spp. survival (%) at 10 localities.

3.3.4 Conclusions

Practical implications of the results are far-reaching. Farmers will not be able to use ACC-ase inhibitors on the farms where resistance has been confirmed. For all practical purposes, they will not be able to control any ryegrass during wheat production, because all the grass weed herbicides, registered for the control of ryegrass, are ACC-ase inhibitors. Practices of crop rotation or bare fallow must be taken into consideration to eliminate the resistant weed. Further studies in this regard must be encouraged to implement management strategies for the prevention of ryegrass resistance and its spread to deal with the situation.

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CHAPTER IV

DIFFERENTIAL EFFICACY OF TRALKOXYDIM AND DICLOFOP-METHYL ON A SUSPECTED RESISTANT RYEGRASS (*LOLIUM RIGIDUM* GAUD.) BIOTYPE.

4.1 Abstract

The efficacy of herbicides belonging to the aryloxyphenoxy propionic acids and the cyclohexanedione groups was determined on a suspected resistant ryegrass biotype (*Lolium rigidum* Gaud.). Ryegrass seed were collected in the Southern Cape and germinated in a glasshouse with a day/night temperature regime of 20°C/15°C. Herbicide application was done at the third leaf stage of ryegrass, and tralkoxydim (2-(1-[ethoxyimo)propyl]-3-hydroxy-5-(2,4,6-trimethylphenyl)cyclohex-2-enone), (125, 250, 500 & 1250 g a.i. ha^{-1}) and diclofop-methyl (±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid (177.5, 355, 710 & 1775 g a.i. ha^{-1}) were applied. Forty-four days after herbicide application, percentage control was measured by means of fresh plant mass. The data show that less than 20% control was obtained with diclofop-methyl at rates up to 1775 g a.i. ha^{-1} (seven times the registered rate). All the formulations of tralkoxydim provided excellent ryegrass control at 250 g a.i. ha^{-1}. Resistance to diclofop-methyl and susceptibility to tralkoxydim within a single *L. rigidum* biotype was established.

Keywords: Diclofop-methyl, *Lolium rigidum*, resistance, susceptibility, tralkoxydim

4.2 Introduction

Ryegrass (*Lolium rigidum* Gaud.) is an annual cross-pollinated diploid grass weed, which occurs as a weed in small grain in the winter rainfall area (Bromilow, 1995; Grabandt, 1985). It is a very competitive weed in wheat lands, and yield losses as a result of competition with a *Lolium* spp. can be as much as 75%, depending on sowing date, cultivar, climate, seeding rate, etc. (Lemerle, Verbeek & Coombes, 1995; Reeves 1976). Sawicki (1987) as cited by Moss & Rubin (1993) defined resistance as a genetic change in response to selection by toxicants that may impair control in the field. Recent research indicated that resistance occurs differentially to the ACCase inhibitors throughout the winter rainfall region, where ryegrass is a problem weed (Smit & De Villiers, 1998).

Tralkoxydim is a postemergence graminicide, registered for the control of ryegrass and wild oats (*Avena fatua* L.) in wheat (*Triticum aestivum* L.), (Vermeulen, Dreyer, Grobler & Van Zyl, 1996). The herbicide belongs to the cyclohexanedione group and the mode of action is the inhibition of the acetyl-CoA carboxylase enzyme (Anonymous, 1997; Rendina & Felts, 1988; Secor & Cseke, 1988). Currently, two tralkoxydim formulations eg. a 250 g a.i. l^{-1} suspension concentrate, and a 100 g a.i. l^{-1} emulsifiable concentrate are registered in South Africa (Vermeulen *et al.*, 1996). An 800 g a.i. l^{-1} water dispersible granule is currently under development (Dreyer, 1998, Zeneca, Johannesburg, RSA). Herbicides belonging to the aryloxyalkanoic acids, such as diclofop-methyl have the same mode of action as tralkoxydim (Holtum, Matthews, Hausler, Liljegran & Powles, 1991). Both herbicide groups are selective postemergence graminicides and produce similar effects on grass weeds (Retzinger & Mallory-Smith, 1997). ACCase is a complex enzyme where three functional sites have been described, eg: a biotin carboxyl carrier site, an ATP-dependent biotin carboxylase and a carboxyltransferase (Devine, Duke & Fedtke, 1993).

Aryloxyphenoxypropanoates and cyclohexanediones are very different in structure and it has been speculated that, due to similarities in symptomology and selective properties, both groups of compounds share the same mode of action (Caseley, Cussans & Atkin, 1991; Anonymous, 1997). In most cases only a single "target site" within the plant could be found, which is the position where the herbicide binds or with which it interferes, resulting in death of the plant. Recent studies have provided evidence for this speculation (Caseley, *et al.*, 1991). Devine, *et al.*, (1993) on the other hand found that some extreme differences in susceptibility might occur. Preston, Tardif & Powles (1996a) indicated that some biotypes of *Lolium rigidum*

indicate resistance to the aryloxyphenoxy propionates, but are susceptible to sethoxydim and tralkoxydim. This indicates that there are several possible mechanisms within ACCase that can provide resistance to herbicides. The aim of this research was to establish whether a specific biotype of *L. rigidum* was resistant to diclofop-methyl, as well as to tralkoxydim.

4.3 Materials and Methods

The study was conducted in a glasshouse with a day/night temperature regime of 20°C/15°C respectively. *L. rigidum* seed was collected from mature surviving plants in the southern Cape and packed in paper bags to be transported to the Small Grain Institute (Bethlehem, Republic of South Africa). Ryegrass seed was germinated in one-litre pots filled with a sandy loam soil with a pH of 5.5. After emergence, at the one leaf stage of the plants, the seedlings were thinned to three plants per pot to obtain plants with similar size and vigour. A water-soluble nutrient medium (6.5% N, 2.8% P, 13.0% K, 7.0% Ca, 2.2% Mg and 7.5% S) was supplied with regular irrigation during the trial.

4.3.1 Herbicide application

Herbicides were applied 23 days after sowing at the three-leaf stage of the ryegrass plants. Treatments were applied with a pressurized sprayer fitted with two 8001 flat fan nozzles, delivering 310 litre ha^{-1} at a pressure of 200 kPa. Three formulations of tralkoxydim viz. an emulsifiable concentrate, a suspension concentrate and a water dispersible granule, were applied at 125, 250, 500 and 1250 g a.i. ha^{-1}. Diclofop-methyl was applied at 177.5, 355, 710 and 1775 g a.i. ha^{-1}. Registered rates of EC and SC formulations of tralkoxydim are 150 and 225 g a.i. ha^{-1} respectively. The registered rate of diclofop-methyl is 255.6 g a.i. ha^{-1}. Registered adjuvants were added according to registration to obtain optimal efficacy. Tralkoxydim was applied with Embrace (500 g l^{-1} / 500 g. l^{-1} non-ionic surfactant) at 0.5% (v/v). No adjuvant was used with diclofop-methyl.

4.3.2 Evaluation

Plant survival was recorded 44 days after sowing, by means of measuring fresh plant mass.

4.3.3 Statistical analysis

The trial was a factorial experiment, arranged as a completely randomized block design and the treatments were replicated four times. Each pot represented a

replication. Data were expressed as a percentage reduction in fresh plant mass in comparison to an untreated control. Data were subjected to analysis of variance to determine the significance of differences between means at the 5% level of probability, using the Tukey test. After termination of experiments, surviving plants were rouged out by hand and left to dry before they were burned to prevent further seed depositing.

4.3.4 Results

The data (Figure 1) show that efficient control was obtained with all three formulations of tralkoxydim at 125 g a.i. ha^{-1}. At 250, 500 and 1250 g a.i. ha^{-1} 100 % control was obtained with tralkoxydim. No control was obtained with diclofop-methyl at the rates tested up to 710 g a.i. ha^{-1}, and only 20 % control was achieved with 1775 g a.i. ha^{-1} (Figure 2).

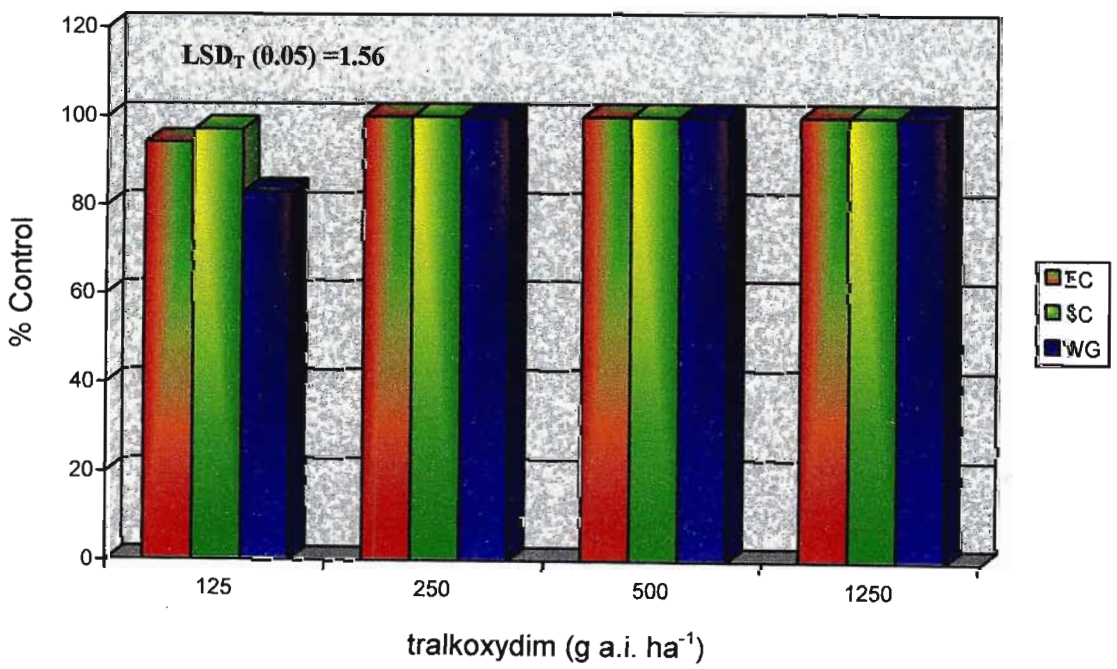

Figure 1: Percentage control of ryegrass achieved with tralkoxydim (Sc, Ec & Wg) formulations.

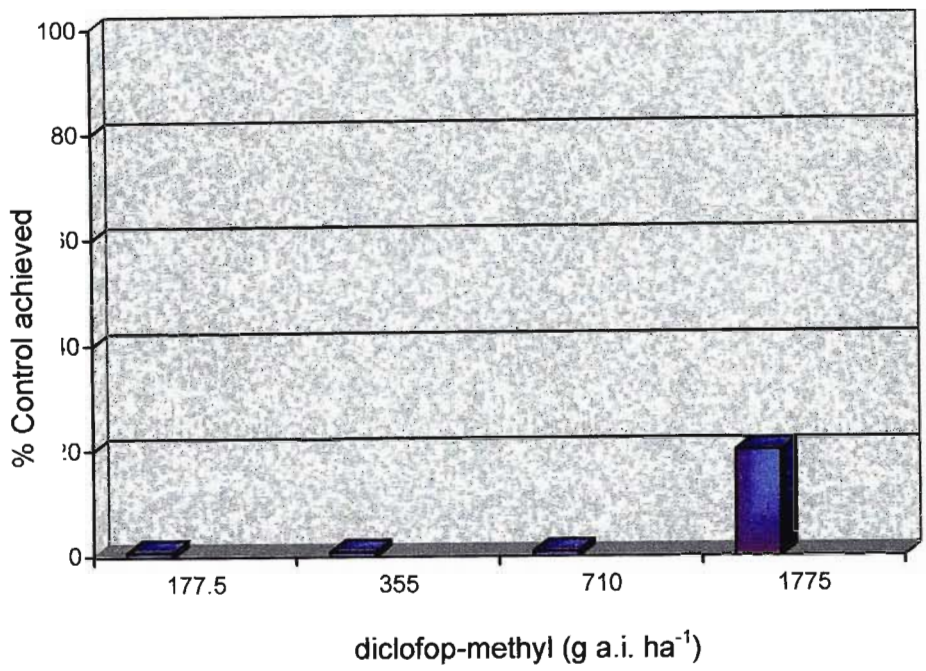

Figure 2: Percentage control of ryegrass achieved with diclofop-methyl

4.3.5 Discussion

It is clear from the data (Plate 1) that resistance occurred at seven times the registered rate of diclofop-methyl, while this specific biotype of *L. rigidum* is susceptible to tralkoxydim (Plate 2). According to Caseley *et al.*, (1991) one would have expected a similar response of the two compounds used in this study, because both are ACC-ase inhibitors. Ryegrass populations are multiple-resistant when the population is resistant to different chemical classes, affecting different target sites (Tardif, Holtum & Powles, 1994; Gressel, 1988) and these different resistance mechanisms can involve a target site and/or a non-target site mechanism (Holtum *et al.*, 1991). On the other hand, cross-resistance is a phenomenon where resistance occurs to herbicide groups to which the population has never been exposed (Preston, Tardif & Powles, 1996). According to Preston, Tardif, Christopher & Powles (1996b), there are several modifications of ACC-ase, which endow resistance to cyclohexanediones and aryloxyphenoxy propionic acids, and the pattern of cross-resistance is determined by the likelihood and dynamics of gene exchange by pollen or type of mutation and could therefore not be predicted. This factor will influence whether cross-pollinated weeds (like *L. rigidum*) will have one or several resistance mechanisms. It is clear from the data that no multiple- or cross-resistance occurred during this study with this specific biotype *of L. rigidum*.

Data from this study indicates that this Ryegrass biotype developed a mechanism to detoxify diclofop-methyl and is therefore resistant towards diclofop-methyl. The fact that this biotype is susceptible to tralkoxydim indicates the complexity of an enzyme / inhibitor interaction and that there are several possible mutations within ACC-ase that can provide resistance (Preston *et al.*, 1996a). According to Preston *et al.*, (1996b) the metabolites of tralkoxydim are not known and any susceptibility to tralkoxydim is due to an altered target site or to the enchange metabolism of tralkoxydim. A possible explanation for the results from this study could be that this specific Ryegrass biotype has only a single mechanism of resistance, which led to resistance to diclofop-methyl.

Plate 1: Control achieved with minimum rates of tralkoxydim and diclofop-methyl.

Plate 2: Control achieved with rates up to seven times registration requirements.

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CHAPTER V

RESISTANCE OF *RAPHANUS RAPHANISTRUM* TO CHLORSULFURON IN THE REPUBLIC OF SOUTH AFRICA

5.1 Abstract

Herbicide resistance poses a substantial threat to the agricultural industry throughout the world and during the last decade several reports regarding herbicide resistance have been published. *Raphanus raphanistrum* L. (wild radish) from two wheat (*Triticum aestivum* L.) farms located in the winter rainfall region of South Africa, showed indications of resistance to chlorsulfuron. Seed from these suspected resistant biotypes as well as seed from a susceptible biotype were collected and transported to the ARC-Small Grain Institute (Republic of South Africa) where herbicide resistant studies were done. Herbicides registered for wild radish control are chlorsulfuron, MCPA and bromoxynil and were used in this study. Significant differences in the degree of control were found between the susceptible and two resistant biotypes, when treated with chlorsulfuron. The LD_{50} values for the resistant biotypes (WR 1 & WR 2) were 45 and 11.3 g a.i. ha^{-1} respectively, whereas the LD_{50} value for the susceptible biotype was 5.6 g a.i. ha^{-1} in the chlorsulfuron experiments. There was an eightfold difference between the susceptible and resistant biotype (WR 1), indicating that resistance has developed to chlorsulfuron. Only twofold resistance was established between the other resistant biotype (WR 2) and the susceptible biotype. Significant differences between herbicide rates were also established with the MCPA and bromoxynil experiments. No significant difference could however, be found between the susceptible and resistant biotypes when treated with MCPA and bromoxynil, indicating the importance of different modes of action of herbicide as a strategy to prevent herbicide resistance.

Keywords: ALS inhibitor, bromoxynil, chlorsulfuron, MCPA, and resistance

5.2 Introduction

According to Ryan (1970) as cited by Bandeen, Stephenson & Cowett (1982), the development of herbicide resistant weeds was first observed in 1960. Since the first report of resistance, various reports of herbicide resistant weeds have been published (Christopher, Powles & Holtum, 1992; Purba, Preston & Powles, 1993; Burnet et al., 1994; Boutsalis & Powles, 1995 and Smit & De Villiers, 1998). These publications include reports of resistance of various weeds species resistant to herbicides with different modes of action.

According to Shaner (1991), two groups of highly potent herbicides have been introduced into the weed control market during the last decade. These are the imidazoliones and Sulfonylureas representing a new generation of low rate herbicides, with extremely low mammalian toxicity. Both imidazoliones and sulfonylureas kill plants by inhibiting the enzyme that catalyzes the first step in branched amino acid biosynthesis, acetolactate synthase (ALS) (Shaner, Anderson & Stidham, 1984, Anonymous, 1977 and Retzinger & Mallory-Smith, 1977). Shaner (1991) stated that the most widely used of the sulfonylureas were chlorsulfuron and metsulfuron. According to Sarmah, Kookana & Alston (1998), chlorsulfuron is a weak acid with pKa of 3.6 and James, Holland & Rahman (1998) stated that chlorsulfuron is present as an anion in soils with a low pH. Both herbicides are potent to susceptible weeds and persist in the soil for several months. According to Vermeulen, Grobler & Van Zyl (1998) chlorsulfuron is a water dispersible granule postemergent herbicide, acting through the roots and foliage, controlling broad-leaved weeds in wheat (*Triticum aestivum* L.). According to Senekal (1997) the total usage of herbicides for dicot weed control in wheat production systems in the winter rainfall region of the Republic of South Africa, was about 18.5 million Rand during 1996. A total of 63% of this market during the same year belonged to sulfonylurea herbicides and sulfonylurea popularity is still increasing, due to low doses, broad weed spectrum and ease of application. The rapid and widespread use of ALS-inhibiting herbicides in South Africa for the control of a wide range of annual broad-leaved weeds could therefore result in the development of herbicide resistance. According to Thill, Mallory, Saari & Cotterman (1989) as cited by Shaner (1991), such a pattern is ideal for the selection of sulfonylurea resistant weed populations. Selection pressure imposed by sulfonylurea herbicides is likely to increase in South Africa by the marketing of two new ALS-inhibiting herbicides, iodosulfuron and sulfosulfuron. The imidazolinone, imazamox 2-[4.5dihydro-4-methyl-4- (1-methylethyl) -5-oxo-iH-imidazol-2-ul] -5-(methoxymethyl) -3-pyridenecarbolic acid is

used in legume pastures for control of dicot and monocot weeds and would also contribute to the selection of ALS-inhibiting herbicide resistant weeds. According to Christopher *et al.*, (1992) the first cases of sulfonylurea resistance was reported by Mallory-Smith, Thill & Dial (1990) and Primiani & Saari (1990) and involved weeds such as *Kochia scoparia* L. and *Lactuca serriola* L.

To date no report of resistance to sulfonylureas or imidazolinone herbicides has been recorded in the RSA. The aim of this study was to determine if a biotype of *Raphanus raphanistrum* L. (wild radish) is resistant or susceptible to chlorsulfuron.

5.3 Materials and Methods

5.3.1 Plant Culture

Wheat production in the RSA can be divided into two main production areas viz. the winter rainfall area (Western and Southern Cape) and the summer rainfall area (Free State Province). Suspected resistant *R. raphanistrum* seed from the Paarl district (hereafter referred to as WR 1) and seed from the Malmesbury area (WR 2) were collected from mature surviving plants and transported to the ARC-Small Grain Institute (Bethlehem, RSA) to be tested for resistance. Both of these towns are situated in the Western Cape (RSA). Complete herbicide records were not available, but the landowners confirmed that chlorsulfuron had been used frequently during the previous seven to nine years. Seed from the WR 1 and WR 2 biotypes were survivors of 9.75 and 13.12 g a.i. ha^{-1} chlorsulfuron treatment in a wheat production system during the winter of 1998. According to Kremer & Kropff (1998) a correct comparison of growth should be made under optimal growing conditions, using populations of both susceptible and resistant biotypes originating from several geographical origins to determine the variation in growth parameters within and between populations. Therefore, seed of a susceptible population (hereafter referred to as S) with no history of sulfonylurea use, was used as the sensitive control during this study. Seed of WR 1, WR 2 and S biotypes were germinated in two-litre pots filled with a sandy loam soil with a pH of 5.5. The seedlings were thinned out at the one leaf stage to three plants per pot to ensure plants with similar size and vigour. The study was conducted in a glasshouse with a day/night temperature regime of 20°C/15°C respectively. To increase seed for later studies, plants of S, WR 1 & WR 2 biotypes were grown in a glasshouse during the summer of 1998. Resistant plants used for seed multiplication were survivors of a 12.8 g a.i. ha^{-1} chlorsulfuron application.

5.3.2 Herbicide application

Three experiments were conducted, one with an ALS-inhibitor herbicide, one auxin growth regulator and one with an inhibitor of photosynthesis at photosystem II. The following herbicides were included in the experiments, chlorsulfuron (Glean, 750 g a.i. kg^{-1} Du Pont De Numerous Int. SA), MCPA (MCPA 400 g a.i. L^{-1} Zeneca Agrochemicals SA (Pty) Ltd) and bromoxynil (225 g a.i. L^{-1} Sanachem (Pty) Ltd). Chlorsulfuron represented an ALS-inhibitor, MCPA an auxin growth regulator and bromoxynil an inhibitor of photosynthesis at photosystem II. Chlorsulfuron was applied at rates between 1.4 and 90 g a.i. ha^{-1}, MCPA at rates between 100 and 6400 g a.i. ha^{-1} and bromoxynil at rates between 42 and 2700 g a.i. ha^{-1}. Registered rates of chlorsulfuron, MCPA and bromoxynil for the control of *R. raphanistrum* are 11.3, 800 and 337 g a.i. ha^{-1} (Vermeulen *et al.*, 1998). Treatments were applied with a pressurized sprayer fitted with two 8001 flat fan nozzles, delivering 250 liter ha^{-1} at a regulated pressure of 150 kPa. During herbicide application, no substance was used to cover the soil surface to allow maximum root- and foliar absorption from plants, resulting in optimum absorption and translocation of the herbicides. Following herbicide applications, the pots were watered daily to avoid water stress.

5.3.3 Evaluation

According to Holt & LeBaron (1990) as cited by Moss & Rubin (1993), many studies relating to resistant weeds have concentrated on the evaluation of parameters as indicators of fitness such as growth rate or plant biomass. Silvertown (1987) stated that the two fundamental components of fitness are survival and reproduction. Therefore, percentage survival was recorded 30 days after herbicide application, by means of measuring plant biomass. The aboveground parts of plants were harvested, oven-dried (60°C for 48h) and weighed on a high decimal precision scale. The plant biomass data were also used to determine the respective LD_{50} values for the S, WR1 and WR2 biotypes. After termination, subsoil plant remains were roughed out by hand and left to dry before they were burned to prevent regrowth and further seed depositing. This precaution was necessary to prevent possible escape of sulfonylurea resistant weeds into the summer rainfall area where resistance to these herbicides has not yet been reported.

5.3.4 Statistical analysis

Experiments with chlorsulfuron, MCPA and bromoxynil were repeated and the data were combined for statistical purposes. The trials were factorial experiments; arranged as a completely randomized design and the treatments were replicated four

times. Data were subjected to analysis of variance to determine the significance of differences between means at the 5% level of probability, using the Tukey test. Data obtained from plant biomass are expressed as a percentage reduction in plant biomass in comparison to a treated susceptible control.

5.3.5 Results

Results of percentage survival obtained from plant biomass, of the three different biotypes (WR 1, WR 2 & S) compared with herbicide rates of chlorsulfuron, MCPA and bromoxynil, are presented in Figures 1, 2 and 3 respectively.

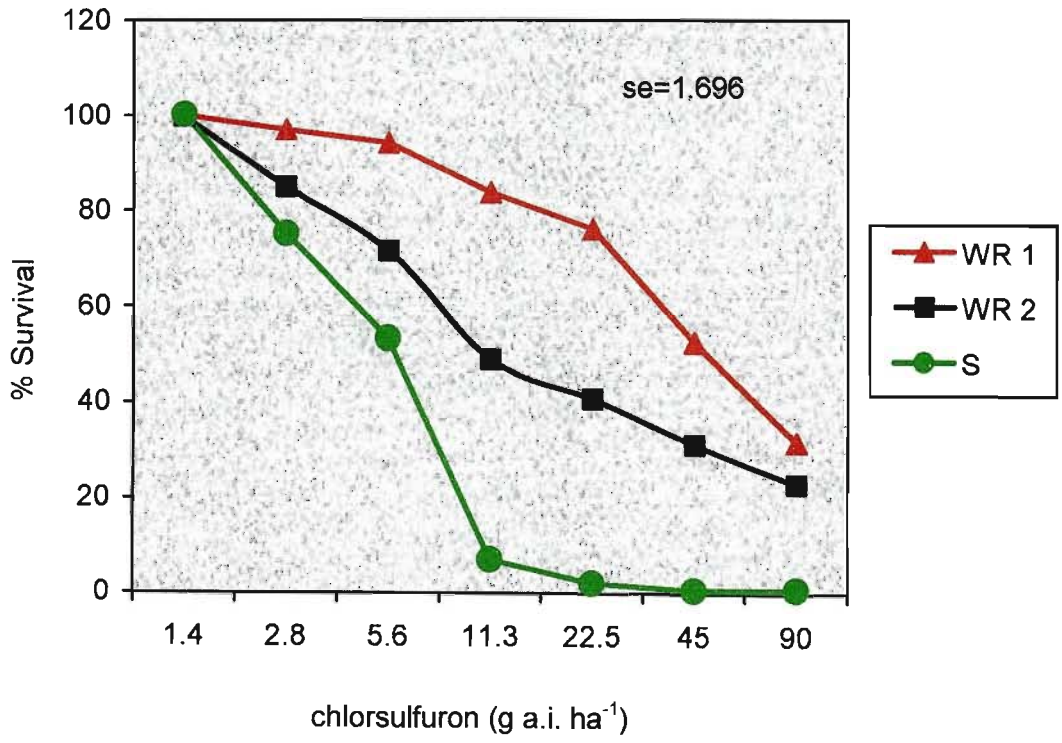

Figure 1: Survival (%) of WR 1, WR 2 and S biotypes of *R. raphanistrum* 30 days after treatment with chlorsulfuron. Each point represents the average percentage survival of 24 plants.

The WR 1 biotype of *R. raphanistrum* showed 52% survival to chlorsulfuron when applied at 45 g a.i. ha^{-1}. In contrast, the S biotype suffered 93% mortality when chlorsulfuron was applied at 11.3 g a.i. ha^{-1}. On the other hand, the WR 2 biotype showed 49% survival to chlorsulfuron when applied at 11. 3 g a.i. ha^{-1} (Figure 1). The calculated LD_{50} for the WR 1 and WR 2 biotypes were 43.3 and 11.5 g a.i. ha^{-1} respectively, whereas that for the S biotype was 5 g a.i. ha^{-1}. Therefore, there is an eightfold difference between the WR 1 and S biotype and a twofold difference between the WR 2 and the S biotype when treated with chlorsulfuron. Significant differences were also achieved between the WR 1 and the WR 2 biotypes when treated with chlorsulfuron, except when chlorsulfuron was applied at 1.4 and 2.8 g a.i. ha^{-1}.

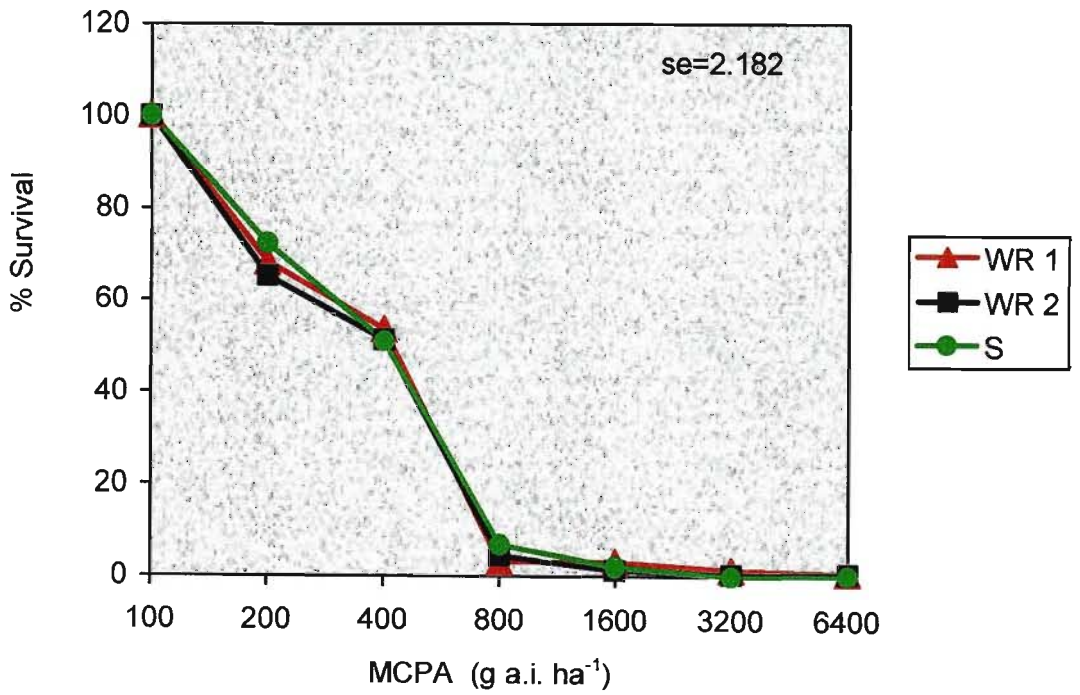

Figure 2: Survival (%) of WR 1, WR 2 and S biotypes *of R. raphanistrum* 30 days after treatment with MCPA. Each point represents the average percentage survival of 24 plants.

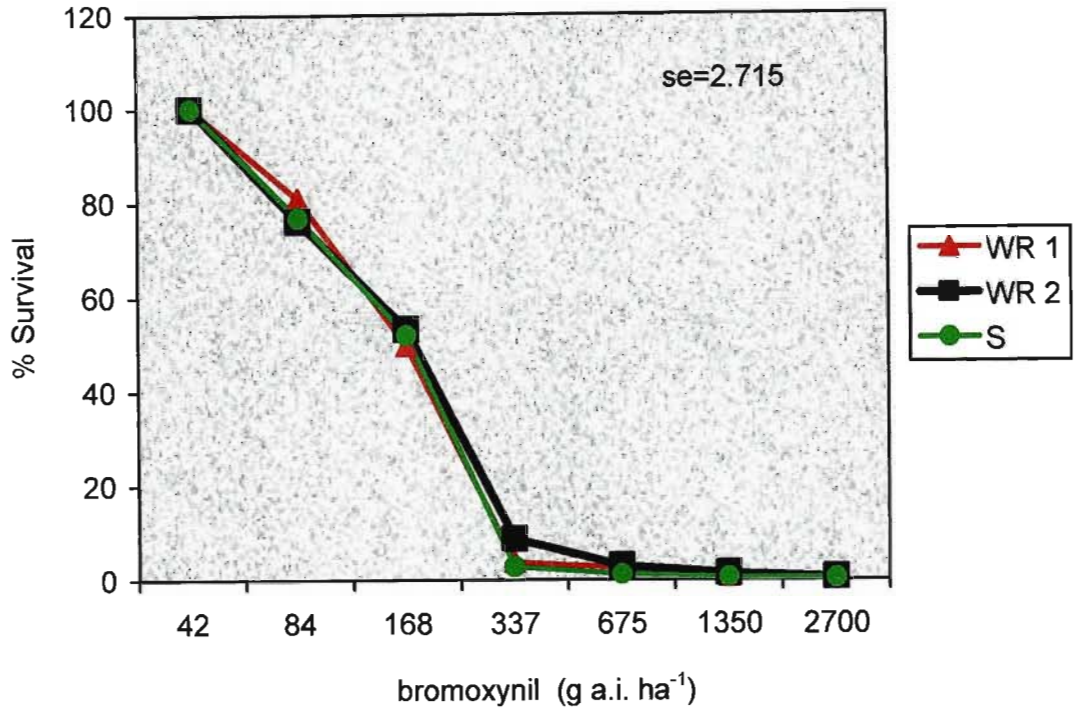

Figure 3: Survival (%) of WR 1, WR 2 and S biotypes of *R. raphanistrum* 30 days after treatment with bromoxynil. Each point represents the average percentage survival of 24 plants.

In contrast, no significant differences could be found between the WR 1, WR 2 and S biotype when sprayed with MCPA (Figure 2) and bromoxynil (Figure 3). The calculated LD_{50} values for the S, WR 1 and WR 2 biotypes were 394, 376 and 392 g a.i. ha^{-1} respectively and 97% mortality was achieved with 800 g a.i. ha^{-1} (Figure 2) when sprayed with MCPA during this pot trial. With the bromoxynil applications the calculated LD_{50} values were achieved with 169, 158 and 161 g a.i. ha^{-1} respectively with the WR 1, WR 2 & S biotypes, while 96% mortality was achieved with 337 g a.i. ha^{-1}. The only significant difference in Figure 1 and 2 were the difference between herbicide rates. With above results in mind, the conclusion can be made that both the WR 1 and the WR 2 biotypes became resistant to chlorsulfuron but are still susceptible to MCPA and bromoxynil applications.

5.3.6 Discussion

According to Cairns (unpublished observations.) the selection pressure for the development of resistance to herbicides is significantly related to the persistence of the herbicide in the soil and Gressel (1991) found that herbicides which persist in the soil for long periods tend to speed up the development of resistance. Sarmah *et al.*, (1998) found that soil pH plays an important role in the degradation of chlorsulfuron. Shaner (1991) stated that chlorsulfuron persists for several months in the soil. According to Ravelli, Pantani, Calamai & Fusi (1997) the degradation of chlorsulfuron decreases with an increase in soil pH. Groves & Foster (1985) found that the persistency of chlorsulfuron is not influenced by the soil texture, but decreases with an increase in soil organic matter. Kotoula-Syka, Eleftherohorinos, Gagianas & Sficas (1993) also found that the leaching of chlorsulfuron increased with increasing soil pH in both sandy loam and silty clay soils. The persistency of and continuous use of chlorsulfuron could therefore contribute to the selection process of a weed resistant population in the RSA. There are 17 different active ingredients registered for the control of *R. raphanistrum* in the RSA on wheat (Vermeulen *et al.*, 1998). However, according to the classification of herbicides regarding mode of action, 14 of these 17 different active ingredients share the same mode of action, viz. ALS inhibitors (Anonymous, 1997; Retzinger & Mallory-Smith, 1997). Therefore, one can conclude that there are a limited number of herbicides available for the control of *R. raphanistrum.* Regarding information such as annual use of chlorsulfuron, persistency of chlorsulfuron in relative low organic matter soils combined with relative low pH soils and the high percentage use of other sulphonylureas (similar modes of action), it is therefore not surprising that resistance to chlorsulfuron was established in the winter rainfall region of the Republic of South Africa.

In this study it became evident that a *R. raphanistrum* biotype that gained resistance to chlorsulfuron, is still susceptible to MCPA and bromoxynil applications. The main reason for the efficacy of MCPA and bromoxynil is because it does not share the same mode of action of chlorsulfuron (Retzinger & Mallory-Smith, 1997). Powles Preston, Bryan & Jutsum (1997) stated that sulphonylureas have enjoyed spectacular success since their introduction to the weed control market, but resistance is a major threat to their continued efficacy. This is therefore the first report in the Republic of South Africa of a broad-leaved weed resistant to an ALS inhibitor, resulting from the use of sulphonylureas.

To ensure the efficacy of chlorsulfuron and other ALS-inhibitors in the future, special effort must be implemented to inform the farmer of the importance of rotation of mode of action to manage and/or to prevent the development of herbicide resistance.

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CHAPTER VI

LITTLE SEEDED CANARY GRASS (*PHALARIS MINOR* RETZ.) RESISTANT TO ACC-ASE INHIBITORS

6.1 Abstract

Since 1996, several incidents of poor weed control regarding little seeded canary grass (*Phalaris minor* Retz.) in wheat (*Triticum aestivum* L.) production have been reported from the Western Cape. Little seeded canary grass showed indications of resistance towards ACC-ase inhibitors. Seed from these suspected resistant biotypes as well as seed from a susceptible biotype, were collected from mature surviving plants and transported to the Small Grain Institute, Bethlehem where herbicide resistance studies were done. Herbicides used during the study were diclofop-methyl, clodinafop-propargyl and iodosulfuron. Both diclofop-methyl and clodinafop-propargyl are ACC-ase inhibitors and iodosulfuron is an ALS-inhibitor. Significant differences in the degree of control were found between the susceptible and four resistant biotypes when treated with diclofop-methyl and clodinafop-propargyl. When treated with diclofop-methyl, the LD_{50} values for the resistant (PMR 1, PMR 2, PMR 3 & PMR 4) biotypes were 594, 700, 225 & 2673 g a.i.ha^{-1} respectively, whereas the LD_{50} value for the susceptible biotype was 184.4 g a.i.ha^{-1}. The LD_{50} values for the PMR 1, PMR 2, PMR 3 & PMR 4 biotypes treated with clodinafop-propargyl, were 79, 94, 29 & 280 g a.i.ha^{-1} respectively, whereas the LD_{50} value for the susceptible biotype was 24 g a.i.ha^{-1}. No significant differences could be found between the susceptible and resistant biotypes when treated with iodosulfuron. The LD_{50} values for the susceptible and resistant biotypes varied between 5.6 & 6.08 g a.i.ha^{-1}. This is therefore the first report, confirming *P. minor* resistance to diclofop-methyl and clodinafop-propargyl treatments in the Republic of South Africa.

Keywords: clodinafop-propargyl, diclofop-methyl, herbicide resistance, *Phalaris minor*

6.2 Introduction

Depending on the viewpoint of an individual, any plant can be categorized as a weed. To some they are plants growing where they are not wanted, or plants in the wrong quantity at the wrong time and to others they are regarded as plants whose virtues have not yet been discovered. On a global basis, about 250 species are sufficiently troublesome to be termed weeds and need to be controlled to ensure effective crop production (Cobb, 1992). Lacey (1985) found yield losses up to 92% in Venezuela due to weed competition. According to Afentouli & Eleftherohorinos (1999) little seeded canary grass (*Phalaris minor* Retz.) is a common and troublesome grassweed in winter cereals throughout Mediterranean countries. The most likely reasons for its prevalence are its similar growth period to winter cereals, early seed dispersal, and ability of seeds to remain dormant in the soil for several years. Several studies indicate 30 to 60% wheat yield reduction from little seeded canary grass competition related to species, plant density, timing of emergence, wheat (*Triticum aestivum* L.) density as well as environmental factors (Afentouli & Eleftherohorinos, 1996; Cudney & Hill, 1979).

Reports of weeds that are resistant to herbicides are now common in almost every country and in almost all crops where herbicides are routinely used for weed control (Moss & Rubin, 1993). In India, it is reported that over 200 000 ha of prime wheat land are so heavily infested with *P. minor* resistant to isoproturon, that many farmers have had to abandon wheat production for varying time periods. This was done to reduce *Phalaris* populations since effective alternative herbicides have not been available (Sayre, 1998). According to Moss & Rubin (1993) the potential threat of herbicide resistant weeds are considerable in current weed management strategies while Stoltenberg & Wiederholt (1995) stated that the confirmation of resistant weed species is essential before effective management strategies can be adopted to prevent the spreading of herbicide resistant weeds.

In a monoculture wheat production system, the agricultural community relies heavily on the use of herbicides to ensure optimum yields. Unfortunately, herbicides registered for the control of *P. minor* in the past belonged to the Aryloxyphenoxy propionic acids (Vermeulen, Grobler & Van Zyl, 1998) and should therefore share the same mode of action, viz. ACC-ase inhibitors (Anonymous, 1997 and Retzinger & Mallory-Smith, 1997). According to Jutsum & Graham (1995), as cited by Adkins *et al.*, (1997) the adverse consequence of repeated use of herbicides has led to the emergence of resistant weed populations.

Reports of poor *P. minor* control with grass weed herbicides from the Western Cape started during 1997 and the cause of the problem has had to be investigated. To date no scientific confirmation of *P. minor* resistance to grass weed herbicides used in wheat, has been reported in the Republic of South Africa. This study was therefore conducted to establish if *P. minor* biotypes were either resistant or susceptible to registered grass weed herbicides.

6.3 Material and Methods

6.3.1 Plant culture

Seed from *Phalaris minor* plants that survived diclofop-methyl (+)-2-[4-(2,4-dichlorophenoxy)phenoxy]propionic acid and clodinafop-propargyl 2-propynyl (R)-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy-propionate] treatments were collected from mature surviving plants from nine winter wheat fields (Table 1) in the Western Cape, packed in brown paper bags and transported to the Small Grain Institute (Bethlehem, Free State Province) to be tested for herbicide resistance.

Table 1: Origin of suspected resistant *P. minor* populations from the Western Cape and history of herbicide use.

		History of products	
Population	Origin	Mode of action	Years used
PMR 1	Philadelphia	ACC-ase	±9
PMR 2	Durbanville	ACC-ase	±12
PMR 3	Paarl	ACC-ase	±6
PMR 4	Malmesbury	ACC-ase	±10
PMR 5	Paarl	ACC-ase	±7
PMR 6	Durbanville	ACC-ase	±6
PMR 7	Philadelphia	ACC-ase	±9
PMR 8	Philadelphia	ACC-ase	±8
PMR 9	Malmesbury	ACC-ase	±8

To avoid duplication of results, this paper reports the procedures followed, as well as the results of the PMR 1, PMR 2, PMR 3 & PMR 4 biotypes compared to the S biotype. The PMR 1, PMR 2 and PMR 3 biotypes survived a 396 g a.i. ha^{-1} diclofop-methyl (360 g a.i. L^{-1}) treatment. While the PMR 4 biotype survived a 48 g a.i. ha^{-1} clodinafop-propargyl (240 g a.i. L^{-1}) treatment in a wheat production system during the winter of 1998. Although complete herbicide records were not available, landowners confirmed that aryloxyphenoxy propionate and cyclohexanedione herbicides had been used frequently during the previous 6 to 12 years (Table 1). To determine the variation in growth parameters within and between populations, Kremer and Kropff (1998) suggest that a correct comparison of growth should be made under optimal growing conditions, using populations with both susceptible and resistant biotypes that came from several geographical origins. Therefore, seed of a susceptible *P. minor* biotype (hereafter referred to as S) with no history of herbicide exposure was collected to serve as control plants during this study. Seed of PMR 1, PMR 2, PMR 3 and PMR 4 biotypes as well as the S biotype were germinated in two-litre pots filled with a sandy-loam soil (2:1:1 w/w) containing the necessary macro and micro nutrients. The seedlings were thinned to three plants per pot within 10 days of emergence to ensure plants with similar size and vigour. The study was conducted in a glasshouse with a temperature regime of 20°C/15°C (day/night) respectively.

6.3.2 Herbicide application

Three experiments were conducted, one with diclofop-methyl, one with iodosulfuron (Hussar 50 g a.i. ha^{-1}, Agrevo RSA) and the other one with clodinafop-propargyl, all registered for the control of *P. minor* in wheat production (Vermeulen *et al.*, 1998; C. Cummins, Agrevo SA, Personal communication). Retzinger & Mallory-Smith (1997) regard diclofop-methyl and clodinafop-propargyl are ACC-ase inhibitors. Iodosulfuron is a sulfonylurea and should therefore be considered an ALS inhibitor (Anonymous, 1997). Diclofop-methyl was applied at rates between 45 and 2880 g a.i. ha^{-1}, iodosulfuron between 1.25 and 80 g a.i. ha^{-1} and clodinafop-propargyl between 6 and 384 g a.i ha^{-1} (Table 2). An ethoxylate alkylpenol (Agral 90) was added to the iodosulfuron at 0.5% (v/v). Complement (mineral oil + nonyl-phenol ethoxylate surfactant) was added to the clodinafop-propargyl treatments at 0.5 L ha^{-1}. No adjuvant was added to the diclofop-methyl treatments. Herbicides were applied according to registration (Vermeulen *et al.*, 1998) at the two-leaf stage of seedling development. Registered rates of diclofop-methyl, clodinafop-propargyl and iodosulfuron are 360, 48 & 10 g a.i. ha^{-1} respectively (Vermeulen *et al.*, 1998; C.

Cummings. Personal communication). Commercial formulations of herbicides, supplied by chemical companies, were used in this study.

Table 2: Herbicide rates (g a.i. ha^{-1}) used in experiments

Treatment	diclofop-methyl (360 g a.i. l^{-1})	clodinafop-propargyl (240 g a.i. l^{-1})	iodosulfuron (50 g a.i. kg^{-1})
1	45	6	1.25
2	90	12	2.50
3	180	24	5.00
4	360*	48*	10*
5	720	96	20
6	1440	192	40
7	2880	384	80

* Registered field rates

Treatments were applied with a stationary air-pressured potsprayer, fitted with two 8001 flat fan nozzles, delivering 310 litres ha^{-1} at regulated pressure of 200 kPa. Plants remained in the application chamber for three hours before being returned to the glasshouse. Following herbicide application, the soil surface was watered daily to avoid drought stress.

6.3.3 Evaluation

During the study, the percentage survival of the PMR 1, PMR 2, PMR 3, & PMR 4 biotypes was compared with the percentage survival of the S biotype. There was no intention to compare the efficacy of different herbicides against one another. Percentage survival was recorded 28 days after herbicide application, by measuring plant biomass. The above ground parts of plants were harvested, oven-dried (80°C for 24 h) and weighed on a four decimal precision scale. After harvesting, subsoil plant rests were roughed out by hand and left to dry before burning to prevent regrowth and further seed depositing. This precaution was necessary to prevent possible escape of *P. minor* resistant biotypes into the summer rainfall region.

6.3.4 Statistical analysis

Experiments were repeated twice and the data of each treatment were combined for statistical purposes. The trial was a factorial experiment, arranged as a completely randomized design, and replicated four times. Data were subjected to analysis of

variance to determine the significance of difference between means at the 5% level of probability, using the Tukey test. Data obtained from the plant biomass is expressed as a percentage reduction in plant biomass in comparison to a treated susceptible control (S). A computerized program was used to determine the respective LD_{50} values using an exponential regression.

6.3.5 Results

The percentage survival of the four suspected resistant biotypes (PMR 1, PMR 2, PMR 3 & PMR 4) and the S biotype compared to various herbicide rates of diclofop-methyl, clodinafop-propargyl and iodosulfuron are presented in Figures 1, 2, & 3 respectively.

Diclofop-methyl

Comparing the results in figure 1, no significant differences were found between different biotypes when diclofop-methyl was applied at 45 g a.i. ha^{-1}. However, significant differences (P=0.01) in the percentage survival between the S biotype and three suspected resistant (PMR 1, PMR 2 & PMR 4) biotypes were noted at all the higher respective herbicide rates. The PMR 1 and PMR 4 biotypes respectively showed 28% and 47% survival when diclofop-methyl was applied at 2880 g a.i. ha^{-1}, while 42% of the PMR 2 biotype survived a 1440 g a.i. ha^{-1} application. In contrast, the S biotype suffered 99% mortality when diclofop-methyl was applied at 360 g a.i. ha^{-1}. However, only a marginal significant difference was found between the S and PMR 3 biotype when diclofop-methyl was applied at 90 and 360 g a.i.ha^{-1}. No significant difference could be found between the S and the PMR 3 biotype when diclofop-methyl was applied at rates of 720 to 2880 g a.i.ha^{-1}. Both biotypes were controlled efficiently with diclofop-methyl rates above 720 g a.i. ha^{-1}. Significant differences were also found between treatments when diclofop-methyl was applied at rates between 45 to 2880 g a.i. ha^{-1}. The calculated LD_{50} values for the PMR 1, PMR 2, PMR 3 & PMR 4 biotypes were 594, 700, 225 & 2673 g a.i. ha^{-1} respectively, whereas that for the S biotype was 184.4 g a.i. ha^{-1} (R^2=0.9377). There is thus a threefold difference between the PMR 1 biotype and S biotype, a fourteen-fold difference between the PMR 2 biotype and the S biotype and a seven-fold difference between the PMR 4 and S biotype.

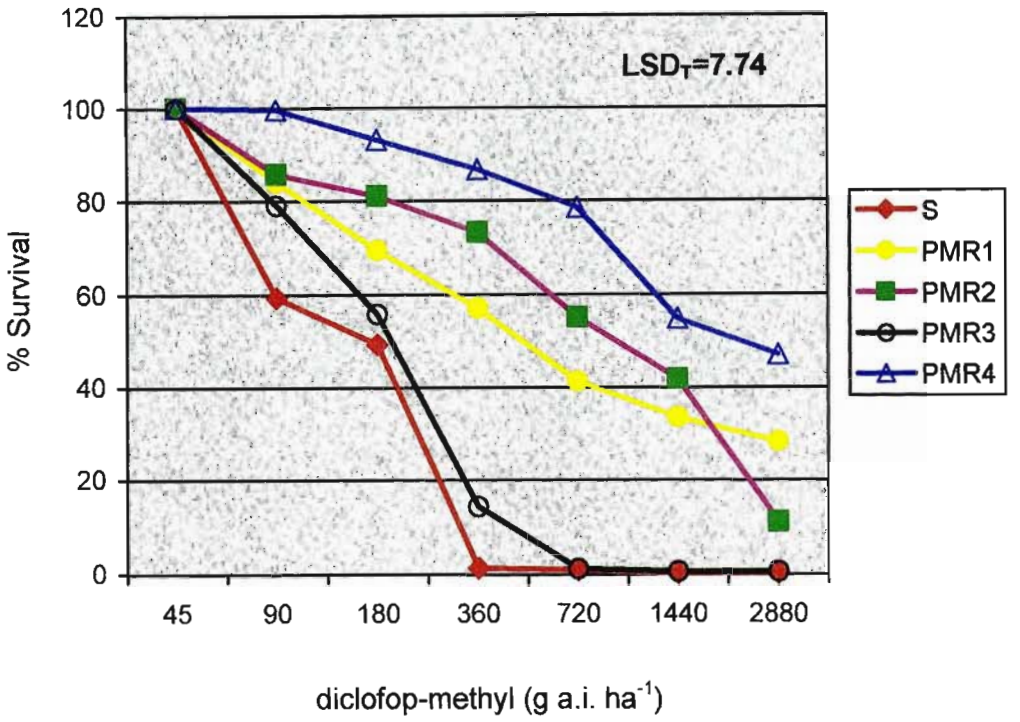

Figure 1: Survival (%) of resistant biotypes (PMR 1, PMR 2, PMR 3 & PMR 4) and a susceptible biotype (S) of *P. minor* 28 days after treatment with diclofop-methyl. Each point represents the average percentage survival of 24 plants.

Clodinafop-propargyl

A similar response to that of diclofop-methyl was observed when the biotypes where treated with clodinafop-propargyl (Figure 2). No significant differences (P=0.01) were found between different biotypes when clodinafop-propargyl was applied at 6 g a.i. ha^{-1}. At 12-384 g a.i. ha^{-1} however, significant differences were found between treatments and biotypes. This was not the case with the PMR 3 and S biotypes when treated with 96 to 384 g a.i. ha^{-1}, since both biotypes suffered 100% mortality. The calculated LD_{50} values for the PMR 1, PMR 2, PMR 3 & PMR 4 biotypes were 79.4, 94, 29 & 280 g a.i. ha^{-1} respectively, whereas that for the S biotype was 24.4 g a.i. ha^{-1} (R^2=0.9806).

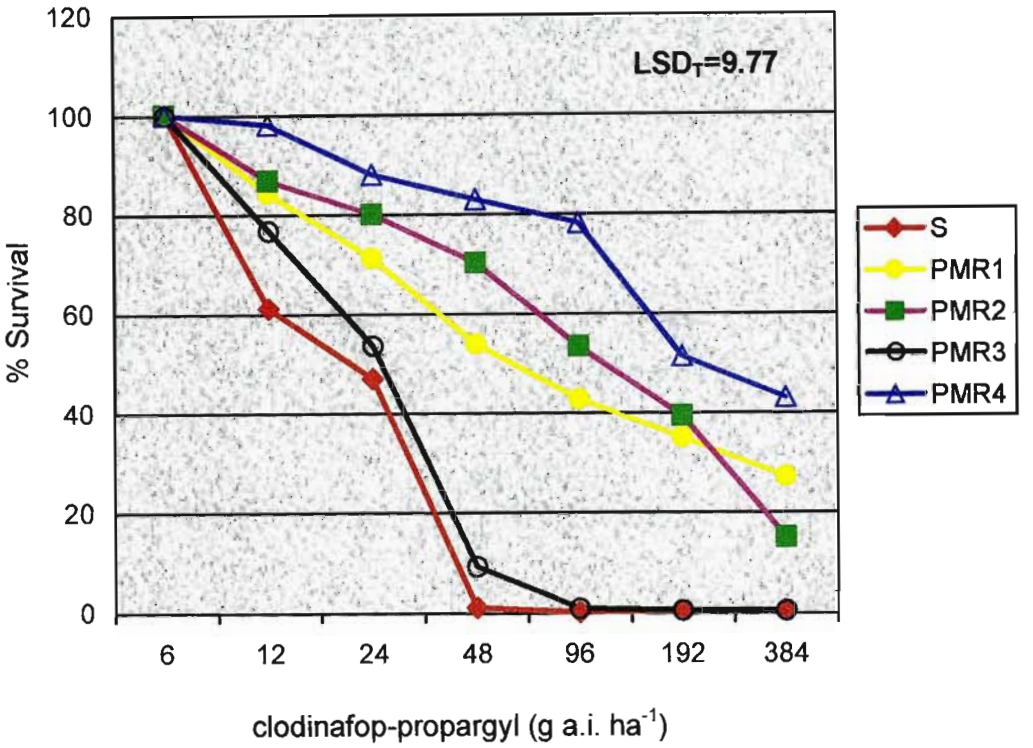

Figure 2: Survival (%) of resistant biotypes (PMR 1, PMR 2, PMR 3 & PMR 4) and a susceptible biotype (S) of *P. minor* 28 days after treatment with clodinafop-propargyl. Each point represents the average percentage survival of 24 plants.

Iodosulfuron

Significant differences (P=0.01) between treatments were obtained when iodosulfuron was applied at concentrations between 1.25 and 10 g a.i. ha^{-1} (Figure 3). However, no significant differences were found at rates between 10 and 80 g a.i. ha^{-1} due to 100% mortality of all the biotypes.

No significant differences were found in percentage survival between the resistant biotypes and the S biotype when treated with iodosulfuron. The calculated LD_{50} values for the PMR 1, PMR 2, PMR 3, PMR 4 and S biotypes were between 5.6 and 6.08 g a.i. ha^{-1} respectively (R^2=0.8196). Confirmation of resistance to ACC-ase inhibitors was found in all the other suspected resistant seed samples (PMR 5, PMR 6, PMR 7 & PMR 9) previously mentioned in Table 1, except for the PMR 8 biotype from Philadelphia. The PMR 8 sample was efficiently controlled with all the herbicides at registered rates. Results of PMR 5 to PMR 8 are not included in this report.

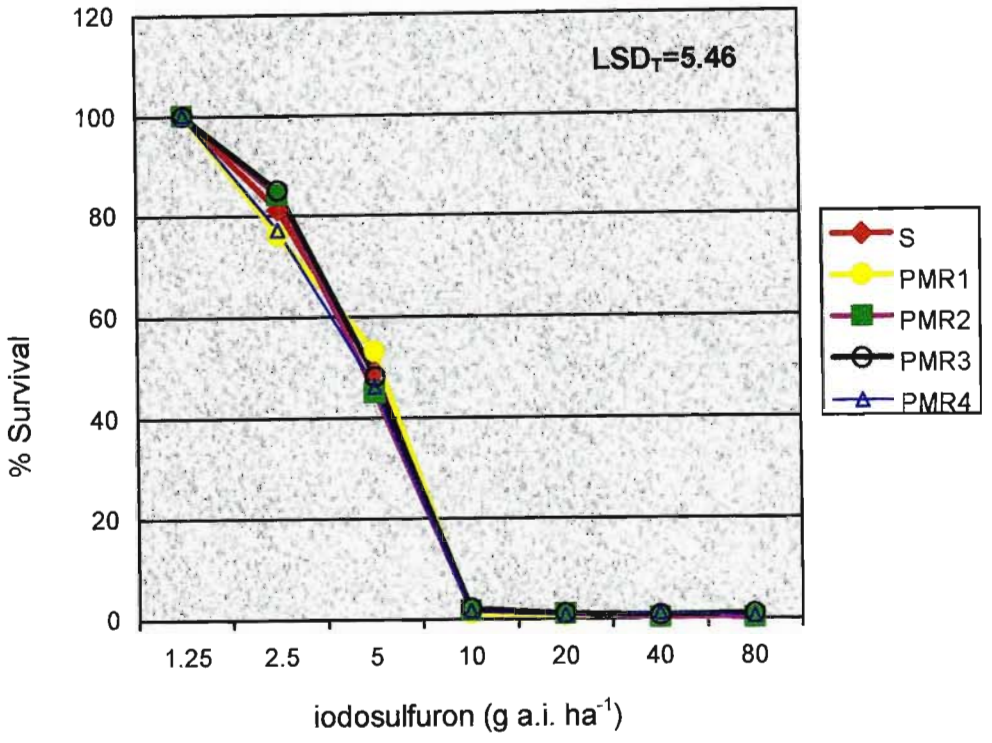

Figure 3: Survival (%) of resistant biotypes (PMR 1, PMR 2, PMR 3 & PMR 4) and a susceptible biotype (S) of *P. minor* 28 days after treatment with iodosulfuron. Each point represents the average percentage survival of 24 plants.

6.3.6 Discussion

According to Martin, Kelly & Gill (1993) herbicide resistance must be taken seriously because the elimination thereof is hardly possible due to inheritance of the seed. In this study it became evident that resistance to diclofop-methyl and clodinafop-propargyl treatments varied at different locations. There appears to be a positive correlation between the level (Fig.1 & 2) of resistance and the total number of applications of similar modes of action (Table 1). It is difficult to explain how resistance had developed in these species because of the different levels of resistance. However, it seems logical to conclude that creeping-resistance developed in the PMR 4 biotype as a result of continuous use of a similar mode of action, conferring a low degree of resistance (Gressel, 1991). However, continuous use of herbicides with similar modes of action has also shown to increase the level of resistance (Gressel, 1993). Iodosulfuron provided excellent control of all four suspected resistant biotypes as well as to the susceptible biotype. The reason for the success of iodosulfuron treatments is because it is a sulfonylureum and should

therefore be an ALS-inhibitor, and does therefore not share the same mode of action of diclofop-methyl and clodinafop-propargyl (Retzinger & Mallory-Smith, 1997). Powles *et al.*, (1977) reported that resistance cannot be overcome by the regular introduction of herbicides with new modes of action, resistance may also develop against the new mode of action resulting in multiple-resistance. Therefore, the development of resistance towards iodosulfuron may also eventually occur with continuous use. This report is the first confirmation that little seeded canary grass is resistant to diclofop-methyl and clodinafop-propargyl treatments in the Republic of South Africa. Alternative methods to control *P. minor* infestations must be introduced to ensure the efficacy of new modes of action for future purposes.

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CHAPTER VII

OCCURRENCE OF RESISTANT WILD OATS *(AVENA FATUA* L.) BIOTYPES IN THE WINTER RAINFALL REGION OF SOUTH AFRICA

7.1 Abstract

Herbicide resistance is a well-known phenomenon throughout the world and occurs in all major crop producing countries. The Republic of South Africa is no exception to the rule and the first case of herbicide resistance was documented when wild oats (*Avena fatua* L.) developed resistance towards diclofop-methyl treatments in the winter rainfall region of South Africa. Despite early confirmation regarding herbicide resistance, the agricultural industry regarded the information as unimportant. The objective of this study was to determine the evolution and severity of ACC-ase herbicide resistant *A. fatua* biotypes in the winter rainfall region of South Africa. Seed from suspected resistant *A. fatua* biotypes were sampled over a period of 24 months and transported to the Small Grain Institute, Bethlehem where herbicide resistance studies were done. The seed was harvested from plants that survived one or more of the following herbicide treatments viz. tralkoxydim, diclofop-methyl, fenoxaprop-P-ethyl or clodinafop-propargyl treatments. Susceptible *A. fatua* seed were obtained from the seedbank of the Small Grain Institute. Significant differences in the degree of control were found between the susceptible and resistant biotypes when treated with diclofop-methyl, fenoxaprop-P-ethyl and clodinafop-propargyl. The GR_{50} values for the AFR 1, AFR 2, AFR 3, AFR 4, AFR 5, AFR 6, AFR 7, AFR 8, AFR 9 and AFR 10 biotypes were between, 353.1 and 1369.3 g a.i. ha^{-1} for diclofop-methyl, 41.7 and 174.6 g a.i. ha^{-1} for fenoxaprop-P-ethyl and between 40.5 and 215.3 g a.i. ha^{-1} for clodinafop-propargyl treatments. The GR_{50} value for the susceptible biotype, when treated with diclofop-methyl, fenoxaprop-P-ethyl and clodinafop-propargyl, were respectively 188.6, 26.1 and 29.6 g a.i. ha^{-1}. However, when treated with tralkoxydim, significant differences in the degree of control were found in all the suspected resistant *A. fatua* biotypes (AFR 1 to AFR 9) except with the AFR 10 biotype. The GR_{50} values for the AFR 1 to AFR 10 tralkoxydim treatments were between 139.1 and 1044 g a.i. ha^{-1}, whereas the GR_{50} value for the susceptible biotype, when treated with tralkoxydim, was 123.9 g a.i.ha^{-1}. No significant differences in the degree of control could be found between the susceptible and AFR 10 biotype when treated with tralkoxydim.

During 1985 only one *A. fatua* biotype was confirmed to be resistant towards diclofop-methyl treatments, whereas today, several *A. fatua* biotypes were confirmed to be resistant towards diclofop-methyl, fenoxaprop-P-ethyl, tralkoxydim and clodinafop-propargyl treatments throughout the winter rainfall region of South Africa.

Keywords: ACC-ase inhibitors, *A. fatua,* resistance, South Africa

7.2 Introduction

Salonen (1992) stated that herbicides are primarily used to avoid yield loss by preventing weeds from interfering with cultivation, harvesting and marketing of grain. *Avena fatua* (L.) is a self-pollinating grassweed and is one of the most troublesome weeds in cereal crops in many countries, and are a major target for herbicide applications (Mansooji, Holtum, Boutsalis, Mathews & Powles, 1992). Sharma and Vanden Born (1978) found that *A. fatua* can cause considerable damage to the crop as a result of competition for nutrients. According to Carlson & Hill (1985) wheat *(Triticum aestivum* L.) yield declined rapidly as the plant density of *A. fatua* increased. *Avena fatua* infestation of 5.5 plants m^2 could result in 20% reduction in grain yield if a crop stand of 100 plants m^2 were attained. The major traits contributing to the survival of *A. fatua* are high seed production and varying degrees of dormancy (Shuma, Quick, Raju & Hsiao, 1995). Sanchez Del Arco, Torner & Fernandez (1995) stated that numerous studies conducted with *A. fatua* have shown that seed persistence in agricultural soils may range from 2-6 years, contributing to the survival of the species.

The introduction of the selective grass weed herbicides, belonging to aryloxyphenoxy propionic acids (APP) and cyclohexanedione oxime (CHD) resulted in much improved control of *A. fatua* in wheat. The target site of the APP and CHD herbicides, is Acetyl-COA carboxylase (ACCase), a key enzyme in fatty acid biosynthesis, which catalyses the conversion of Acetyl-CoA to malonyl-CoA (Hoppe & Zacher, 1985). The structures of aryloxyphenoxypropionates and the cyclohexanediones are given respectively in Figure 1 and 2 (Cobb, 1992). Despite normally efficient weed control with above-mentioned herbicides, certain *A. fatua* populations were found to survive herbicide treatments during 1985 (Cairns & Hugo, 1986). Because at that time this has only arisen on one or two farms, herbicide resistance was regarded as unimportant by the agricultural industry.

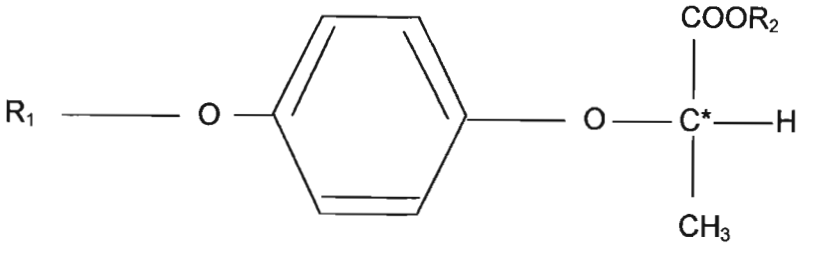

Figure 1: Structure of aryloxyphenoxypropionates (Cobb, 1992).

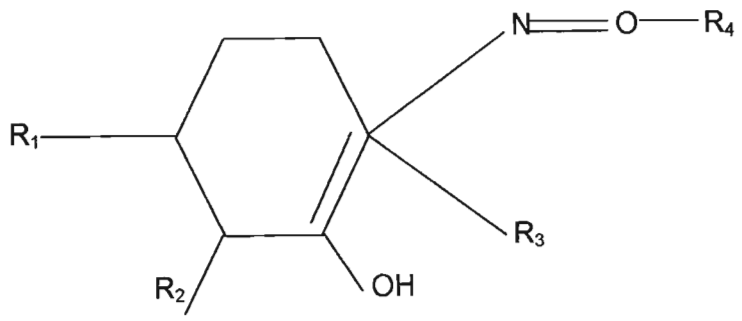

Figure 2: Structure of cyclohexanediones (Cobb, 1992).

Herbicide resistance may occur as a result of one or more mechanisms, including reduction in herbicide uptake, translocation of herbicide or modified target site. In Australia there are currently at least 20 biotypes of two species of wild oats *(A. fatua* and *Avena sterillis* spp. *ludoviciana)* exhibiting resistance to post-emergence Acc-ase-inhibiting herbicides (Maneechote, Holtum, Preston & Powles, 1994). These were first reported by Boutsalis, Holtum & Powles (1990). Most reports regarding *A. fatua* resistance to herbicides involved post-emergence herbicides such as APP and CHD herbicides. However, Malchow, Maxwell & Dyer (1993) and O'Donovan, Sharma, Harker, Maurice, Baig & Blackshaw (1994) confirmed *A. fatua* resistance towards triallate and difenzoquat.

According to Cavan, Biss & Moss (1998) two mechanisms are involved in the increasing incidence of resistance, namely the selection of novel mutations and the movement of *A. fatua* seeds. Betts, Ehlke, Wyse, Gronwald & Somers (1992) stated that the appearance of herbicide resistance in a weed population is due to the increase in numbers of a pre-existing resistant biotype due to the selection pressure exerted by repeated herbicide applications. According to Vermeulen, Dreyer, Grobler & Van Zyl (1996) the only herbicides registered for the control of wild oats in wheat, prior to 1997, belonged to the APP and the CHD herbicides groups. Unfortunately, all these herbicides share the same mode of action, viz, ACC-ase inhibitors (Anonymous, 1997 and Retzinger & Mallory-Smith, 1997).

Prior to 1999 confirmed reports of herbicide resistance in the Republic of South Africa are *Amaranthus hybridus* (L.) resistant to atrazine treatments (Sereda, Erasmus & Coetzer, 1996), *Lolium* spp. resistance towards ACC-ase inhibitors (Smit & De Villiers, 1998) and *Phalaris minor* (Retz) resistance towards ACC-ase inhibitors

(Smit & Cairns, 2000). However, Cairns & Hugo (1986) filed the first report of wild oat biotypes, resistant to diclofop-methyl treatments in the Western Cape. The objective of this study was to determine the evolution and severity of *A. fatua* resistance to ACC-ase inhibitors in the winter rainfall region of South Africa since the first report regarding *A. fatua* resistance in the Republic of South Africa during 1986.

7.3 Material and Methods

7.3.1 Plant culture

Suspected resistant wild oats seed were sampled over a 24-month period and the preliminary experiments were conducted over a period of 16 months on several different suspected resistant *A. fatua* biotypes. To avoid duplication of data, only 10 different experiments (representing specific regions) are discussed in this paper. Seed from suspected resistant *A. fatua* biotypes were collected from several different locations throughout the Western and Southern Cape (Table 1). Seed samples were taken from mature surviving plants, packed in brown paper bags and transported to the Small Grain Institute (Bethlehem, RSA) to be tested for resistance in glasshouse facilities. Seed from the *A. fatua* biotypes were survivors from either diclofop-methyl *(+)*-2-[4-(2,4-d ichlorophenoxy)phenoxy]propanoic acid, tralkoxydim (2-(1-[ethoxyimo) propyl]-3-hydroxy-5-(2,4,6-trimethylphenyl)cyclohex-2-enome, fenoxaprop-P-ethyl (±)-2-[4-[(6-chloro-2-benzoxazolyl)oxy] phenoxy] propanoic acid or clodinafop-propargyl 2-propynyl (R)-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy-propionate] treatments in a wheat production system during the winter of 1997 and 1998 (Table 1). According to Kremer & Kropff (1998) a correct comparison of growth should be made under optimal growth conditions, using populations with both susceptible and resistant biotypes from several geographical origins to determine the variation in growth parameters. Therefore, a susceptible *A. fatua* biotype (hereafter referred to as S biotype) was obtained from the Small Grain Institute seedbank. The seed had been self-pollinated for two generations and confirmed to be susceptible towards ACC-ase inhibiting herbicides in a preliminary study. According to Thurston (1951) *A. fatua* has distinct periods of emergence and the storage of *A. fatua* seed for short periods and in particular at high temperatures (20°C-30°C) can affect dormancy (Quail and Carter 1969). In order to obtain efficient germination, seed of both the S and R biotypes were incubated dry at 30°C in the dark for three weeks. The seeds were then pricked with a dissecting needle and incubated on filter paper moistened with distilled water in a petri dish at 4°C / 48 h dark until coleoptiles had emerged after 3-5 days. Enough seeds from 10 different localities were transplanted at the same day to ensure similar growth. Five germinated seeds were sown in two-liter

pots filled with a sandy loam soil with a pH of 5.5. After emergence at the one leaf stage, the seedlings were thinned out to three plants per pot to ensure plants with similar size and vigour. The study was conducted in a glasshouse with a day/night temperature regime of 20^0C/15^0C respectively.

Table 1: Origin of suspected resistant *A. fatua* populations from the winter rainfall region in South Africa and the history of herbicides used

Population	Nearest Town	Active ingredient	g. a.i. ha^{-1}
AFR 1	Eendekuil	tralkoxydim	225
AFR 2	Piketberg	diclofop-methyl	360
AFR 3	Porterville	tralkoxydim	200
AFR 4	Mooreesburg	diclofop-methyl	432
AFR 5	Riebeeck-Wes	clodinafop-propargyl	60
AFR 6	Malmesbury	fenoxaprop-P-ethyl	55
AFR 7	Paarl	clodinafop-propargyl	52
AFR 8	Philadelphia	tralkoxydim	225
AFR 9	Durbanville	clodinafop-propargyl	48
AFR 10	Caledon	diclofop-methyl	360

7.3.2 Herbicide Application

The herbicides included in the experiments were diclofop-methyl (diclofop-methyl 378 g a.i. L^{-1}, Agrevo SA (Pty) Ltd), tralkoxydim (tralkoxydim 250 g a.i L^{-1}, Zeneca Agrochemigals SA (Pty) Ltd), fenoxaprop-P-ethyl (fenoxaprop-P-ethyl 69 g a.i. L^{-1}, Agrevo SA (Pty) Ltd) and clodinafop-propargyl (clodinafop-propargyl 240 g a.i. L^{-1}, Novartis SA (Pty) Ltd). Diclofop-methyl was applied at rates between 45 and 2880 g a.i. ha^{-1}, tralkoxydim at rates between 28 and 1800 g a.i. ha^{-1}, fenoxaprop-P-ethyl at rates between 6 and 384 g a.i. ha^{-1} and clodinafop-propargyl at rates between 6.5 and 416 g a.i. ha^{-1}. Registered rates of diclofop-methyl, tralkoxydim, fenoxaprop-P-ethyl and clodinafop-propargyl are 360, 225, 48 and 52 g a.i.ha^{-1} respectively (Vermeulen, Grobler & Van Zyl, 1998). All the herbicides were applied at seven different rates (Figure 3-6). According to Hatzios and Penner (1985) adjuvants enhance the efficacy of a number of herbicides. Therefore, adjuvants were added to spray mixtures according to registration. Complement was added to the clodinafop-propargyl treatments at 0.5 L ha^{-1}, while Embrace was added to the tralkoxydim

treatments at 0.5% (v/v). Both Complement and Embrace are mineral oils + nonyl-phenol ethoxylate surfactants. According to registration, no adjuvants were added to the diclofop-methyl and fenoxaprop-P-ethyl treatments. According to Maneechote *et al.*, (1994) resistance may occur as a result of one or more mechanisms including herbicide uptake and / or translocation. Therefore, herbicide treatments were applied in a pesticide application chamber and plants were not subjected to environmental conditions that could influence the uptake and translocation of herbicides. Plants therefore remained in the application chamber for three hours before being returned to the glasshouse. Before herbicide application, the pots were positioned in such a way, that only one herbicide solution of each treatment was used to spray all 10 localities as well as the susceptible biotype. This ensured that all the treatments received exactly the same herbicide solution of each treatment. Glasshouse treatments were applied with a pressurized sprayer fitted with two 8001 flat fan nozzles, delivering 250-liter ha^{-1} at a regulated pressure of 150 kPa. Following herbicide application, the pots were watered daily to avoid drought stress.

7.3.3 Evaluation

According to Holt & LeBaron (1990) as cited by Moss & Rubin (1993) many studies relating to resistant weeds have concentrated on the evaluation of parameters as indicators of fitness such as growth rate or plant biomass. Percentage survival was recorded 30 days after herbicide application, by means of measuring plant biomass. During the study, suspected resistant biotypes were compared with the S biotype. No effort was made to compare the efficacy of different herbicides against one another. The above-ground parts of plants were harvested, oven-dried (60°Cfor 48h) and weighed on a high decimal precision scale. The plant biomass data were also used to determine the respective LD_{50} values of all the *A. fatua* biotypes tested. After harvesting, subsoil plant rests were roughed out by hand and left to dry before they were burned to prevent regrowth and further seed depositing. This precaution was necessary to prevent distribution of resistant seed in the Summer rainfall region were experiments were done.

7.3.4 Statistical analysis

Experiments were performed twice and the data of each treatment were combined for statistical purposes. The trials were factorial experiments, arranged as a completely randomized design, and treatments were replicated four times. Data were subjected to analysis of variance to determine the significance of difference between means at the 5% level of probability, using the Tukey test. Data obtained

from plant biomass are expressed as a percentage reduction in plant biomass in comparison to a treated susceptible control. A computerized program was used to determine the respective GR_{50} values using an exponential regression. The formula ($y=994.62e^{-0.0337X}$; $R^2=0.9483$) was used to determine the respective GR_{50} values.

7.3.5 Results

This paper reports only the results obtained from experiments performed on suspected resistant *A. fatua* seed, originating from fields in the top ten wheat producing districts. It became evident that resistance towards ACC-ase inhibiting herbicides occurs throughout the winter rainfall region and that different levels of resistance occurs between different *A. fatua* biotypes. Results of percentage survival of different R & S biotypes, when treated with tralkoxydim, diclofop-methyl, fenoxaprop-P-ethyl & clodinafop-propargyl, are presented in Figures 3a and 3b, 4a and 4b, 5a and 5b and 6a and 6b respectively. No significant differences (P=0.001) were found between suspected resistant and susceptible biotype when herbicides were applied at the lowest herbicide rates. This could have been the result of a too low rate, resulting in 100% survival of both the R & S biotypes (Figure 3 - 6).

When treated with tralkoxydim, significant differences (P=0.001) in percentage survival between the S and all the R biotypes were noted at all the herbicide rates (Figure 3a), except for the AFR 10 biotype (Figure 3b). Tralkoxydim supplied efficient control of the AFR 10 biotype at all the rates when compared with the S biotype. However, this was not the case when the AFR 10 biotype was treated with diclofop-methyl, clodinafop-propargyl and fenoxaprop-P-ethyl (Figures 4b, 5b and 6b). Significant differences (P=0.001) were found between all the R and S biotypes when treated with diclofop-methyl, fenoxaprop-P-ethyl and clodinafop-propargyl (Figures 4a and 4b, 5a and 5b and 6a and 6b).

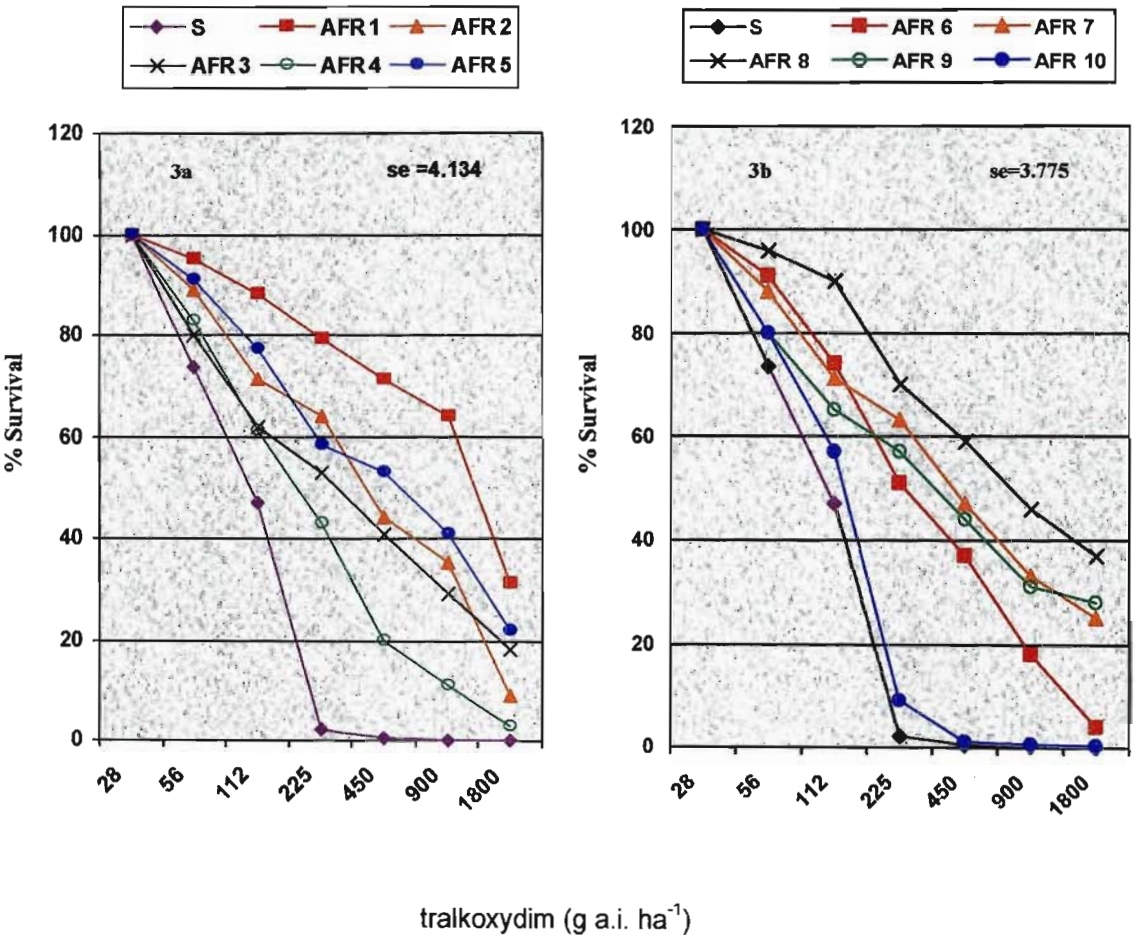

Figure 3 (a and b): Survival (%) of *A. fatua* biotypes (R and S) when treated with tralkoxydim at different herbicide rates. Each point represents the average survival (%) of 24 plants.

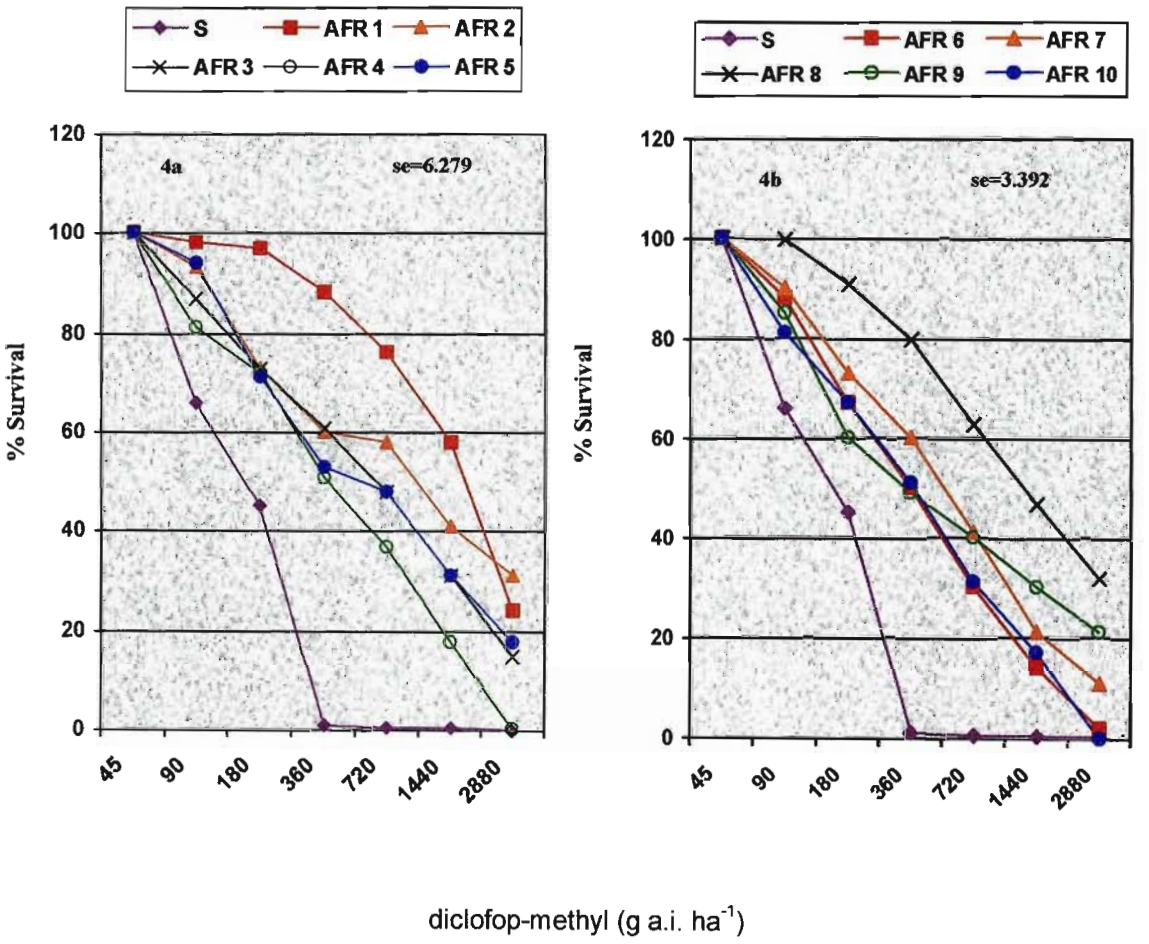

Figure 4 (a and b): Survival (%) of *A. fatua* biotypes (R and S) when treated with diclofop-methyl at different herbicide rates. Each point represents the average survival (%) of 24 plants.

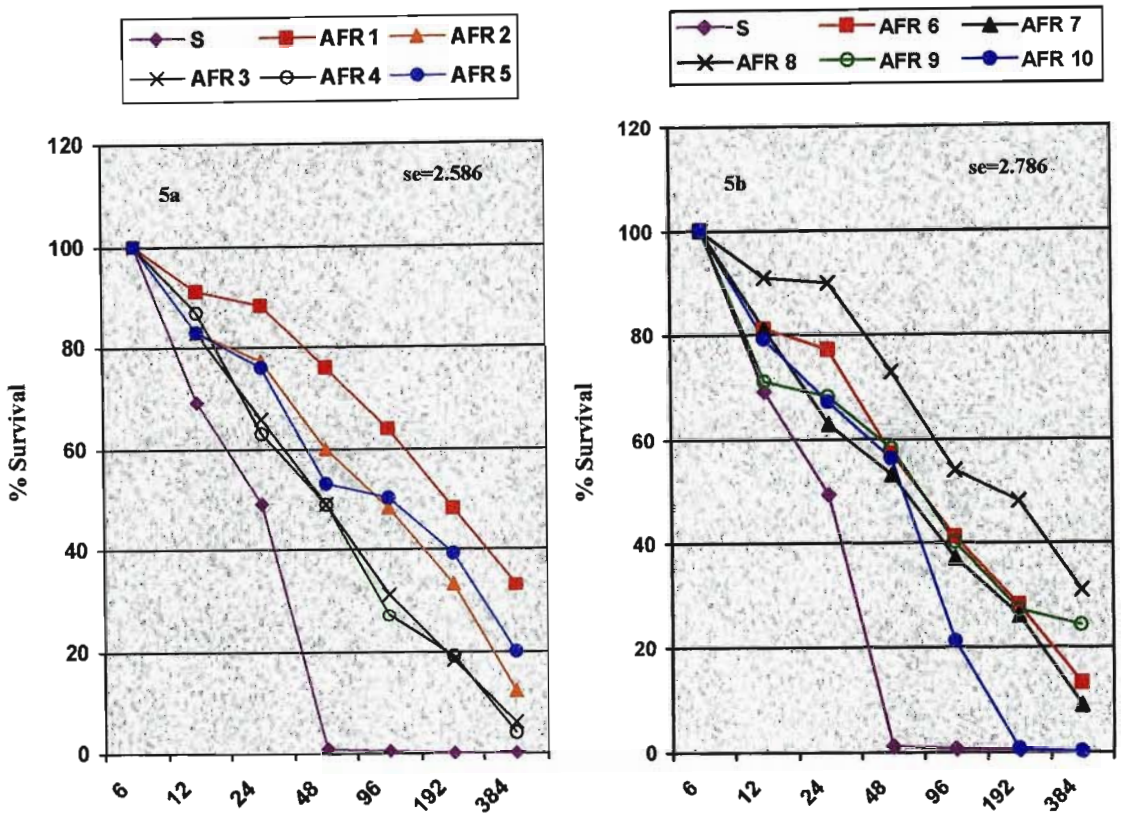

Figure 5 (a and b): Survival (%) of *A. fatua* biotypes (R and S) when treated with fenoxaprop-P-ethyl at different herbicide rates. Each point represents the average survival (%) of 24 plants.

The GR_{50} values for the R & S biotypes are presented in Table 2 and it is clear from the data that there is a variation in the level of resistance between different biotypes. The to GR_{50} values show that there is an eightfold difference between the AFR 1 and S biotype, while only a twofold difference occurs between the AFR 6 and the S biotype when treated with tralkoxydim. The GR_{50} values for the AFR 1 to AFR 10 biotype were between 139.1 – 1044 g a.i. ha^{-1}, whereas the GR_{50} for the S biotype, treated with tralkoxydim was 123.9 g a.i. ha^{-1}. A similar pattern is found when the R biotypes were treated with diclofop-methyl, fenoxaprop-P-ethyl and clodinafop-propargyl.

The GR_{50} values for the AFR 1 to AFR 10 were between 353.1 and 1369.3 g a.i. ha^{-1} for diclofop-methyl, 41.7 to 174.6 g a.i. ha^{-1} for fenoxaprop-P-ethyl and between 40.5 and 215.3 g a.i. ha^{-1} for the clodinafop-propargyl treatments. The GR_{50} values for the susceptible biotype when treated with diclofop-methyl, fenoxaprop-P-ethyl and clodinafop-propargyl were 188.6, 26.1 and 29.6 g a.i. ha^{-1}.

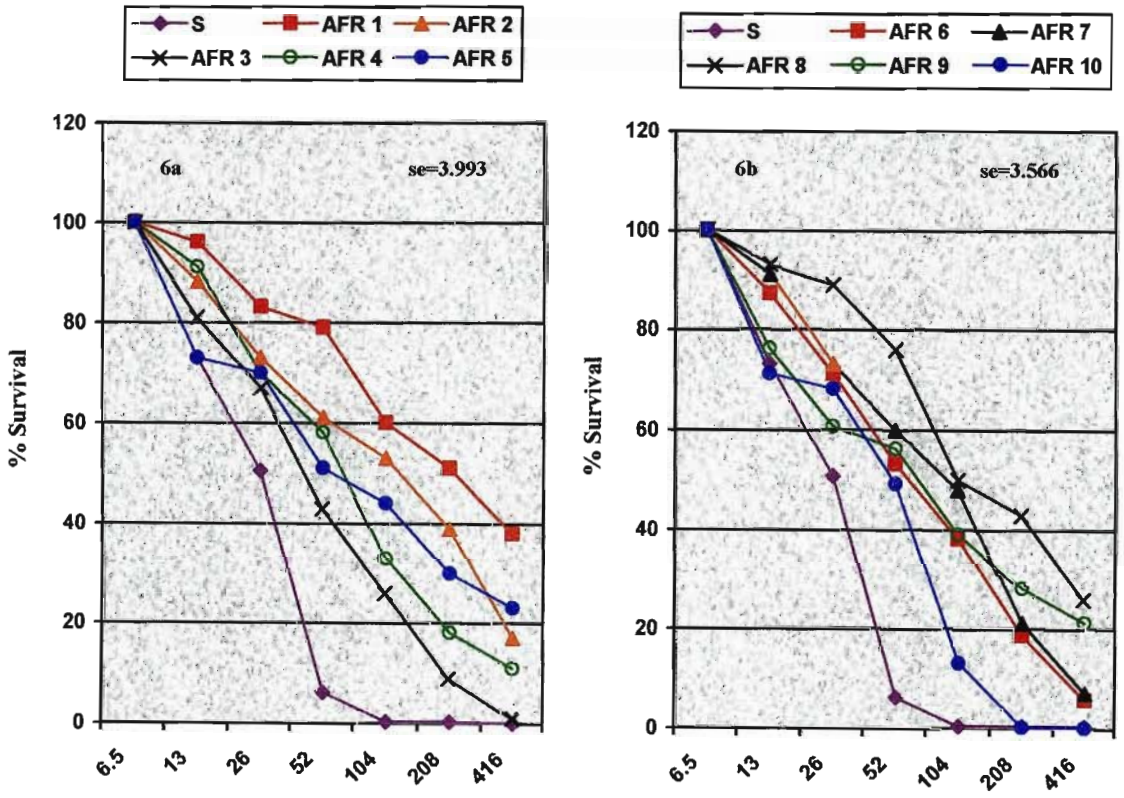

Figure 6 (a and b): Survival (%) of *A. fatua* biotypes (R and S) when treated with clodinafop-propargyl at different herbicide rates. Each point represents the average survival (%) of 24 plants.

Table 2: GR_{50} values of 10 *A. fatua* biotypes as well as the susceptible biotype performed over a period of 16 months using an exponential regression

	GR_{50} values (g a.i. ha^{-1})			
Population	tralkoxydim	diclofop-methyl	fenoxaprop-P-ethyl	clodinafop-propargyl
AFR 1	1044	1369.3	174.6	215.3
AFR 2	307.5	867.8	72.4	94.9
AFR 3	286.8	567.9	48.9	45.5
AFR 4	189.9	396.3	47.7	62.7
AFR 5	453.3	562.9	82.8	71.6
AFR 6	259.7	362.5	66.1	60.1
AFR 7	406.3	408.8	54.7	70.6
AFR 8	785.5	1287.8	145.9	132.3
AFR 9	349.7	463.5	64.1	65.8
AFR 10	139.1	353.1	41.7	40.5
S	123.9	188.6	26.1	29.6

7.3.6 Discussion

It is clear from the data that *A. fatua* resistance occurs at different levels to ACC-ase inhibitors. According to the data, it was found that differences in percentage survival between the APP and CHD herbicides, as presented in Figures 3b, 4b, 5b & 6b, occurred. Anonymous (1997) reports that resistance is not built up against the active ingredient nor the chemical group of a specific herbicide, but against the mode of action of a herbicide. Therefore, according to Caseley, Cussans & Atkin (1991) a similar response with the four compounds used in this study would be expected because all of them are ACC-ase inhibitors. However, according to Preston, Tardiff, Christopher & Powles (1996b) extreme differences in susceptibility between biotypes and related herbicides might occur. The fact that the AFR 10 biotype is susceptible to tralkoxydim indicates the complexity of an enzyme / inhibitor interaction and that there are several possible mutations within ACC-ase that can provide resistance (Preston, Tardiff & Powles, 1996a). According to Cobb (1992) dramatic yield losses will have a profound effect on the economy in terms of the need to import food and the cost of weed control. Weeds form a part of a community in a given area and might therefore be susceptible to many pests and diseases. Because of their close association with crops, they may serve as important food reservoirs or carriers of

pests and pathogens. Cunningham (1981) found that the fungus causing eyespot *(Pseudocercosporella herpotrichoides* Fron) was able to survive on wild oats species. Eyespot is a well-known disease in the winter rainfall region of South Africa, and it is therefore of the utmost importance to effectively control *A. fatua* in wheat production.

Despite early confirmation of *A. fatua* resistance to diclofop-methyl (Cairns & Hugo, 1986), the agricultural industry regarded it as unimportant. Today producers in South Africa have to face reality and the problem has evolved to be more serious than originally considered. Now, 14 years later, *A. fatua* resistant to ACC-ase inhibitors has infested approximately 7000 hectares of wheat production fields. The distribution of these resistant *A. fatua* biotypes occurs throughout the entire winter rainfall region of South Africa. According to Mansooji *et al.*, (1992) it may prove difficult to manage herbicide resistance in *A. fatua* due to relatively few herbicides registered for the control of *A. fatua* and secondly because wild oats has the potential to remain viable in some soils for long periods. *A fatua* resistant to ACC-ase inhibiting herbicides is now a threat throughout the winter rainfall region and if weed control practices are not altered, serious infestations of resistant *A. fatua* biotypes will occur. The agricultural industry should therefore explore alternative methods of weed control, such as crop rotations. According to Powles, Preston, Bryan, Jutsum (1997) and Mansjooji *et al.*, (1992) long-term weed control strategies will require use of non-chemical integrated weed management practices if the grassweed herbicides are to be conserved for use in cereal crops for *A. fatua* control.

In view of the widespread use of ACC-ase inhibitors in the winter rainfall region of South Africa, it is likely that the number of fields infested with ACC-ase resistant *A. fatua* exceed those documented. During this study it became evident that further studies should be implemented to establish if cross- and / or multiple resistance occurs in these *A. fatua* biotypes. The success of management strategies in a crop rotation system depends largely on the absence of cross-resistance.

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CHAPTER VIII

SUMMARY

Sustainable management of weed populations in agriculture is critical. Weeds infesting crops must be controlled or they could reduce crop yields, hinder harvesting and contaminate the product. The farming community has realized the value of herbicides over the last 20 years. Use of herbicides (R91 million Global) now exceeds that of fungicides and insecticides combined. In contrast, the means devoted to weed science is less than either entomology or plant pathology. The use of herbicides has allowed agriculture to expand and to keep pace with developing human populations. However, there is a negative side, crop rotations have been reduced, alternative weed control techniques have been abandoned and all these developments have led to more intensive herbicide use. As a result the intensive use of herbicides has led to the development of herbicide resistance.

At the beginning of 1997, the agricultural industry in South Africa was in total darkness regarding herbicide resistance. Important information such as: what is resistance, how does resistance develop, what is the extent of the problem in South Africa, how do we deal with resistance at present and how do we prevent and/or retard further developments of resistance in the future, was unanswered. These questions were of utmost importance to preserve out precious herbicides for future use. At that stage, the extent of herbicide resistance in South Africa was uncertain and the only cases of confirmed herbicide resistance were reports of resistance to atrazine, simazine and diclofop-methyl resistance observed with certain wild oat biotypes. However, several reports of poor weed control in the winter rainfall area of South Africa, involving several other weed species such as *P. minor*, *L. rigidum* and *R. raphanistrum* suggested that these species might became resistant to herbicide treatments which were effective in the past. Although Cairns & Hugo confirmed *Avena fatua* resistance to diclofop-methyl in 1985, the farming community and agricultural industry rejected it as unimportant. The result of such an ignorant approach led to severe problems regarding chemical weed control for the wheat producing industry in the winter rainfall area of the Republic of South Africa. The situation has deteriorated in the last couple of years and the entire industry depends on solutions provided from the Small Grain Institute (SGI) and the University of Pietermaritzburg.

A research project, funded by the Winter Grain Research Trust was registered by the SGI and in collaboration with the University of Pietermaritzburg certain aspects regarding herbicide resistance were investigated to provide answers and solutions for the agricultural industry in South Africa. As mentioned previously, the primary aims of this study were categorized into four phases:

- To develop a classification system of herbicides registered in the Republic of South Africa
- To confirm resistance to herbicides used in wheat production
- To determine a management strategy to minimize the potential losses caused by herbicide resistant weeds.
- To do a national survey of the occurrence of herbicide resistant weeds.

As a result of a complete study regarding herbicides registered in South Africa, a printed poster was distributed among approximately 1150 grain producers and agricultural role players in South Africa (Chapter II). This poster contained important information in order to provide the industry with the opportunity to manage herbicide resistance. All the registered herbicides in South Africa were classified with regard to their specific mode of action. In order to rotate herbicides to prevent herbicide resistance, one must be able to rotate the mode of action of specific herbicides. Since several herbicides with different active ingredients share a similar mode of action, such a complete survey regarding different modes of action was of immense importance.

In summarizing the results obtained from the study thus far, it is obvious that herbicide resistance is no longer just a threat to the grain producers in South Africa, but a devastating reality. During a congress held in 1997 in the Western Cape, 6000 (0.6% of area) hectares were speculated to be infested by herbicide resistant *Lolium* biotypes. In the past four years several studies regarding *Lolium* spp. resistance were performed and as a result of intensive studies over a four year period, *Lolium* spp. resistance to ACC-ase inhibitors was confirmed (Chapter III). Unconfirmed reports regarding *Lolium* resistance strongly indicates that approximately 50 000 hectares (5.5% of area) are involved. However, during the study it became evident that depending on the specific *Lolium* biotype, it is sometimes possible to achieve effective control with tralkoxydim (Chapter IV). Since tralkoxydim is also an ACC-ase inhibitor it is rather strange to achieve such results. However, according to literature such results are possible, but is definitely not a long-term solution. These results are

strongly dependent on the reaction of a specific biotype to the herbicide treatment. It seems that the only possible explanation for this reaction can be the fact that tralkoxydim and other ACC-ase inhibitors do not share a similar binding site with this specific biotype. Unfortunately, nobody can predict such reaction of a biotype in advance, and therefore it is unacceptable for recommendations for future applications. This reaction was also an exception to the rule. Out of all the resistance tests done during the past three years, only a small percentage (±1.5%) *Lolium* biotypes reacted in this manner.

Chapter V revealed that *R. raphanistrum* is, at this time the only broad-leaved weed resistant to herbicide applications in the winter rainfall region. Resistance to chlorsulfuron treatments was confirmed. However, in this study it also became evident that alternative herbicides (MCPA & bromoxynil) can be used with enormous efficacy for the control of *R. raphanistrum*. This information is of great value and it is strongly advised to incorporate these products into the management strategies for the control of *R. raphanistrum*. Both MCPA and bromoxynil do not share the same mode of action as chlorsulfuron and should therefore control ALS resistant *R. raphanistrum* biotypes.

For the first time, this study revealed that *P. minor* is also resistant to ACC-ase inhibitors (Chapter VI). However, iodosulfuron provided efficient control of these resistant biotypes. This information is of great importance, however, it provides only a short-term solution to the resistance problem. Iodosulfuron is an ALS-inhibitor and according to literature ALS-inhibitors are the group of herbicides with the largest risk to develop resistance. Resistance to iodosulfuron can develop within three to five years of consecutive use. If iodosulfuron can be utilized in a crop rotation system, with effective mode of action rotation, the efficacy of the product can be preserved for future uses in *T. aestivum* production. Studies regarding *P. minor* resistance also revealed that these resistant biotypes are at this point restricted to a specific region in the western Cape. However, as a result of cross-pollination and harvesting practices, one should expect that spreading of the dominant resistant gene should probably occur within the near future.

Although resistance of *A. fatua* was confirmed way back in 1985, not much was done to prevent the spreading and further development of resistance among *A. fatua* populations. For the first time since 1985 a thorough study regarding the evolution of *A. fatua* resistance was completed (Chapter VII). Results of this long-term study

revealed that *A. Fatua* resistance occurs throughout the Western Cape and is no longer restricted to certain areas or farms. *A. fatua* is a self-pollinating weed and therefore the spreading of the resistance gene is much slower than in the case of a cross-pollinating weed. However, harvesting equipment is largely responsible for the spreading of the resistant *A. fatua* biotypes. This information is of immense value since it is the latest complete database regarding *A. fatua* resistance. This information will be of great value in the monitoring of future development and spreading of resistant *A. fatua*.

Unfortunately, studies completed at this stage do not reveal the complete situation regarding herbicide resistance in South Africa. Recent preliminary studies revealed even more important information. Firstly, it appears that herbicide resistance is no longer restricted to the winter rainfall region of South Africa. Studies done in the summer rainfall region strongly indicate that herbicide resistance is on the brink of establishing itself in the summer rainfall region. This will however be no surprise, as herbicide resistance will develop in a region were the producer relies heavily on the efficacy of a herbicide for optimum crop production. In the current economical situation, the producer is forced to rely on chemical weed control. Alternative methods such as cultivation / mechanical control are too expensive and the promotion of minimum tillage is on the increase. Both practices leave the producer with only one effective alternative method for weed control and that is to make extensive use of herbicides. Furthermore, products such as paraquat, a non-selective contact herbicide and glyphosate a non-selective systemic herbicide, appear to loose their efficacy against certain weed species. The possible development of resistance towards non-selective herbicides is worthwhile investigating. Both paraquat and glyphosate are products that are extensively used during minimal- or no-tillage practices. If the industry losses these products as a result of resistance, the entire industry will be forced to revert 15 years in cropping practices. Something the farming industry cannot afford.

In the past two years three new products have been introduced into the agricultural market. They are Hussar (iodosulfuron 50 g a.i. kg^{-1}), Monitor (sulfosulfuron 750 g a.i. kg^{-1}) and Cysure (imazamox 40 g a.i. L^{-1}). These products took their rightful place just in time in the agricultural market. According to registration, these products do control either *Lolium* spp, *A. fatua* and / or *P. minor* ACC-ase resistant biotypes. All three products share a different mode of action (ALS inhibitor) to the original graminicides (ACC-ase inhibitors). Unfortunately the industry has relied heavily

during the past three years on these products. To complicate the situation further, these products have been applied with phenomenal success. According to literature, one could predict that the successes obtained with these products are directly correlated to their future downfall. Already, reports of poor weed control regarding these products have been made. Up to date no reports regarding cross and multiple-resistance are documented in South Africa. That does not mean that either or both of these types of resistance do not occur in South Africa. To ensure the future of these newly released products it is of the utmost importance to inform the industry of the limitations of newly released products and guide them with thorough knowledge to manage these products in a proper way. Given al theses factors it is obvious that the industry will have to continue to opt for herbicides as their preferred method of weed control. One repercussion from a reliance on herbicides is the appearance of herbicide resistant weed biotypes. Suggested recommendations to prevent or reduce the occurrence of resistant weed biotypes include: increased cultivation, crop rotation, rotation of herbicides with different modes of action, use of herbicide mixtures with different modes of action and to prevent seed-depositing of resistant biotypes.

The primary aims of this study were completed, but in the meantime the resistance problem has proved to be much more complicated and devastating (13% of area in Western Cape) than it was originally thought to be. As mentioned earlier in this chapter, it seems that more weed species and more herbicides are involved than previously thought. It also appears that the problem has multiplied and is now also making its appearance in the summer rainfall region, even in the irrigation areas. It is therefore of utmost importance to continue this important study to provide the correct answers to the questions posed, and to be able to successfully manage herbicide resistance. If the study is terminated at this stage, the industry will loose valuable time and knowledge regarding herbicide resistance in South Africa. Therefore the importance of immediate ongoing studies is of great importance. Ongoing studies should involve aspects such as cross- and multiple-resistance, confirmation of new resistant weed species as well as herbicides involved. After completion of these studies, and only then, the most appropriate answers and recommendations regarding herbicide resistance can be made.

The key message that can be learned from this chapter is the realization that herbicides are a precious resource of great importance to agricultural productivity and thorough knowledge regarding herbicide resistance is necessary in order to ensure optimum efficacy with herbicide applications.

CHAPTER IX

ANEXURE A

ANOVA'S FOR EXPERIMENTS DONE DURING STUDY

A1: Chapter 3: *Lolium* spp. resistance to ACC-ase inhibitors in wheat (*Triticum aestivum* L.) within the RSA: a preliminary study

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BEH	40	13453.9158	336.3479	385.31	<.001
Residual	123	107.3715	0.8729		
Total	163	13561.2873			

Standard errors of means

Table	BEH
rep.	4
d.f.	123
e.s.e.	0.4672

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
BEH.REP	123	0.9343	8.5

A2: Chapter 4: Differential efficacy of tralkoxydim and diclofop-methyl on a suspected resistant ryegrass (*Lolium rigidum* Gaud.) biotype

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
HER.BEH stratum					
BEH	19	78.111	4.111	1.78	0.047
Residual	60	138.354	2.306		
Total	79	216.465			

Standard errors of means

Table	BEH

rep.	4
d.f.	60
e.s.e.	0.759

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
HER.BEH	60	1.519	11.1

A3: Chapter 5: Resistance of *Raphanus raphanistrum* to chlorsulfuron in the Republic of South Africa

A3.1: Survival (%) of WR1, WR2 and S biotypes of *R. raphanistrum* 30 days after treatment with chlorsulfuron (Figure 1)

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rate	6	67655.310	11275.885	3919.34	<.001
Locality	2	25059.024	12529.512	4355.09	<.001
Rate.Locality	12	9459.976	788.331	274.01	<.001
Residual	63	181.250	2.877		
Total	83	102355.560			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	12	28	4
d.f.	63	63	63
e.s.e.	0.490	0.321	0.848

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Local	63	1.696	3.0

A3.2: Survival (%) of WR1, WR2 and S biotypes of *R. raphanistrum* 30 days after treatment with bromoxynil

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rate	6	127491.810	21248.635	2883.50	<.001

Locality	2	27.214	3.607	1.85	0.166
Rate.Locality	12	133.619	1.135	1.51	0.144
Residual	63	464.250	7.369		
Total	83	128116.893			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	12	28	4
d.f.	63	63	63
e.s.e.	0.784	0.513	1.357

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Local	63	2.715	8.0

A3.3: Survival (%) of WR1, WR2 and S biotypes of *R. raphanistrum* 30 days after treatment with MCPA

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rate	6	118990.333	19831.722	4164.66	<.001
Locality	2	29.786	14.893	3.13	0.051
Rate.Locality	12	160.881	13.407	2.82	0.004
Residual	63	300.000	4.762		
Total	83	119481.000			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	12	28	4
d.f.	63	63	63
e.s.e.	0.630	0.412	1.091

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	63	2.182	6.7

Chapter 6: Little seeded canary grass (*Phalaris minor* Retz.) resistant to ACC-ase inhibitors in South Africa

A4.1: Survival (%) of resistant biotypes (PMR1, PMR2, PMR3 & PMR4) and a susceptible biotype (S) of *P. minor* 28 days after treatment with diclofop-methyl

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rate	6	112299.303	18716.550	2423.26	<.001
Locality	4	47280.843	11820.211	1530.38	<.001
Rate.Locality	24	18161.988	756.749	97.98	<.001
Residual	105	810.990	7.724		
Total	139	178553.123			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	20	28	4
d.f.	105	105	105
e.s.e.	0.621	0.525	1.390

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	105	2.779	5.2

A4.2: Survival (%) of resistant biotypes (PMR1, PMR2, PMR3 & PMR4) and a susceptible biotype (S) of *P. minor* 28 days after treatment with clodinafop-propargyl

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rate	6	112468.40	18744.73	1521.59	<.001
Locality	4	45734.36	11433.59	928.12	<.001
Rate.Locality	24	16911.26	704.64	57.20	<.001
Residual	105	1293.51	12.32		
Total	139	176407.53			

Standard errors of means

Table	Rate	Locality	Rate Locality

rep.	20	28	4
d.f.	105	105	105
e.s.e.	0.785	0.663	1.755

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	105	3.510	6.6

A4.3: Survival (%) of resistant biotypes (PMR1, PMR2, PMR3 & PMR4) and a susceptible biotype (S) of *P. minor* 28 days after treatment with iodosulfuron

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rate	6	222988.665	37164.777	9684.14	<.001
Locality	4	50.813	12.703	3.31	0.013
Rate.Locality	24	284.268	11.845	3.09	<.001
Residual	105	402.958	3.838		
Total	139	223726.704			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	20	28	4
d.f.	105	105	105
e.s.e.	0.438	0.370	0.980

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	105	1.959	5.9

Chapter 7: Evolution of resistant wild oats (*Avena fatua* L.) biotypes in the winter rainfall region of the Republic of South Africa

A5.1: Survival (%) of *A. fatua* biotypes (R & S) when treated with tralkoxydim at different rates (Figure 3a)

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Rate.Locality stratum					
Rate	6	136359.31	22726.55	1329.85	<.001
Locality	5	30757.30	6151.46	359.96	<.001
Rate. Locality	30	12893.09	429.77	25.15	<.001
Residual	126	2153.28	17.09		
Total	167	182162.98			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	24	28	4
d.f.	126	126	126
e.s.e.	0.844	0.781	2.067

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	126	4.134	7.5

A5.2: Survival (%) of *A. fatua* biotypes (R & S) when treated with tralkoxydim at different rates tralkoxydim (Figure 3b)

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Rate.Locality stratum					
Rate	6	153256.11	25542.68	1792.44	<.001
Locality	5	32589.45	6517.89	457.39	<.001
Rate.Locality	30	14507.36	483.58	33.93	<.001
Residual	126	1795.53	14.25		
Total	167	202148.45			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	24	28	4
d.f.	126	126	126
e.s.e.	0.771	0.713	1.887

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	126	3.775	7.3

A5.3: Survival (%) of *A. fatua* biotypes (R & S) when treated with diclofop-methyl at different rates (Figure 4a)

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Rate.Locality stratum					
Rate	6	133230.62	22205.10	563.29	<.001
Locality	5	34152.25	6830.45	173.27	<.001
Rate.Locality	30	14947.69	498.26	12.64	<.001
Residual	126	4966.98	39.42		
Total	167	187297.54			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	24	28	4
d.f.	126	126	126
e.s.e.	0.912	0.837	2.043

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	126	6.279	11.1

A5.4: Survival (%) of *A. fatua* biotypes (R & S) when treated with diclofop-methyl at different rates (Figure 4b)

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Rate.Locality stratum					
Rate	6	157569.56	26261.59	2282.58	<.001
Locality	5	26316.42	5263.28	457.47	<.001
Rate.Locality	30	10650.18	355.01	30.86	<.001

Residual	126	1449.66	11.51
Total	167	195985.82	

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	24	28	4
d.f.	126	126	126
e.s.e.	0.692	0.641	1.696

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	126	3.392	6.5

A5.5: Survival (%) of *A. fatua* biotypes (R & S) when treated with fenoxaprop-P-ethyl at different rates (Figure 5a)

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rate	6	147763.023	24627.170	3682.76	<.001
Locality	5	22446.705	4489.341	671.34	<.001
Rate.Locality	30	11969.229	398.974	59.66	<.001
Residual	126	842.580	6.687		
Total	167	183021.536			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	24	28	4
d.f.	126	126	126
e.s.e.	0.528	0.489	1.293

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	126	2.586	7.3

A5.6: Survival (%) of *A. fatua* biotypes (R & S) when treated with fenoxaprop-P-ethyl at different rates (Figure 5b)

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.DOSIS.LOKAL stratum					
Rate	6	147535.463	24589.244	3166.87	<.001
Locality	5	22639.954	4527.991	583.16	<.001
Rate.Locality	30	11806.688	393.556	50.69	<.001
Residual	126	978.330	7.765		
Total	167	182960.435			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	24	28	4
d.f.	126	126	126
e.s.e.	0.569	0.527	1.393

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Date.Locality	126	2.786	5.4

A5.7: Survival (%) of *A. fatua* biotypes (R & S) when treated with clodinafop-propargyl at different rates (Figure 6a)

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rate	6	142483.99	23747.33	1489.52	<.001
Locality	5	25645.83	5129.17	321.72	<.001
Rate.Locality	30	11608.68	386.96	24.27	<.001
Residual	126	2008.81	15.94		
Total	167	181747.31			

Standard errors of means

Table	Rate	Locality	Rate Locality

rep.	24	28	4
d.f.	126	126	126
e.s.e.	0.815	0.755	1.996

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	126	3.993	7.4

A5.8: Survival (%) of *A. fatua* biotypes (R & S) when treated with clodinafop-propargyl at different rates (Figure 6b)

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rate	6	164514.60	27419.10	2156.79	<.001
Locality	5	20101.91	4020.38	316.24	<.001
Rate.Locality	30	11563.98	385.47	30.32	<.001
Residual	126	1601.83	12.71		
Total	167	197782.32			

Standard errors of means

Table	Rate	Locality	Rate Locality
rep.	24	28	4
d.f.	126	126	126
e.s.e.	0.728	0.674	1.783

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
REP.Rate.Locality	126	3.566	6.9