The use of Flucarbazone-sodium to control wild oats (*Avena* spp.) in cultivated wheat fields of the Western Cape of South Africa

By

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

Wild oats (*Avena* spp.) is a prominent weed in cultivated wheat fields of the Western Cape Province of South Africa. For the sustainable production of wheat, it is crucial to apply the correct herbicide rates at the correct growth stages of both wild oats and wheat in order to achieve maximum weed control. Flucarbazone-sodium has shown to provide excellent activity against wild oats when applied as a post-emergence herbicide to wheat in field experiments conducted in Canada and the USA. Flucarbazone-sodium acts as an inhibitor of the enzyme acetolactate synthase (ALS) also known as acetohydroxyacid synthase (AHAS), an important enzyme that acts as a catalyst in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine. This product, known as Everest® 2.0, is a new sulfonylaminocarbonyltriazoline herbicide on the South African market. Flucarbazone-sodium has not yet been tested scientifically under South African conditions. The environment, soil, wheat cultivars and cultivation techniques between South Africa and North America differ immensely. In this study flucarbazone-sodium was evaluated under South African conditions in the Western Cape.

The first part of the study aimed to evaluate the phytotoxic effect of flucarbazone-sodium on the locally grown wheat cultivars of the Western Cape, as herbicides have no use if they reduce yield potential, regardless of how effectively they control weeds. For this study the internationally accepted BBCH scale was used to describe the different growth stages of the crop. Flucarbazone-sodium was applied at half, one and double the recommended rates at three different plant growth stages ([12-13], [14-15] and [16-17]; BBCH scale on five cultivars (SST 88,

SST 027, SST 056, SST 015 and Pannar 3408) in a phytotoxicity field trial. Yield and grain quality (protein content, hectolitre mass and thousand kernel mass) was examined at the end of the growing season. Wheat yield was not negativly affected by flucarbazone-sodium applications, however, double the recommended rate showed a significant ($p < 0.05$) reduction in yield and hectolitre mass for cultivar SST 056. Appart from SST 056 there is no statistical evidence to discard flucarbazone-sodium in a weed management system, as long as the application guidelines on the product label are followed.

To date, no studies have been published on the effectiveness of flucarbazone-sodium in controlling wild oats biotypes in South Africa. For this study, three sites in the Swartland area (Moorreesburg and Piketberg) with severe wild oats infestations were chosen. Flucarbazone-sodium was applied at half, recommended and double the recommended rates at three different growth stages ([12-13], [14-15] and [16-17]; BBCH scale of the wheat. The results showed that flucarbazone-sodium was very effective in controlling wild oats in cultivated fields at all sites except one. At the Pools site near Piketberg in the Western Cape very low levels of wild oats control by flucarbazone-sodium was recorded. Resistance was suspected and seeds were harvested for further investigation.

Glasshouse and molecular trials were conducted in order to prove that the biotype present at Pools is resistant to flucarbazone-sodium and to investigate the possible mechanism responsible for this resistance. Results showed that the sampled wild oats biotype at Pools is clearly resistant to flucarbazone-sodium. Even at eight times the recommended rate, flucarbazone-sodium could not control a single wild oats plant. Flucarbazone-sodium appeared to have some effect on the wild oats biotype sampled at Pools, since the dry mass (DM) of the wild oats was negatively

affected as the dosage rate was increased. PCR results showed the sampled wild oats biotype had homozygous resistance mutations Ala-205-Val and Trp-574, and heterozygous resistance mutations Pro-197 and Ser-653. This is the first documented case of Ala-205-Val, Trp-574 and Pro-197 mutations present in *Avena* spp. worldwide.

Uittreksel

Wildehawer (*Avena* spp.) is ‘n prominente onkruid in koring produksie areas van die Wes-Kaap van Suid-Afrika. Vir die volhoubare produksie, van koring is dit belangrik dat die korrekte onkruiddoder-konsentrasie toegedien word tydens die korrekte groeistadium van beide koring en wildehawer vir optimale onkruidbeheer. In Kanada en Amerika het flukarbason-natrium uitstekende beheer getoon teen wildehawer wanneer dit as ‘n na-opkoms middel toegedien is. Flukarbason-natrium dien as ‘n inhibeerder van die ensiem asetolaktaat sintase (ALS) ook bekend as asetohidroxysuur sintase (AHAS). Hierdie belangrike ensiem dien as ‘n katalis vir die biosintese van aminosure leusien, isoleusien en valien. Die produk genaamd Everest® 2.0 is ‘n nuwe sulfonielaminokarbonieltriasolinoon-onkruiddoder op die Suid-Afrikaanse mark. Flukarbason-natrium is nog nooit wetenskaplik getoets onder Suid-Afrikaanse toestande nie. Daar is geweldige groot verskille tussen die omgewing, grondtipes, koring kultivars en bestuurspraktyke van Suid-Afrika en Noord-Amerika. In hierdie studie is flukarbason-natrium getoets onder die Suid-Afrikaanse toestande van die Wes-Kaap.

Die eerste gedeelte van hierdie studie was gefokus op om vas te stel of flukarbason-natrium enige fitotoksiese effek sal hê op die koringkultivars wat in die Wes-Kaap geplant word, want daar is geen nut vir ‘n onkruiddoder wat die opbrengspotensiaal van ‘n gewas benadeel nie, ongeag hoe effektief dit onkruid beheer. Vir hierdie studie is die internasionaal aanvaarde BBCH skaal gebruik om die verskillende groeistadiums van beide koring en wilde hawer te beskryf. Flukarbason-natrium is teen half, aanbevole en dubbel die aanbevole dosis op drie verskillende groeistadiums ([12-13], [14-15] and [16-17]; BBCH skaal) van vyf

koringkultivars (SST 88, SST 027, SST 056, SST 015 en Pannar 3408) toegedien. Opbrengs en graankwaliteit (proteïeninhoud, hektolitermassa en duisendkorrelmassa) is aan die einde van die groeiseisoen gemeet. Die resultate het gewys dat koringopbrengs nie negatief beïnvloed is deur flukarbason-natrium nie. Dubbel die aanbevole dosis van flukarbason-natrium het egter ‘n beduidende ($p < 0.05$) afname in opbrengs en hektolitermassa veroorsaak op die SST 056 kultivar. Al is daar ligte tendense wat dui op ‘n afname in koringobrengs en -kwaliteit, is dit nie beduidend genoeg om produsente te ontmoedig om die produk deel te maak van ‘n geïntegreerde onkruidbestuursplan nie. Dit is egter van uiterse belang dat die riglyne op die etiket gevolg word.

Tot op hede is daar nog geen studies gepubliseer wat die beheer van wildehawer biotipes in Suid-Afrika met flukarbason-natrium bestudeer nie. Vir hierdie studie is daar drie persele in die Swartland (Moorreesburg en Piketberg) geïdentifiseer waar hoë wildehawer populasies teenwoordig was. Flukarbason-natrium is teen half, aanbevole en dubbel die aanbevole dosis toegedien op drie verskillende wildehawer groeistadiums ([12-13], [14-15] en [16-17]; BBCH skaal). Die resultate het getoon dat flukarbason-natrium wildehawer uiters effektief beheer op ‘n vroeë groeistadium by alle persele, behalwe vir die perseel by Pools naby Piketberg in die Wes-Kaap. ‘n Uiterste lae vlak van beheer deur flukarbason-natrium is waargeneem en moontlike weerstand is vermoed.

Wildehawersaad wat by die Pools perseel geoes was is gebuik in glashuis en molukulêre studies om weerstand te bevestig. Die moontlike meganisme wat verantwoordelik is vir die weerstand was ook bestudeer. Resultate het getoon dat die biotipe wat by die Pools perseel teenwoordig is wel weerstandbiedend is teen flukarbason-natrium. Selfs teen agt keer die aanbevole dosis was daar nie ‘n enkele

plantsterfte aangeteken nie. Flukarbason-natrium het wel ‘n effek gehad op die wildehawer biotipe teenwoordig by Pools, aangesien die droëmassa afgeneem het soos die konsentrasie van flukarbason-natrium verhoog het. PCR resultate het ook aangedui dat die wildehawerpopulasie by Pools homosigotiese weerstand mutasies Ala-205-Val en Trp-574 bevat, asook heterosigotiese weerstand mutasies Pro-197 en Ser-653. Dit is die eerste gedokumenteerde geval wêreldwyd waar Ala-205-Val, Trp-574 and Pro-197 mutasies in *Avena* spp. waargeneem is.

Table of Contents

List of tables

List of figures

List of abbreviations

0.5R	Half the recommended rate
2R	Double the recommended rate
AHAS	Acetohydroxyacid synthase
ALS	Acetolactate synthase
ANOVA	Analysis of variance
BBCH	Scale used to identify phenological development stages of a plant
DM	Dry mass
EC	Emulsified concentrate
GA	Gibberellic acid
GSTs	Glutathione S-transferases
HLM	Hectolitre mass
PCR	Polymerase chain reaction
R	Recommended
SC	Suspension concentrate
TKM	Thousand kernel mass
WGD	Water dispersible granular herbicide

Chapter 1

General introduction

1.1 Introduction

Wheat (*Triticum aestivum*) is one of the main staple foods of South Africa. The demand for wheat in South Africa cannot be met by the country's current wheat production system, therefore 1.5 million tons were imported during the 2014/2015 marketing season. South Africa produces 1.75 million tons on a total area of 476 570 hectares resulting in an average yield of 3.67 tons per hectare. The Western Cape Province of South Africa produced 899 000 tons of wheat in the 2014/2015 season, contributing to 51% of the total crop (www.sagl.co.za). However, with the rising cost of fuel, fertilisers, labour and agrochemicals the input costs to produce wheat is increasing annually. This progressive narrowing of the profit margin forces wheat producers in South Africa to optimise all aspects of wheat production, including weed control. According to Pieterse (2010), wild oats (*Avena* spp.) are one of the most prominent pests in wheat, second only to ryegrass (*Lolium* spp.). Wild oats are known to reduce yields by directly competing for resources such as nutrients and light, thus causing considerable damage to wheat yields if left uncontrolled (Lockhart and Howatt 2004).

With the increased reports of wild oats resistance to the available Group A and Group B herbicides in South Africa, producers are requesting alternative active ingredients for weed control. Flucarbazone-sodium (Everest® 2.0) is a new Group B herbicide that has proven to have strong and consistent activity against wild oats in Canada and the USA (Santel et al. 1999). Flucarbazone-sodium forms part of the sulfonylaminocarbonyltriazoline herbicides. Flucarbazone-sodium acts as an inhibitor of the enzyme acetolactate synthase (ALS) also known as acetohydroxyacid

synthase (AHAS), an important enzyme that acts as a catalyst in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine (Cobb and Reade 2010). Currently, there are no reports of wild oats resistance to flucarbazone-sodium worldwide, making this product an attractive prospect to control wild oats in South Africa.

The aims of this study were to:

1. Investigate the possible phytotoxicity of flucarbazone-sodium on wheat cultivars commonly grown in the Western Cape of South Africa.

2. Investigate the effect of flucarbazone-sodium on some of the wild oats *(Avena* spp.*)* biotypes found in the Western Cape of South Africa.

3. Determine whether there is wild oats (*Avena* spp.) resistance to flucarbazone-sodium, and if found, to further investigate the possible mechanism responsible for the observed resistance.

1.2 References

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Chapter 2

Literature review

2.1 Possible origin of wild oats (*Avena* spp.) in the Western Cape of South Africa

Wild oats (*Avena* spp.) has been a prominent weed in agricultural systems over many centuries and still is today (Täckholm et al. 1941). Täckholm et al. (1941) found two species of wild oats, *Avena fatua* and *Avena sterilis,* in wheat samples from the 12th Egyptian Dynasty (2000 – 1788 B.C). The exact origin of wild oats is unclear although most researchers agree it originated in south west Asia. Natural and artificial selection for certain physiological characteristics forced wild oats on an evolutionary path that made it harder to detect and control among cultivated crops and it consequently spread within traded cereal mixes to Western Europe (Cairns 1984).

In Western Europe local climatic conditions dictated which of the wild oats species flourished in certain areas. For instance, *Avena ludoviciana* is mainly found in southern England whereas *A. fatua* is found throughout Europe, Asia and North America. With global trade and the growing interest of European countries in the spice route around the tip of Africa, wild oats spread to the Cape Colony (Western Cape) of South Africa (Cairns 1984).

2.2 Wild oats in the Western Cape

Cairns (1974) collected and identified four main species of wild oats (*Avena* spp.) in the winter rainfall area of the Western Cape, namely, *A. barbata, A. fatua, A. sterilis* spp. *ludoviciana* (*A. ludoviciana*) and *A. sterilis* spp. *macrocarpa* (*A. sterilis*). He found that the compilation of seeds collected from cultivated fields were 70% *A.*

fatua, 20% *A. ludoviciana* and 5% *A. sterilis* with the remaining 5% being reported to be interspecies crosses. Murray et al. (2002) stated that pollen-mediated gene flow is a rare event and pollen transfer among wild oats appears to be restricted to relatively short distances as outcrossing. Cairns (1974) found that *A. barbata* was not as dormant as *A. fatua*, *A. ludoviciana* and *A. Sterilis,* and was found mainly next to roads and not in cultivated lands. According to Cairns (1974), *A. fatua* had a very low initial germination percentage, as opposed to *A. barbata* where 100% of seeds germinated within the first 12 months, while only 17.3% of *A. fatua* germinated during the same time period. He concluded that this probably was the reason why *A. barbata* is not found in cultivated fields. All seeds would germinate within the first growing season, causing the population to be eradicated with a single effective herbicide application. However, with the radical changes in cultivation practices in the Western Cape since 1974, it is to be expected that the species compilation of wild oats has also changed.

2.3 Identification of wild oats

Identification of wild oats in cereal crops during the vegetative stage can be done by looking at two of the main morphological aspects, namely auricle formation and the twist direction of leaves. As illustrated in Table 2.1, the auricles can be used to easily distinguish wild oats (or cultivated oats) from wheat and barley. The auricles of wild oats are absent when compared to the curly hairy auricles of wheat and the long smooth auricles of barley. The lower half of wild oats (and cultivated oats) leaves tend to twist in an anti-clockwise direction when viewed from above and wheat and barley leaves tend to twist clockwise when viewed from above. Identifying different species of wild oats at the vegetative stage can be difficult and unreliable (Moss 2015).

Table 2.1: Auricle differences between wild oats, cultivated oats, wheat and barley (Moss 2015)

Wild oats	Oats	Wheat	Barley
			
No auricles on wild-oats, but leaf margins are often hairy.	No auricles on oats and leaf margins are hairless.	Well-developed hairy auricles. Hairs restricted to the auricles.	Large, hairless auricles.

Species identification of wild oats is mainly done by observing specific structures and traits on the seeds (Baum 1977). Table 2.2 is a compact identification key for wild oats species and subspecies present in the winter rainfall region of the Western Cape (South Africa). The most common way to identify wild oats is by observing the primary and secondary seeds and their physiological relation to each other (Smit 1993). For the purpose of this study only wild oats found in the Western Cape will be discussed.

Different wild oats species can also be identified by examining the chromosome number and using genome karyotyping (Coffman 1977). Karyotyping is a method where chromosomes, in their condensed form (metaphase), are classified into seven groups, A to G, according to length and centromere position (Tseng 1995) (Figure 2.1). This method can be used to distinguish between different wild oats species as well. Coffman (1977) stated that wild oats can be categorised according to their chromosome number. He also stated that polyploidy plays a big role in the differentiation of the *Avena* family. The *Avena* genus comprises diploid ($2n = 2x =$

14; A and C genomes), tetraploid (2n = 4x = 28; AB and AC genomes) and hexaploid (2n = 6x = 42; ACD genomes) species (Leggett and Thomas 1995; Loskutov 2008; Tomás et al. 2016). The diploids and tetraploids are considered minor species and the hexaploids tend to be more economically significant. *A. fatua, A. ludoviciana* and *A. sterilis* are all hexaploids, while *A. barbata* is a tetraploid (Coffman 1977).

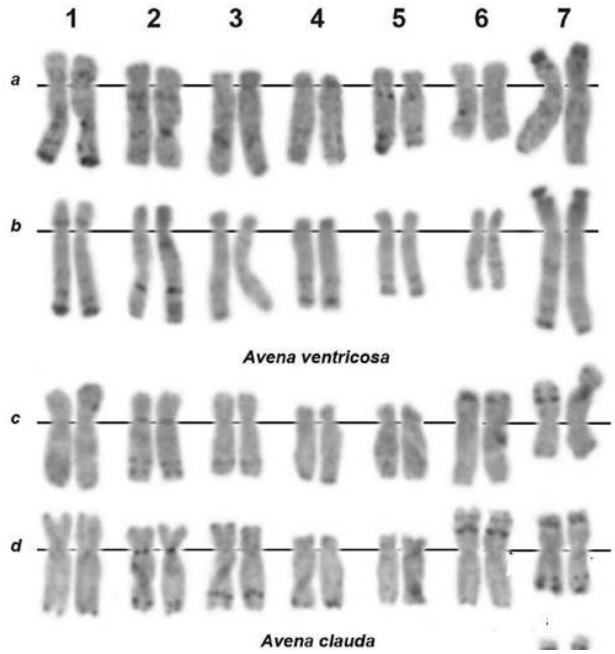
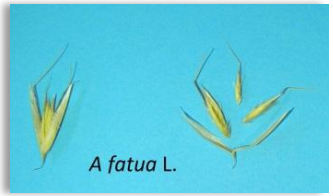
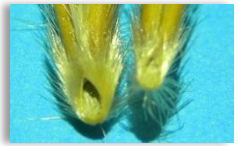


Figure 2.1: Karyotypes of diploid *Avena* species (*A. ventricosa* and *A. clauda*) with different variants of the C genome (Badaeva et al. 2010)

Table 2.2: Key to identify wild oats (*Avena* spp.) species and subspecies of the Western Cape's winter rainfall region (Cairns 1974) (Photo: DW Bester)

Seed characteristics	***Avena fatua***	***Avena barbata***	***Avena sterilis* spp. *macrocarpa* (*A. sterilis*)**	***Avena sterilis* spp. *ludoviciana* (*A. ludoviciana*)**
Spikelet dissected				
Abscission scars	 Seeds articulate on axis and separate easily Abscission scars are found on all seeds	 Seeds articulate on axis and separate easily Abscission scars are found on all seeds	 Only lower seed articulates thus second separates with pinnacle axis Abscission scar only on the lower seed	 Only lower seed articulates thus second separates with pinnacle axis Abscission scar only on the lower seed
Colour	Light brown to black	Light brown to fawn	Light straw to dark grey	Light straw to black
Spikelet size	15 – 20 mm	15 – 20 mm	20 - 30 mm	15 – 20 mm
Floret number	2 – 3	2	2 – 5	2 – 3
Awns	All seeds have awns	Both seeds have awns	Only the first two seeds have awns	Only the first two seeds have awns

2.4 Seed dormancy

The success of wild oats can be attributed to its periodic germination and lengthy period of dormancy under a wide range of climatic conditions (Cairns 1984; Cavers et al. 1992; Fennimore et al. 1999; Naylor 1983).

Beginning in 1972, Cairns conducted a three-year experiment in which he planted *A. fatua* seeds. The germination percentage under natural conditions was recorded after harvest at six months. In year one (1972) 28.3% germination was recorded and in year two (1973) and year three (1974) 18.7% and 10% respectively was recorded. This meant a total of 57% germination over the three-year period, leaving 43% of the seeds, both viable and non-viable, in the soil (Cairns 1974). Seed dormancy may vary within a population due to interactions between environmental conditions during seed development, germination environment and plant genotypes (Fennimore et al. 1999).

Simpson (2007) stated that wild oats seed dormancy can be affected by temperature in four ways:

(i) temperature during seed development influenced the level of dormancy;
(ii) temperature determined the persistence of dormancy in the dry seed;
(iii) temperature is able to induce dormancy of non-dormant seed; and
(iv) after seeds absorb water, temperature can determine whether it germinates or not.

Cairns (1974) showed that temperature plays an important role in the breaking and initiation of dormancy of wild oats seeds. His results indicated that there was a dramatic loss in dormancy when wild oats seeds were subjected to moist conditions at a constant temperature of 5 °C. Similar studies around the world resulted in

conflicting results. For instance, Friesen and Shebeski (1961) found the optimum germination temperature of *A. fatua* in Canada to be 21 °C and that dormancy was broken under warm and dry conditions. On the other hand, Quail and Carter (1969) working in Australia, found that moist and warm temperatures were best for breaking dormancy in wild oats. It is clear that each *A. fatua* biotype has developed a different relationship with its environment and the temperature and climatic conditions required to break seed dormancy.

According to Hsiao and Simpson (1971), it might appear to be a logical assumption that water is essential for the germination of wild oats, but with wild oats it is not always that simple. They found that both small and large quantities of water restricted the germination of *A. fatua* when compared to moderate volumes of water. They also found that light, together with low quantities of water, inhibited germination compared to germination under darkness and low quantities of water. This study also showed that large quantities of water together with white light promoted germination significantly more than wild oats seeds that had been subjected to darkness and large quantities of water. Thus, light promoted germination of wild oats seeds. Cairns (1984) concluded that white light inhibited the germination of both Canadian and South African *A. fatua* biotypes, whereas the germination of U.K. biotypes were stimulated by white light. Water and light clearly has an influence on the breaking and initiation of dormancy in *A. fatua* but the relation between these factors varies greatly between biotypes.

Gibberellic acid (GA) plays an important role in breaking dormancy of wild oats (*A. fatua*) seeds. The germination of dormant wild oats seeds can be stimulated through GA application (Adkins et al 1986). Adkins et al. (1986) applied GA to developing panicles of *A. fatua* and found that it was far more effective in preventing

dormancy in seed than a foliar application of the hormone. They found that both GA_3 and $GA_{4/7}$ were the only plant regulators that stimulated the germination of dormant *A. fatua* seeds when it was applied exogenously to mature seeds. Cairns (1984) found that azide, hydroxylamine and daminozide complemented gibberellic acid in stimulating germination of *A. fatua* seeds. However, Fennimore and Foley (1998) concluded that GA is not the primary regulator of seed dormancy in wild oats and that other factors independent of GA, may regulate dormancy.

Cairns (1984) fumigated dormant *A. fatua* seeds with 'Phostoxin' tablets and found that the seeds became non-dormant. These tablets produced three gases namely NH_3, PH_3 and CO_2. Of these three gases it was found that only NH_3 caused the loss of dormancy in *A. fatua* seeds. Cairns (1984) explained that an application of NH_3 to *A. fatua* seeds was successful in breaking dormancy by causing a combined effect of increased permeability and water uptake, increased peroxidase activity, lower catalase level and increased respiratory rate. The same rate of NH_3 gas is however not equally effective in breaking dormancy of semi- and deeply dormant wild oats seeds (Cairns and de Villiers 1986). According to Agenbag and de Villiers (1989), ammonium-containing fertilisers are also effective in stimulating germination and emergence of wild oats in sandy and loamy soils. In their trials wild oats seedling emergence increased by between 5 and 35% compared to the no-nitrogen treatments.

Jana and Naylor (1980) stated that seed dormancy in wild oats is a heritable trait, where genetic factors account for 50% of the germination variation and the remaining 50% is due to environmental influences. There is no clarity on which specific genes are responsible for seed dormancy in wild oats. However, Fennimore et al. (1999) found at least three loci that appear to regulate dormancy in wild oats.

They found two loci (*G*1 and *G*2) that promote early germination and a third locus (D) that promotes late germination.

2.5 The effect of wild oats on wheat yield and quality

Wild oats (*Avena* spp.) is one of the most competitive and harmful weeds resulting in large yield losses in wheat (Khan et al. 2010). It causes a yield loss of an estimated 6.4 million tons of grain worldwide annually and is considered one of the world's worst weeds (Lockhart and Howatt 2004). Wheat yield declines as wild oats plant density increases, due to the fact that wild oats are more competitive than wheat for the same resources (Carlson and Hill 1985). Wild oats and wheat have been shown to have near-identical growth stages after emergence. In Alberta (Canada) wild oats reduced wheat yield by at least 55% and as much as 85% when wild oats emerged before the wheat crop (Howatt 2009). This indicates that wheat and wild oats are always in direct competition for resources such as nutrients, space and light, with light being one of the most important of these resources (Cudney et al. 1989a). It was reported that wild oats caused 14 to 39% wheat yield loss in Saskatchewan, Canada, depending on the size of wild oats populations (Howatt 2009). About 90% of the hard red spring wheat hectares in Minnesota (USA) are sprayed each year to control wild oats (Wiersma et al. 2009). For the sustainable production of wheat it is crucial to apply the correct herbicide dosage at the correct growth stages of both wild oats and wheat in order to achieve maximum control (Cudney et al. 1989b).

It has been proven that wild oats also has an allelopathic effect on wheat, especially root growth (Pérez and Ormeño-Nuñez 1991). This study collected root exudates (scopoletin) from the roots of wild oats between emergence and the first leaf stage. Scopoletin has been reported to inhibit growth of tobacco and sunflowers

at concentrations of 0.1 mM. They found scopoletin in small amounts (10 µM), which is too small to cause inhibition of root and coleoptile growth of spring wheat. However, it was suggested that as the wild oats population increases the higher the concentration of root exudates in the soil will become, which can lead to the inhibition of root and coleoptile growth in spring wheat.

Dew and Keys (1976) created a prediction model for wheat yields loss for crops that are infested with wild oats. This equation can be written as:

$$L=ab^{1}\sqrt{wo} \qquad (1)$$

L = Loss in yield,
a = Estimated yield if weed free (g m^{-2})
b^{1} = Competitive index (0.0339) of wild oats in wheat
wo =Absolute density (wild oats m^{-2})

The loss in yield (L) will be calculated in g m^{-2} which can then be converted to ton ha^{-1}. This proposed equation can be used to evaluate how economically viable the use of certain herbicides are.

Hamman (1979) confirmed that Drew's equation gave good predictions for spring wheat production in Canada. However, Carlson and Hill (1985) found that Drew's equation underestimated wild oats effect on dwarf hard red spring wheat (Anza) which they used in their studies. They suggested that a relative density (RW) of wild oats was more effective than an absolute value (wo) in predicting wheat yield loss due to competition with wild oats. The addition of wild oats plants to a constant wheat density increased the total plant density of the system, hence creating a higher level of competition.

$$RW = \frac{wo}{(wo+wh)} \quad (2)$$

wh = total density of wild oats plus wheat

$$y' = 100 - \left[\frac{83.5\ RW}{0.164+RW}\right] \quad (3)$$

y' = Wheat yield percentage of expected weed-free yield.

Carlson and Hill (1985) found the competition index of wild oats in wheat to be 0.0473 ± 0.0034. They suggested that the difference between competition indices may be the result of several plausible factors such as different cultural practices, the different wheat cultivars used in the studies, and fertiliser applications. They also suspected that nitrogen fertiliser and available moisture could increase the competition of wild oats with wheat. Blackshaw and Brandt (2008) found that wild oats was slightly more competitive than wheat in terms of soil nitrogen uptake at all fertiliser rates. Lack et al. (2011) showed that by increasing the nitrogen application the negative effect of wild oats on wheat grain yield also increased.

2.6 Flucarbazone-sodium

In the 1980s five new herbicide classes emerged that share the same site of action. They proved to be very effective and selective with a broad spectrum of activity. These five classes, sulfonylureas (SU), imidazolinones (IM), triazolopyrimidines (TP), sulfonylaminocarbonyltriazolines and pyrimidinyloxybenzoates (PTB), are all chemically different but have the same site of action. They act as inhibitors of the enzyme acetolactate synthase (ALS) also known as acetohydroxyacid synthase (AHAS), an important enzyme that acts as a catalyst in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine. ALS inhibitors are very successful herbicides on cereal crops and currently represent the second largest

class of herbicidal active ingredients (Frihauf et al. 2010). ALS inhibitors are highly selective at low dosages. This is however not due to herbicide uptake, movement or sensitivity to ALS, but is correlated to rapid rates of metabolism in tolerant crops (Cobb and Reade 2010).

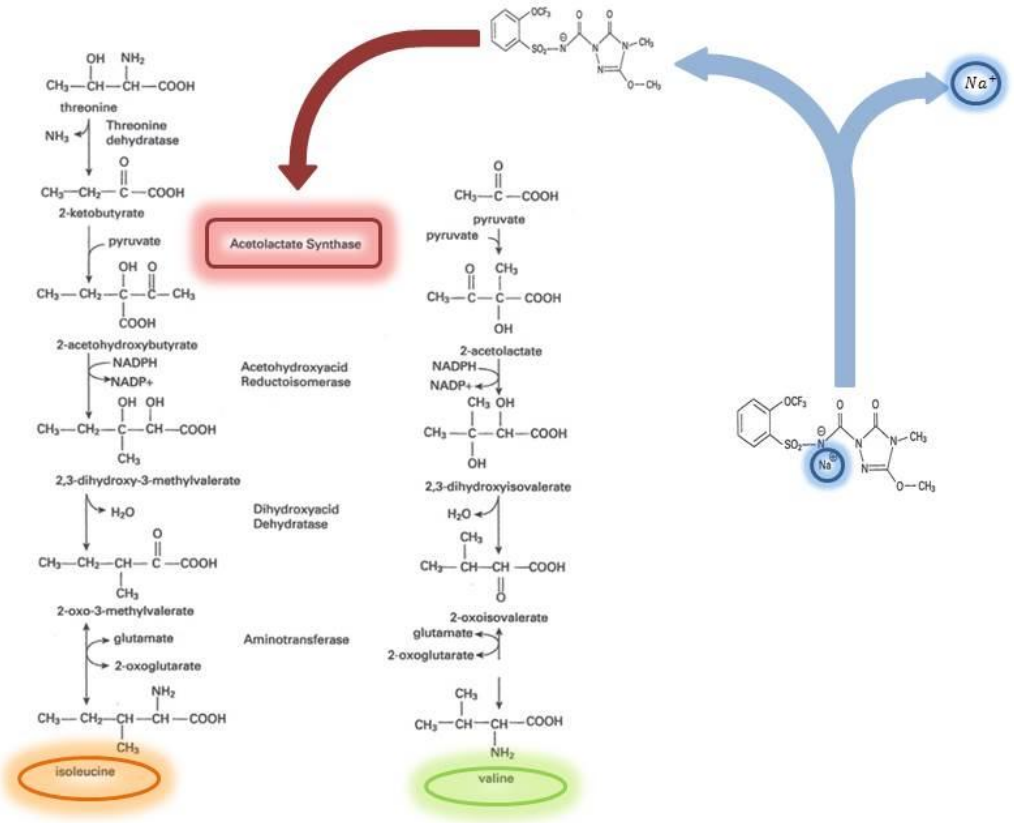


Figure 2.2: Biosynthesis of amino acids (alanine, isoleucine, valine and leucine) affected by the inhibition of enzyme acetolactate synthase (ALS) also known as acetohydroxyacid synthase (AHAS) by flucarbazone (Cobb and Reade 2010)

Leucine, isoleucine and valine are important amino acids which form the bases of functional proteins in the plant cell. For example, the CLAVATA 1 gene in plants encodes for a protein which has a leucine-rich repeat. This protein and many similar proteins enable cellular responses to various types of extra cellular signal (Taiz and

Zeiger 2010). It is however not clearly understood how treated plants die after ALS inhibition (Cobb and Reade 2010).

Flucarbazone-sodium is a new sulfonylaminocarbonyltriazoline herbicide. Flucarbazone-sodium is a commercially formulated sodium salt, and because it is a relatively weak acid (pK_a=1.9) it will dissociate to flucarbazone anion and a sodium cation at environmental pH levels (Eliason et al. 2009). See Figure 2.2.

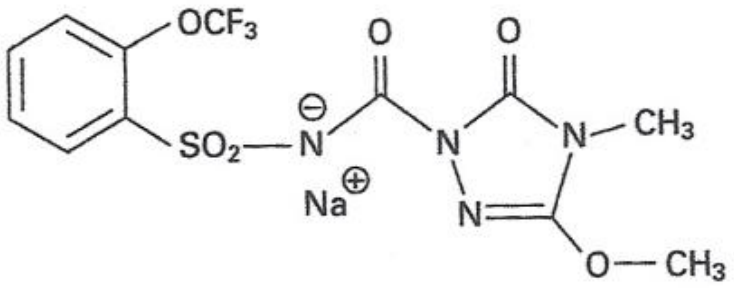


Figure 2.3: Flucarbazone-sodium (Cobb and Reade 2010)

Flucarbazone-sodium provides excellent activity against grass weeds and several important broadleaf weeds when applied as a post-emergence herbicide to wheat. In field experiments conducted in Canada and the USA, this herbicide has demonstrated strong and consistent activity against wild oats (*Avena fatua*) (Santel et al. 1999). Due to flucarbazone's high level of selectivity, it is important to note that flucarbazone-sodium can have residual activity on crops in the following growing season. Eliason et al. (2009) studied the behaviour of flucarbazone-sodium in six western Canadian soils. They found that flucarbazone-sodium concentrations as low as 1 µg kg^{-1} caused root inhibition of mustard. It was concluded that the phytotoxicity of flucarbazone-sodium is greatly related to soil organic matter and/or soil pH (Eliason et al. 2009).

Geisel et al. (2008) tested the residual activity of ALS-inhibiting herbicide residues in soils (Saskatchewan, Canada). Imazamethabenz, flucarbazone-sodium, sulfosulfuron, and florasulam each in combination with imazamox and imazethapyr was tested. They found that all the herbicides reduced mustard root length when compared with the control, except flucarbazone-sodium and florasulam. The residual activity of flucarbazone-sodium has not been tested in South Africa. Due to the vast number of climatic, soil and cultivation differences between South Africa and North America one can expect to see great differences in residual activity of flucarbazone-sodium.

Not only is it important to understand the residual activity of a new type of herbicide, it is also important to understand how the herbicide will react to other agro-chemicals when mixed together in the same application. Howatt (2009) showed that herbicides that control broadleaf weeds can antagonise the efficacy of graminicides. This can lead to a decrease in grassy weed control and eventually yield loss. A general perception is that broadleaf herbicides like MCPA and bromoxynil mixed in a tank with graminicides for broad spectrum weed control in wheat, often cause a decrease in grass weed control. Howatt (2009) found that flucarbazone-sodium together with carfentrazone-ethyl (broadleaf herbicide) gave no more than 65% control of wild oats. This indicates that care should be taken when mixing flucarbazone-sodium with other agro-chemicals.

Due to the fact that wild oats has an uneven germination rate, one application per season may not be enough for effective control. Lockhart and Howatt (2004) conducted a study on how split applications of herbicides at reduced rates can effectively control wild oats in wheat. They found that in areas where environmental conditions favour wild oats germination for an extended period of time, a second

herbicide application may be needed to control later emerging wild oats. Controlling wild oats with flucarbazone-sodium tends to be more effective at the three-leaf growth stage compared with the four- to five-leaf stages. (Lockhart and Howatt 2004)

2.7 Resistance

The extensive use of ALS-inhibiting herbicides in winter wheat has resulted in the development of ALS-resistant weed species (Frihauf et al. 2010). Currently there are more species resistant to ALS-inhibiting herbicides than any other herbicide group (Tranel and Wright 2002). Species with confirmed resistance to ALS inhibitors in South Africa are ryegrass (*Lolium rigidum*), small-seeded canary grass (*Phalaris minor*), wild radish (*Raphanus raphanistrum*), common chickweed (*Stellaria media*) and wild oats (*Avena fatua*) (http://weedscience.org/). There are different mechanisms that cause herbicide resistance such as target-site resistance, non-target-site-resistance, metabolism-based resistance and multiple resistance.

Target-site resistance occurs when a mutation in the DNA sequence chemically alters the target binding site of a specific herbicide. Alternatively, the target enzyme can be over-expressed by means of gene amplification or alterations in the gene promoter (Powles and Yu 2010). Target-site mutations have caused weeds to become resistant to herbicides that inhibit Photosystem II (PSII), microtubule assembly and also enzymes such as acetolactate synthase (ALS), acetyl-CoA carboxylase (ACCase) and 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. The resistance most often is caused by a point mutation. A point mutation can result from a single nucleotide change in the DNA sequence that encodes for a specific amino acid. The target-site on the functional protein then changes due to the alteration of the amino acid. This means that the herbicide can no longer bind to the

specific target-site and plants with this mutation will not be affected by the herbicide. Although many weed species are resistant to the same herbicide, resistance may not be due to the same mutation (www.passel.unl.edu).

In recent years non-target site resistance became an increasingly common phenomenon. Some studies report that by using only one mode-of-action herbicides might increase the selection of non-target-site-based resistance genes and therefore cause resistance to other unrelated herbicides (Beckie et al. 2012; Delye et al. 2011). A single mechanism caused by a single gene mutation can result in cross-resistance between two or more herbicides in a resistant weed biotype, although it is possible for more than one gene to contribute to the same resistance mechanism (Preston and Mallory-Smith 2001). The mechanism can either be target-site based or non-target-site based, like metabolism-based resistance. Non-target-site based resistance, such as decreased herbicide penetration into the plant, decreased rates of herbicide translocation, increased rates of herbicide sequestration or enhanced levels of metabolic breakdown of the active ingredient, prevents phytotoxic levels of the herbicide from reaching the site of action. The origin of weed biotypes that are cross-resistant to herbicides from different mode-of-action groups are generally a result of enhanced plant metabolism. However, an altered herbicide binding-site or a reduction in herbicide translocation most often only causes cross-resistance to herbicides within the same mode of action group (Beckie and Tardif 2011).

Metabolism-based resistance is caused by the biochemical breakdown and detoxification of the herbicide and does not involve alterations in the binding site of the herbicide. Most plants have the ability to break down herbicides to some extent, but when plants are selected for increased rates of detoxification the weed population becomes resistant to the herbicide (www.passel.unl.edu). Beckie et al.

(2012) found that metabolism-based resistance was much more prevalent than target-site resistance in ALS-inhibiting resistant wild oats populations in Canada.

There appear to be two main enzyme systems implicated in metabolism-based resistance: the glutathione S-transferases (GSTs) and the cytochrome P450 mono-oxygenases (P450s). To date, the cytochrome P450 mono-oxygenases are the most common group of enzymes responsible for metabolism-based resistance (Powles and Yu 2010). Studies have shown that when resistance is caused by cytochrome P450 mono-oxygenase detoxification there is usually more than one P450 gene involved, which can be expected if one takes into account the amount of P450 isozymes that have been identified (Letouzé and Gasquez 2001; Preston 2004). A wide variety of reactions within plant metabolism is catalysed by P450 enzymes and usually either cause hydroxylation or de-alkylation of the herbicide. Thus, some P450s will cause certain herbicides to be metabolised to products with a reduced or modified phytotoxicity (Powles and Yu 2010). These metabolised products are then further inactivated, often by conjugation to glucose and subsequent transport into the vacuole (Kreuz et al. 1996). P450-based herbicide resistance is a very menacing resistance mechanism due to the fact that P450 enzymes can metabolise herbicides with different modes-of-actions, potentially including herbicides that have never been used before (Powles and Yu 2010).

Glutathione S-transferases (GSTs) are multifunctional enzymes families that catalyse the conjugation of glutathione to a variety of electrophilic of hydrophobic substrates. GSTs have a particular role of interacting with active oxygen species as a protective mechanism to oxidative stress (Dixon et al. 1998). GSTs are most often part of a stress response within the plant, and in some weed species herbicides are detoxified by glutathione conjugation. Glutathione-conjugated herbicides can be

sequestered in the vacuole (Martinoia et al. 1993) or exuded via root tips (Schröder et al. 2007).

Multiple resistance is when a resistant weed biotype acquires two or more resistance mechanisms, either as a result of repeated herbicide mode-of-action group selection, or the accumulation of different resistance alleles through pollen flow in species subjected to outcrossing (Beckie and Tardif 2011).

2.8 Using the BBCH scale

A standard code or scale is often used in research to describe growth stages of both agricultural crops and weeds. The BBCH scale is an extension of the cereal scale (Zadoks scale) developed by Zadoks et al. in 1974. The BBCH scale distinguishes between monocotyledons, dicotyledons, perennial plants, gramineae and vegetative propagated plants (Figure 2.4). This scale then gives a more specific indication of growth stages for individual crops and weeds (Table 2.3).

Table 2.3: Relative codes from the BBCH scale (Lancashire et al. 1991)

BBCH code	Description	BBCH code	Description
0	Dry seed	**10**	First true leaf emerged from coleoptile
1	Beginning of seed inhibition	**11**	First leaf unfolded
2	-	**12**	2 leaves unfolded
3	Seed inhibition complete	**13**	3 leaves unfolded
4	-	**14**	4 leaves unfolded
5	Radicle (root) emergence from seed	**15**	5 leaves unfolded
6	Elongation of radicle, formation of root hairs and/or lateral roots	**16**	6 leaves unfolded
7	Coleoptile emerged from caryopsis	**17**	7 leaves unfolded
8	-	**18**	8 leaves unfolded
9	Emergence: coleoptile breaks through soil surface	**19**	9 or more leaves unfolded

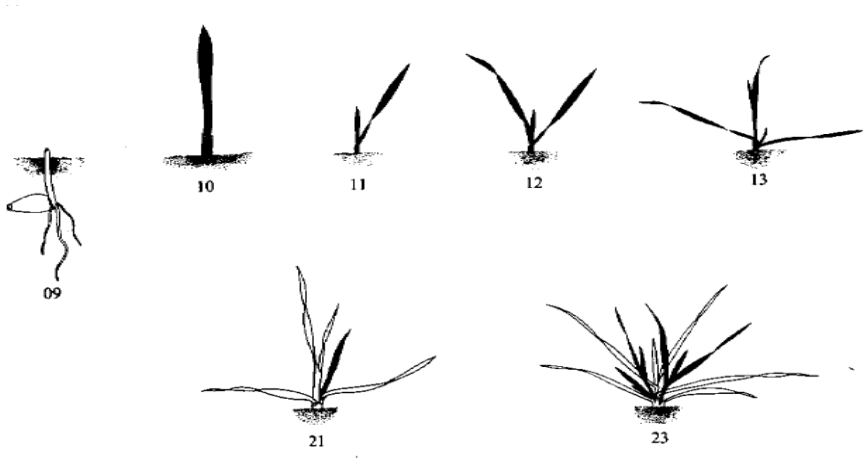


Figure 2.4: Visual representation of the BBCH scale used in this study (Lancashire et al. 1991)

2.9 Conclusions

The selectivity of flucarbazone-sodium at low rates (30 g ai ha^{-1}), especially for wild oats in wheat, makes it an attractive proposition for use by the South African agricultural community. There are no documented reports of resistance of wild oats to flucarbazone-sodium and very few incidents of phytotoxicity, thus making flucarbazone-sodium an attractive option for the control of wild oats. There is no way to predict how flucarbazone-sodium will react in South African soils or to local climatic conditions. Hence, the effectiveness of wild oats control by using flucarbazone-sodium needs to be investigated as well as its effect on wheat cultivars grown in the Western Cape of South Africa.

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Chapter 3

Evaluating phytotoxicity of flucarbazone-sodium on popular wheat cultivars of the Western Cape in South Africa

3.1 Introduction

Wild oats (*Avena* spp.) has been a prominent weed in agricultural systems over centuries (Täckholm et al. 1941). The success of wild oats can be attributed to its periodic germination and lengthy period of dormancy under a wide range of climatic conditions (Cairns 1984). The irregular germination of wild oats is the main factor contributing to its success as a weed (Adkins and Ross 1981). Flucarbazone-sodium has shown to provide excellent activity against wild oats when applied as a post-emergence herbicide to wheat (*Triticum aestivum* L.) field experiments conducted in Canada and the USA (Santel et al. 1999). Flucarbazone-sodium acts as an inhibitor of the enzyme acetolactate synthase (ALS), also known as acetohydroxyacid synthase (AHAS), an important enzyme that acts as a catalyst in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine (Cobb and Reade 2010).

Herbicides drastically influence all aspects of primary and secondary metabolism in crops (Alla et al. 2008). Although wheat can metabolise flucarbazone-sodium, some studies suggest that care should be taken when applying flucarbazone-sodium on wheat (Howatt 2009). Spring wheat treated with flucarbazone-sodium appeared stunted compared with wheat treated with other grass herbicides namely clodinafop-propargyl, tralkoxydim and imazamethaben (Howatt 2009). Flucarbazone-sodium caused a 23% injury on wheat, and injured wheat more in spring than in the fall (Hoskins et al. 2009). However, another study by Wiersma et al. (2009) tested the tolerances of eight hard red spring wheat

cultivars to flucarbazone-sodium at two locations in Minnesota (USA) at recommended, half, and twice the recommended rates. Under these conditions flucarbazone-sodium caused intermediate injury, a slight height decrease and a slight decrease in grain yield to the cultivars tested. Other ALS-inhibiting herbicides such as thifensulfuron, chlorsulfuron, prosulfuron, and tribenuron are all registered on soft red winter wheat, however, their labels contain warnings about potential wheat injury (Grey et al. 2012).

Everest® 2.0 is a suspension concentrate (SC) formulation and will only be available to local South African producers from 2017 (personal communication, S. Nolte, 2016, Arysta LifeScience, Paarl). Currently Everest® 70 WGD (water dispersible granular herbicide) is available on the South African market. This study made use of an SC formulation of flucarbazone-sodium which has built-in safener technology. It must be noted that crop tolerance can differ between formulations of the same herbicide active ingredient. SC formulations of oxyflurofen caused less injury to broccoli (Gast et al. 2004), cabbage (Hatterman-Valenti and Auwarter 2007), and onions (Richardson et al. 2006), compared with equivalent rates of an EC (emulsified concentrate) formulation. Other examples include glyphosate resistant soybean response to different glyphosate formulations (Reddy and Zablotowicz 2003), rice response to propanil (Baltazar and Smith 1994) and leaf necrosis of wheat was greater at 3 to 6 days after treatment with the EC formulation compared with the WGD formulation of saflufenacil (Frihauf et al. 2010).

The degree of injury to wheat caused by flucarbazone-sodium applications can be affected by biotic and abiotic factors. The product label states that unacceptable injury symptoms may occur as a result of unfavourable growing conditions such as waterlogged or water saturated soils, temperature extremes, drought, low fertility,

freezing weather or plant disease at the time of application (Wiersma et al. 2009). Soil temperature has an effect on the availability of herbicides in soil, and influences plant metabolic processes such as transpiration, membrane permeability, and water uptake (McMullan and Nalewaja 1990). Increased plant metabolic activity at high compared to low temperatures may cause crop injury as a result of increased herbicide uptake (Adams 1973). Hence, the weather conditions during the trial site were taken into account when analysing the results of this study.

For this study, the internationally-accepted BBCH scale was used to describe the different growth stages (Lancashire et al. 1991). By using an international scale subjectivity is limited in the description of the growth stages. The BBCH scale is an extension of the Zadoks code commonly used to describe growth stages in cereals. The BBCH scale gives a decimal code for all agricultural crops and weeds (Lancashire et al. 1991). However, for this study the decimal code used in the BBCH scale and the Zadoks code was the same.

Many studies quantify herbicide phytotoxicity by means of visual inspection of the crop after herbicide application, and then expressing it as a percentage compared to an untreated control (Frihauf et al. 2010; Grey et al. 2012; Howatt 2009; Masiunas 1989). McMullan and Nalewaja (1990) on the other hand used a scale to evaluate foliar necrosis and growth reduction (stunting) of winter wheat, from 0 (no injury) to 100 (plant death). Other common ways to record herbicide phytotoxic effect is the reduction in grain yield (Degenhardt et al. 2005; Frihauf et al. 2010; Grey et al. 2012; Khan et al. 2003; Newhouse et al. 1992; Trusler et al. 2007), biomass (Anderson and Nielsen 1991; Degenhardt et al. 2005; Khan et al. 2003; Masiunas 1989; McMullan and Nalewaja 1990) and plant height (Khan et al. 2003; Newhouse et al. 1992). The effects on grain quality as a result of herbicide application are

uncommon but some studies do evaluate this aspect (Martin et al. 1990; Ries et al. 1970). This study uses grain yield and grain quality parameters to evaluate the effect of flucarbazone-sodium on local wheat cultivars.

Bailey et al. (2004) stated that for a herbicide to be adopted by producers, crop tolerance must be established and the potential for increased sensitivity to a herbicide on certain crop cultivars must be defined. A study by Grey et al. (2012) found no statistical ($p < 0.5$) difference in yield between different plots (imidazolinone-resistant soft red winter wheat) treated with chlorsulfuron (ALS inhibitor) and the untreated control. This is in contrast with the findings of Grey and Bridges (2003) where 10% wheat injury from chlorsulfuron was recorded at 151 days after application for the wheat cultivar “Dozier”. Regardless, whether flucarbazone-sodium effectively controls wild oats in the cultivated wheat fields of the Western Cape, the product has to be tested for possible phytotoxicity. Flucarbazone-sodium has never been analysed scientifically under South African field conditions. By examining the variation in the results from different studies done in Canada and the USA it becomes apparent that flucarbazone-sodium has to be tested on the local wheat cultivars before any clear conclusions can be made—hence the aim of this study.

3.2 Materials and methods

3.2.1 Trial site layout and design

A site in the Swartland area (-33.161499, 18.508260) was chosen to study the effect of flucarbazone–sodium (Everest® 2.0) applications on the yield and grain quality of local spring wheat (*Triticum aestivum* L.) cultivars. The trial site was laid out as a

5x4x3 factorial design, and replicated four times in a randomised block design. Four randomly placed blocks (A, B, C and D) were placed over a total area of 2000 m^2.

Within each block five different cultivars were planted in random rows. Cultivars chosen for this study were SST 88, SST 027, SST 056, SST 015 and Pannar 3804. These cultivars were chosen due to their popularity among wheat producers in the area. According to a list compiled by the South African Grain Laboratory (SAGL), SST 056 (21.67%) and SST 88 (16.79%) dominated the market in the Western Cape. SST 015 (9.45%) and SST 027 (13.93%) were also popular cultivars in the 2013 growing season. Pannar 3408 was chosen because it was a relatively new cultivar at the time and according to the SAGL it was the most popular Pannar cultivar (0.88%) (www.sagl.co.za).

Each of the rows consisted of twelve 1.36m x 4m plots, wherein a set of treatment applications were randomised. Each set of applications contained the following treatments: Control, 0.5R, R and 2R, where R is the recommended rate (70 $m\ell\ ha^{-1}$) for flucarbazone-sodium (Everest® 2.0) as stipulated by Nolte (personal communication, S. Nolte, 2013, Arysta LifeScience, Paarl). Each treatment was applied at three different growth stages: [12-13], [14-15] and [16-17] (BBCH scale) to examine whether there was any variation in susceptibility between the different growth stages.

Wheat was planted on 6th June 2013 with a Wintersteiger “Plotman” planter. The Wintersteiger “Plotman” planter has eight discs, each with a rubber pressing wheel. The distance between rows was 17 cm, thus making the total width 1.36 m. Wheat was planted at a density of 250 plants m^2. Blocks D and C were planted 2.5 m from Blocks A and B to allow a tractor and a spreader to pass through. This was

done to simulate the fertilising conditions of commercial farming. The first fertiliser application was done on 13th June 2013 (Yara: Alpha 33; 24N, 10.6P, 2.6K) and the second on 1st August 2013 (Yara: Cura A433; 40N, 5P). Two fungicide treatments were applied with a knapsack sprayer. Fungicide applications were made on all plots at the same rates to limit the possible effect of pathogens on the wheat yield. The first fungicide application was done on 11th July 2013 (Tebuconazole® 750 mℓ ha^{-1}) and the second on 23rd August 2013 (Duett® 1 ℓt ha^{-1}) (Figure 3.1).

3.2.2 Treatment application procedure

The treatments were formulated and mixed before moving to the trial site in order to achieve greater accuracy and consistency in treatment concentrations. Rain water (pH 5.8; EC 890 µS cm^{-1}) was used, to eliminate or limit the effect of pH and anion immobilisation on the activity of flucarbazone-sodium. A Matabi “Super Agro 16”, knapsack sprayer with a 2 m application boom was used to spray all the plots in the trial. The Matabi knapsack sprayer was fitted with a flow regulator for the even application of treatments. The knapsack sprayer was calibrated at 150 ℓt ha^{-1}, at 180 kPa (1.8 bar) with a walking speed of 1 m s^{-1} (3.6 km h^{-1}). “Teejet 110 02 vp” nozzles were used with an interspacing of 500 mm. These nozzles were designed to be used at 500 mm above the target area, thus slight adjustments to height above ground level were made according to plant growth stage at the time of each application. At each growth stage the treatment application was done in an ascending order from 0.5R to 2R. After each treatment application the knapsack sprayer, pipes and spray nozzles were thoroughly washed and cleaned with hydrogen peroxide. Treatment applications were done on 30th June 2013, 25th July 2013 and 10th August 2013 for growth stages [12-13], [14-15] and [16-17] respectively (Figure 3.1).

3.2.3 Climatic conditions during treatment application

Data was obtained from the weather databases of Langewens experimental farm situated 22.3 km from the trial site. The data was obtained via e-mail (email communication, I.O. Joubert, 25 March 2014, Langewens experimental farm, Moorreesburg). Average temperatures varied between 18 °C by day and 6.5 °C by night during the application period and temperature extremes varied between 25.6 °C and 4 °C. All applications were done on rain- and wind-free days during the late morning when the dew had evaporated from the leaves.

From the planting date (06/06/2013) to the first application (30/06/2013) 43 mm precipitation was recorded with maximum temperatures ranging between 25.6 °C and 13.6 °C (17.1 °C average) and minimum temperatures ranging between 13 °C and 5.4 °C (8.4 °C average). Between the first and second application (25/07/2013) 50.6 mm precipitation was recorded with maximum temperatures ranging between 25.3 °C and 13.7 °C (17.3 °C average) and minimum temperatures ranging between 13 °C and 5.3 °C (8.5 °C average). Between the second and third application (10/08/2013) 7mm precipitation was recorded with maximum temperatures ranging between 25.2 °C and 12 °C (18 °C average) and minimum temperatures ranging between 12.3 °C and 4 °C (7.7 °C average). Figure 3.1 gives a more detailed

account of the climatic conditions over the treatment application period.

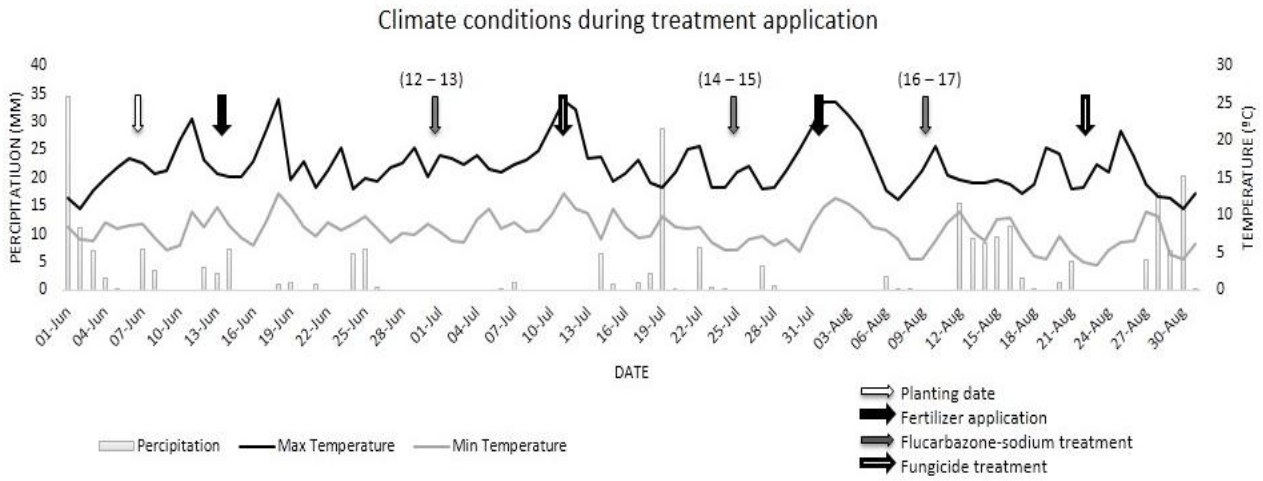

Figure 3.1: Schematic representation of the climatic conditions during the treatment application period

3.2.4 Harvesting and measurements

Yield was measured by harvesting a 0.25 m^2 sub-plot sample of each plot and was done to account for possible unforeseen irregularities in the trial site. The use of sub-plot harvesting to evaluate yield in phytotoxicity trials is supported by Frihauf et al. (2010) and McMullan and Nalewaja (1990). Inter-cultivar competition, row effect and small differences in the plot length could all influence the yield data if the whole plot was harvested. These sub-plot samples were harvested by hand and threshed with a threshing machine at Welgevallen farm (University of Stellenbosch). The rest of the plot area was harvested by a Hege B25 trial combine with a 1.5 m front and this wheat was used for quality analysis, by measuring the hectolitre mass, thousand kernel mass and protein content (%). Protein content and hectolitre mass was calculated by using a Perten Inframatic grain analyser at the Koperfontein wheat testing station. The thousand kernel mass (TKM) was calculated by weighing a thousand kernels with two 500-kernel slot plates.

3.2.5 Statistical analysis

Data were analysed using analysis of variance (ANOVA) and repeated-measures analysis of variance. Where appropriate and applicable, second and third-order interactions were calculated. Mean comparisons were achieved by means of Fisher's least significant difference ($P < 0.05$) using Statistica (STATISTICA 11.0, Statsoft Inc., Tulsa, Oklahoma, USA)

3.3 Results and discussion

3.3.1 Yield

There does not appear to be a significant ($p < 0.05$) interaction between any of the main factors in this 5x4x3 factorial experiment. However, the data did show significant differences within both the treatments and cultivars with relation to wheat (*Tritcum aestivum* L.) grain yield. The analysed data does not indicate that the growth stage of wheat at flucarbazone-sodium application has a significant effect on wheat yield (Table 3.1).

Table 3.1: Interaction between main factors cultivar, treatment and growth stage with regards to wheat yield as a result of four different flucarbazone-sodium application rates (Control, 0.5R, R and 2R)

	F-value	Pr > F	**Significance**
Cultivar	12,710	<0,000000	**
Treatment	3,224	<0,023965	*
Growth stage	0,159	0,853083	ns
Cultivar * Treatment	0,664	0,784399	ns
Cultivar * Growth stage	0,218	0,987345	ns
Treatment * Growth stage	0,595	0,733677	ns
Cultivar * Treatment * Growth stage	0,837	0,686294	ns

* $p < 0.05$; ** $p < 0.01$; ns, not significant at $p = 0.05$

On average, the recommended (R) flucarbazone-sodium application rates had no significant effect on the yield of the cultivars tested in this study. Grey et al. (2012)

tested ALS-inhibiting herbicides chlorsulfuron, mesosulfuron, thifensulfuron, tribenuron, prosulfuron, and pyroxsulam and found no yield differences between treated and untreated plots of imidazolinone-resistant soft red winter wheat. When Fenoxaprop was applied at the recommended and twice the recommended rates it did not significantly reduce the yield of any of the eight hard red spring wheat cultivars tested (Wiersma et al. 2003). In this study, however, there were a significant ($p < 0.05$) reduction in the average wheat yield when double the recommended rate (2R) was applied in comparison with the control (C) (Figure 3.2). In comparison, Wiersma et al. (2003) found that plant heights of wheat cultivars Ingot, Ivan, McVey, HJ98, P2375, and Verde were decreased when double the recommended rate of flucarbazone-sodium was applied. This suggests that wheat can become less tolerant when the recommended rate for flucarbazone-sodium application is exceeded.

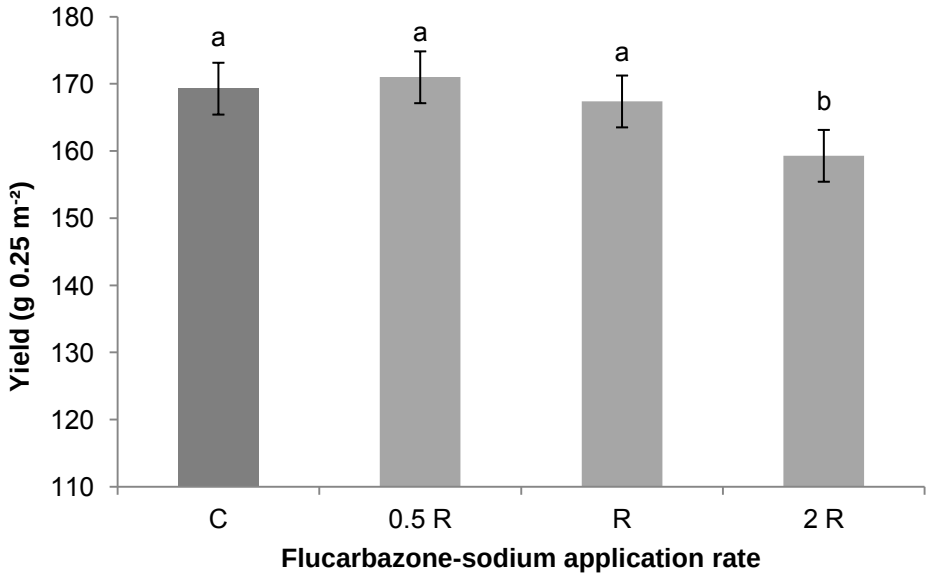

Figure 3.2: The collective average yields of five wheat cultivars (Pannar 3804, SST 056, SST 88, SST 015, and SST 027) used in this study and their response to flucarbazone-sodium (Everest® 2.0). C (control) and R (recommended rate). Treatment combinations with different letter symbols differ significantly ($p < 0.05$). Error bars = standard error (3.86)

In Table 3.1 the data shows a significant ($p < 0.05$) difference in yields between different cultivars. This can be attributed to natural cultivar yield potential differences. In 2013 the ARC Small Grain Institute of South Africa found that there were average yield differences in the Swartland area for the cultivars Pannar 3804 (4.96 ton ha^{-1}), SST 015 (4.94 ton ha^{-1}), SST 056 (4.78 ton ha^{-1}), SST 88 (4.76 ton ha^{-1}) and SST 027 (4.76 ton ha^{-1}). This is why the cultivars in this study were analysed separately with regards to flucarbazone-sodium tolerance, and it was found that only SST 056 showed a significant reduction ($p < 0.05$) in yield, compared to the control when double the recommended rate (2R) of flucarbazone-sodium was applied (Figure 3.3). The data therefore suggests that not all local wheat cultivars are equally tolerant to flucarbazone-sodium at increased rates. This variation in susceptibility between cultivars to herbicides is not uncommon as McMullan and Nalewaja (1990) reported variation in tolerance between wheat cultivars when the active ingredient triallate was incorporated during spring pre-plant applications. Shaw and Wesley (1991) also observed variation in cultivar tolerance to herbicides applied on winter wheat. Susceptible cultivars appear to absorb and translocate the herbicide similarly, but they metabolise the herbicide more slowly, resulting in crop injury (Wiersma et al. 2003).

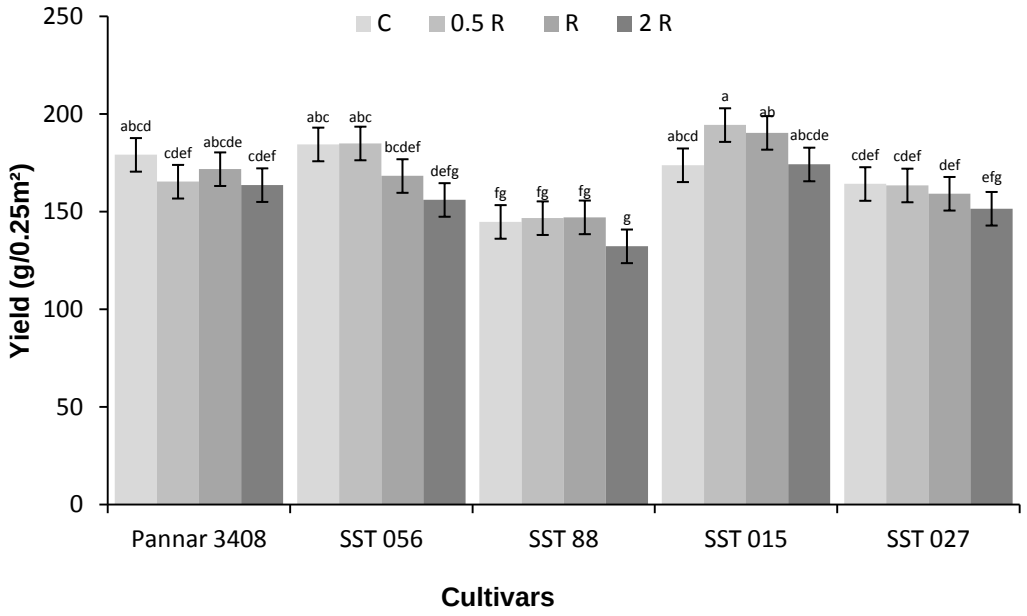

Figure 3.3: The average yields of the five wheat cultivars (Pannar 3804, SST 056, SST 88, SST 015, and SST 027) for each flucarbazone-sodium (Everest® 2.0) application rates respectively. C = Control and R = recommended rate. Treatment combinations with different letter symbols differ significantly ($p < 0.05$). Error bars = standard error (8.6)

The literature suggests that in many cases where initial phytotoxicity or stunting occurs as a result of ALS-inhibitor herbicide application, the crop appears to recover to such an extent that yields are not significantly affected. Wiersma et al. (2003) documented initial crop injury, as a result of flucarbazone-sodium applications, to be between 9% to 13% for label recommended rates (0.03 kg ai ha^{-1}) and 13% to 16% for double recommended rates applied on eight different hard red spring wheat cultivars, but no reduction in grain yield. Grey et al. (2012) reported that mesosulfuron caused significant stunting of soft winter wheat from 7 right through to 34 days after application, however, there was no significant yield reduction when compared to the untreated control. Similarly, Bailey et al. (2004) reported that mesosulfuron applications resulted in a 29% stunting 21 days after application on a non-imidazolinone-resistant cultivar (AGS 2000), however, at harvest no injury was

observed. Thus, it appears that wheat tends to outgrow initial injury sustained from ALS-inhibiting herbicides. However, this does not always hold true for all cultivars, especially at increased application rates.

3.3.2 Protein content (%)

No significant ($p < 0.05$) interaction between any of the main factors in this 5x4x3 factorial experiment with regards to protein content was recorded. There were no significant ($p < 0.05$) differences between the four different flucarbazone-sodium applications with respect to protein content, neither did the wheat growth stage present at the time of application have any influence on protein content. However, the data did show significant differences between the different cultivars tested in this study (Table 3.2). Polymeric proteins may vary in amount, size and distribution among different wheat cultivars and this can be attributed to differences in structure, number of cysteine residues and hydrophobic interaction between different protein subunits (Malik 2009).

Table 3.2: Interaction between main factors cultivar, treatment and growth stage with regards to wheat protein content as a result of four different flucarbazone-sodium application rates (Control, 0.5R, R and 2R)

	F-value	**Pr > F**	**Significance**
Cultivar	14,36	<0,000000	**
Treatment	0,42	0,741656	ns
Growth stage	2,38	0,095151	ns
Cultivar * Treatment	0,41	0,959450	ns
Cultivar * Growth stage	0,33	0,954514	ns
Treatment * Growth stage	0,72	0,635116	ns
Cultivar * Treatment * Growth stage	1,03	0,435092	ns

* $p < 0.05$; ** $p < 0.01$; ns, not significant at $p = 0.05$

Protein content may differ naturally among different cultivars (Terman et al. 1969). Variation in activity and presence of different genes that influence

characteristics of wheat quality causes significant phenotypic differences among wheat cultivars with regards to grain and flour quality (Payne et al 1979). Mladenov et al. (2001) conducted a study where they investigated wheat quality traits as a result of environment and cultivar interactions and found highly significant differences among the environments and cultivars with regards to wheat protein content. During the 2013 growing season the ARC Small Grain Institute of South Africa found average differences in protein content for cultivars Pannar 3804 (12.24%), SST 015 (12.4%), SST 056 (11.94%), SST 88 (11.94%) and SST 027 (13.07%) in their Swartland cultivar evaluation trials. Thus, the variation in protein content among the cultivars evaluated in this study is not unusual and can be explained by genetic variation between cultivars.

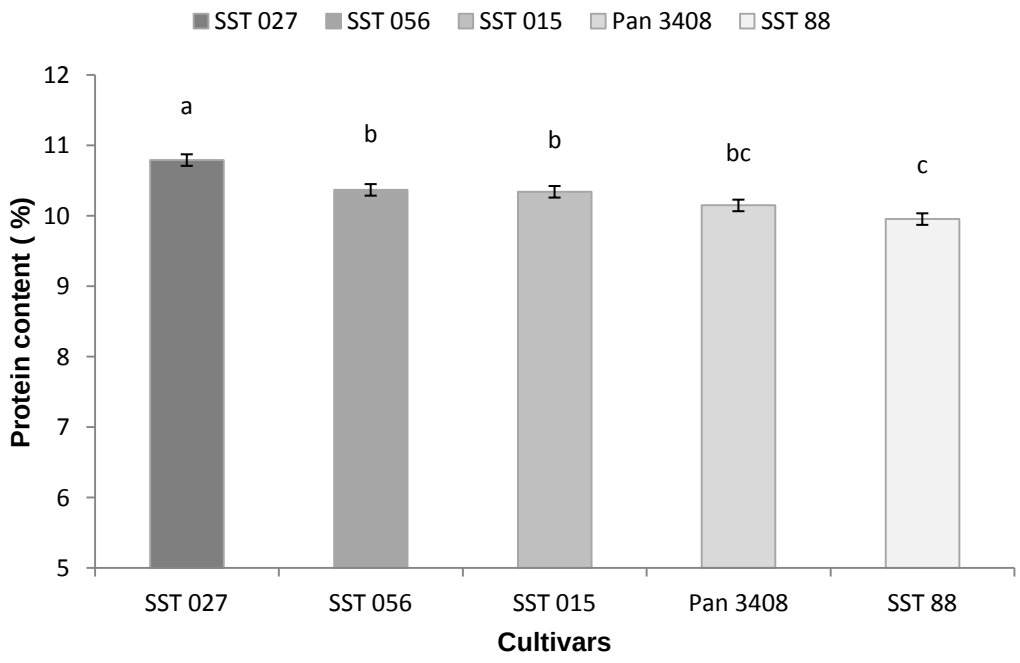

Figure 3.4: The average wheat grain protein content of five wheat cultivars (SST 027, SST 056, SST 015, Pannar 3408 and SST 88) as a result of four different flucarbazone-sodium application rates (Control, 0.5R, R and 2R) applied at three different growth stages ([12-13], [14-15] and [16-17]). Treatment combinations with different letter symbols differ significantly ($p < 0.05$). Error bars = standard error (0.082)

The fact that this study did not find any significant effect of flucarbazone-sodium applications on wheat grain protein content does not mean that protein content should not be taken into account when evaluating phytotoxicity of herbicides on wheat. Martin et al. (1990) used 'Oslo' spring wheat to evaluate 10 broadleaf herbicide treatments at three growth stages. They found that dicamba, dicamba plus 2,4-D and dicamba plus MCPA reduced wheat yield 28, 21 and 24%, and interestingly increased seed protein 8, 10 and 13%, respectively, when applied at BBCH stage 44. Alternatively they documented that chlorsulfuron, an ALS inhibitor, together with MCPA did not affect grain protein content and yield at any of growth stages. Ries et al. (1970) also found an increase in wheat grain protein content in all of their trials, when they applied suboptimal rates of simazine and terbacil. However, Alla et al. (2008) found that the ALS inhibitor chlorimuron-ethyl decreased enzymes (valine, leucine and isoleucine) used to form plant proteins in plant tissues of young 10 day old wheat plants. Whether flucarbazone-sodium has the same effect on young wheat plants is unclear, if it did however, it does not appear to significantly affect grain protein content at time of harvest.

3.3.3 Hectolitre mass (HLM)

No significant ($p < 0.05$) interaction between any of the main factors in this 5x4x3 factorial experiment, with regards to hectolitre mass (HLM) was recorded. The data showed significant ($p < 0.05$) differences between the HLM of the different cultivars in this study (Table 3.3). Shuey (1960) showed that cultivars can differ in HLM by as much as 13.1 kg hL^{-1} without differing in flour yield. Wheat cultivars also appear to differ in their response to the environment and other factors that affects HLM. Results from a two year study in Canada, where five cultivars were used to investigate the reduction in HLM as a result of moderate rainfall, showed that

cultivars differed in HLM reduction (Czarnecki and Evans 1986). The ARC Small Grain Institute of South Africa found average differences in HLM for cultivars Pannar 3804 (76.27 kg), SST 015 (76.74 kg), SST 056 (77.63 kg), SST 88 (77.10 kg) and SST 027 (77.63 kg) in their Swartland cultivar evaluation trials (2013). Cultivar effect appears to be the reason for these significant differences between cultivars.

Table 3.3: Interaction between main factors cultivar, treatment and growth stage with regards to wheat hectolitre mass (HLM) as a result of four different flucarbazone-sodium application rates (Control, 0.5R, R and 2R)

	F-value	Pr > F	**Significance**
Cultivar	104	<0,000000	**
Treatment	2	0,064961	ns
Growth stage	0	0,640296	ns
Cultivar * Treatment	1	0,430983	ns
Cultivar * Growth stage	2	0,053150	ns
Treatment * Growth stage	1	0,519386	ns
Cultivar * Treatment * Growth stage	1	0,494431	ns

* $p < 0.05$; ** $p < 0.01$; ns, not significant at $p = 0.05$

There were no significant ($p < 0.05$) differences between the four different flucarbazone-sodium applications with respect to HLM. However the p-value was very low ($p = 0.065$) (Table 3.3). Upon further investigation of the data, it appears that on average across all the cultivars, double the recommended rate (2R) resulted in a significant decrease ($p < 0.05$) in HLM compared to the control (C) (Figure 3.5). There are other accounts where herbicides were recorded to influence HLM. Marinkovic et al. (1997) found that double rates of MCPP plus 3,6-D caused significant decreases in wheat hectolitre mass. When all the cultivars were analysed separately Pannar 3408 and SST 056 had a HLM value significantly ($p < 0.05$) lower than that of the control, at double the recommended rate (2R) (Figure 3.6).

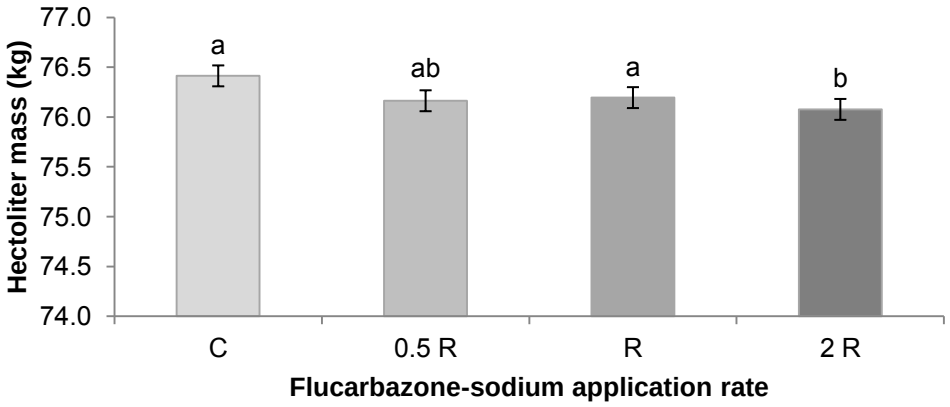

Figure 3.5: The average wheat hectolitre mass (HLM) of five wheat cultivars (Pannar 3804, SST 056, SST 88, SST 015, and SST 027) treated with different rates of flucarbazone-sodium (Everest® 2.0). C = Control and R = recommended rate. Treatment combinations with different letter symbols differ significantly ($p < 0.05$). Error bars = standard error (0.106).

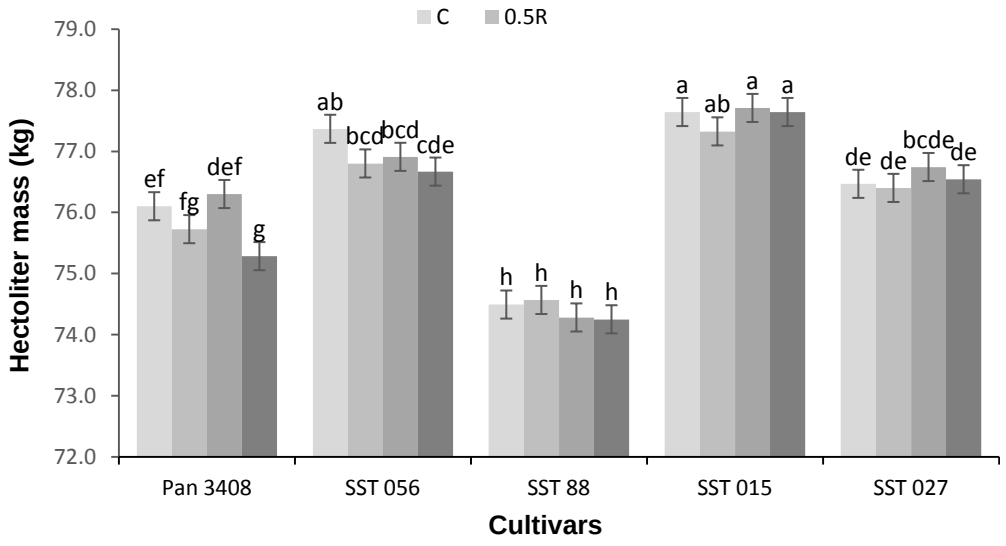

Figure 3.6: The average wheat hectolitre mass (HLM) of wheat cultivars SST 056 and Pannar 3408 treated with different rates of flucarbazone-sodium (Everest® 2.0). Treatment combinations with different letter symbols differ significantly ($p < 0.05$). Error bars = standard error (0.237)

3.3.4 Thousand kernel mass (TKM)

No significant ($p < 0.05$) interaction between any of the main factors in this 5x4x3 factorial experiment, with regards to thousand kernel mass (TKM) was recorded. The data showed significant ($p < 0.05$) differences between TKM of the different cultivars

and the different flucarbazone-sodium treatments. The statistical analysis did not show any significant effect with regards to TKM and growth stage sensitivity to flucarbazone-sodium (Table 3.4).

Table 3.4: Interaction between main factors cultivar, treatment and growth stage with regards to wheat thousand kernel mass (TKM) as a result of four different flucarbazone-sodium application rates (Control, 0.5R, R and 2R).

	F-value	Pr > F	**Significance**
Cultivar	231,78	0,000000	**
Treatment	4,28	0,006088	**
Growth stage	0,19	0,824791	ns
Cultivar * Treatment	1,05	0,405313	ns
Cultivar * Growth stage	1,25	0,275314	ns
Treatment * Growth stage	0,75	0,606460	ns
Cultivar * Treatment * Growth stage	0,90	0,596975	ns

* p < 0.05; ** p < 0.01; ns, not significant at p = 0.05

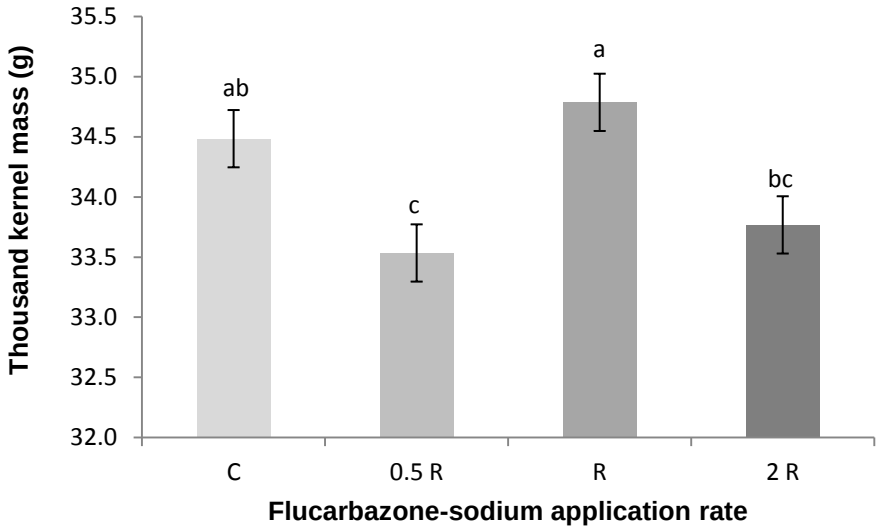

Figure 3.7: The average wheat thousand kernel mass (TKM) of five wheat cultivars (SST 015, SST 056, SST 027, SST 88 and Pannar 3408) treated with different rates of flucarbazone-sodium (Everest® 2.0). C = Control and R = recommended rate. Treatment combinations with different letter symbols differ significantly (p < 0.05). Error bars = standard error (0.237)

The data indicated significant ($p < 0.05$) differences between different treatments (Figure 3.7). However, the differences do not appear to be logical. Thus, no clear conclusions can be made with regards to flucarbazone-sodium applications on wheat grain TKM.

3.4 Conclusion

The concept of phytotoxicity is a complex one. In many cases with ALS-inhibiting herbicides there appears to be an initial phytotoxic effect, but it does not necessarily manifest in grain yield loss. In the discipline of agriculture the priority should be to express phytotoxicity as a reduction in yield and yield quality, which will ultimately have financial implications for producers.

This study showed that wheat yield was not negatively affected by flucarbazone-sodium at recommended rates or lower. However, double the recommended dosage rate (2R) showed a significant reduction in yield, and on closer inspection of the data only SST 056 appear to show a significant ($p < 0.05$) reduction. The HLM of SST 056 was also negatively affected by increased flucarbazone-sodium rates, and only SST 056 appears to show some form of phytotoxicity to this active ingredient. This cultivar-specific (wheat) phytotoxicity effect to herbicides does not appear to be priority with agro-chemical suppliers, agro-chemical distributors, wheat plant breeders or regulating govermental research institutes in South Africa. However, the immense amount of resources and funding needed to evalute phytotoxicity accurately might be the main reason for this oversight. Thus, future research should focus on investigating possible alternatives to quantify and simplify wheat phytotoxicity with the aim of making herbicide

phytotoxic screening more feasible financially and this should also form part of wheat breeding programmes.

Even though there are slight trends that indicate a reduction in the yield and quality of wheat, there is no statistical evidence to discard this product in a weed management system, as long as the application guidelines on the product label are followed. It should also be mentioned that flucarbazone-sodium applications are subject to weather restrictions (Wiersma et al. 2003). Soil type, clay content and organic matter has a varying effect on ALS-inhibiting herbicides with respect to possible phytotoxic effect and weed control (Wiersma et al. 2009).

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Chapter 4

The use of flucarbazone-sodium to control wild oats (*Avena* spp.) biotypes in the Western Cape of South Africa

4.1 Introduction

Wild oats (*Avena* spp.) is a prominent weed in agriculture systems throughout the world (Dexter et al. 1981; Fennimore et al. 1999; Li et al. 2007; Muur 1999; Willenborg et al. 2005). Producers in western Canada spent more money on herbicides for wild oats control than on any other weed species (Harker et al. 2016). In the United States wild oats infests more than 11 million ha of cropland, causing more than $1 billion in crop losses (Beckie et al. 2012). In South Africa wild oats is the second most prominent grass weed, second only to ryegrass (*Lolium* spp.) in the cultivated wheat fields of the Western Cape (Pieterse 2010). Four different species of wild oats can be found in the Western Cape of South Africa namely *A. fatua, A. sterilis, A. ludoviciana* (*Avena sterilis* spp. *ludoviciana*) and *A. barbata* (Cairns 1974). The ability of wild oats seed to germinate from relatively deep under the soil surface and stay dormant in soils for many years makes it a very strenuous weed to control (Sharma and Vanden Born 1978; Smit 1993). Dormancy and multiple emerging flushes throughout the growing season results in the persistence and continual re-infestation of this weed in the soil seed bank (Beckie et al. 2012; Fuerst et al. 2011). Wild oats compete directly with wheat for nutrients and sunlight, causing substantial losses in grain yields (Carlson and Hill 1985).

For the sustainable production of wheat, it is crucial to apply the correct herbicide rate at the correct weed growth stage in order to achieve maximum control (Holm et al. 2000). The majority of post-emergence herbicides available to wheat producers are ALS inhibitors (Bellinder et al. 1994; Frihauf et al. 2010). ALS

inhibitors tend to be popular among producers because of their broad weed control spectrum, low application rates, high levels of crop safety and their relatively long activity in soils, thus helping to control weed species such as wild oats that have multiple flushes per growing season (Beckie and Tardif 2011; Mazur and Falco 1989). However, the continuous use of ALS-inhibiting herbicides has resulted in several herbicide-resistant weed species (Merotto et al. 2009; Vidal et al. 2005; Whaley et al. 2007). There are many reported cases world wide of wild oats resistance to ALS inhibitors (Beckie et al. 1999; Heap et al. 1993; Joseph et al. 1990; www.weedscience.org). In South Africa, resistance in wild oats to ALS inhibitors was reported as far back as 1986 (Cairns and Laubscher 1986).

In field experiments conducted in Canada and the USA, flucarbazone-sodium has shown to provide excellent activity against wild oats when applied as a post-emergence herbicide to wheat (Santel et al. 1999). Flucarbazone-sodium acts as an inhibitor of the enzyme acetolactate synthase (ALS), also known as acetohydroxyacid synthase (AHAS), an important enzyme that acts as a catalyst in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine (Cobb & Reade 2010). The product known as Everest® 2.0 is a new sulfonylaminocarbonyltriazoline herbicide (ALS inhibitor) on the South African market. Currently there are over 20 different products on the South African market registered to control wild oats in wheat, but due to varying levels of resistance to some of these products the demand grew for alternative herbicide action groups to aid in the ongoing battle to control wild oats. However, Beckie and Tardif (2011) stated that choosing among classes of ALS inhibitors to manage ALS resistance can be considered as a risky short-term proposition. No studies to date have been

published on flucarbazone-sodium effectiveness in controlling wild oats under South African conditions.

The main aim of this study was to evaluate flucarbazone-sodium on different wild oats biotypes present in the Western Cape. However, the South African label for Everest® 2.0 states that the product controls resistant wild oats, small-seeded canary grass (*Phalaris* spp.) but only suppresses ryegrass (*Lolium* spp.). Thus, for the sake of completeness of this study, a spectrum trial was conducted over a single growing season to evaluate the activity of flucarbazone-sodium on small-seeded canary grass and ryegrass.

4.2 Methods and materials

4.2.1 Location and trial layout

For the wild oats trials, three sites in the Swartland area (Moorreesburg and Piketberg) with high wild oats infestations were chosen. Bester, a field specialist, was consulted (personal communication, F. Bester, 2013, Moorreesburg) and great care was taken to find trial sites where wild oats was almost the only weed present. These sites were at Glen Peter (-33.164363, 18.509043) 2013, Pools (-2.809598, 18.847044) in 2013 and Doringdam (-33.163928, 18.515528) in 2014. All the sites were laid out in a completely randomized block design. Each site had 48 (2 m x 4 m) plots. For the spectrum study two trial sites were selected to evaluate the efficacy of flucarbazone-sodium on canary grass and ryegrass. These sites were at Baronskop (-33.169685, 18.491714) and Klein Tweevlei (-33.133228, 18.504871) for ryegrass and small-seeded canary grass respectively, in 2014. Each site had 16 (2 m x 4 m) plots.

All sites were laid out in a manner to prevent cross contamination with other herbicides, hence a 1.5 m buffer zone was laid out around each trial site. Paraquat (Gramoxone®) was used to clear pathways around each plot. The trial sites were placed in the various fields in such a way to not only avoid cross-contamination but also ensure that all sites received the recommended commercial application of fertilisers and fungicides. In other words, herbicides were sprayed around the trial sites and fertilisers and fungicides were applied over the trial sites. This was to create an environment which simulated as closely as possible current commercial practices.

4.2.2 Treatments and treatment application procedure

For the trials on wild oats there were four treatments, a control (C), 0.5R, R and 2R where R is the recommended application rate (70 mℓ ha^{-1}) for flucarbazone-sodium (Everest® 2.0). Each treatment was replicated four times at three different growth stages, [12-13], [14-15] and [16-17], according to the BBCH scale (Lancashire et al. 1991). The completely randomized block design was therefore arranged in a 4x3 factorial with four application treatments and three growth stages. The same four treatments (C, 0.5R, R and 2R) were replicated four times at both the ryegrass and canary grass trial sites. The growth stage of ryegrass and small-seeded canary grass were [13-15] at the time of application.

Since very small amounts of the product were used, treatments were formulated and mixed before travelling to the trial site. This was done to achieve maximum accuracy in the concentration. An adjuvant (Wet-all®) was added to all application mixtures at the recommended rate of 50 mℓ ha^{-1}. Rain water (pH 5.8; EC 890 µS cm^{-1}) was used, to eliminate or limit the effect of pH and anion immobilization on the activity of flucarbazone-sodium. For the spraying of all the trials a Matabi,

"Super Agro 16", knapsack sprayer with a 2 m application boom was used. A flow regulator was also fitted to the Matabi knapsack for the even application of treatments. The knapsack was calibrated at 150 ℓ ha^{-1}, at 180 kPa (1.8 bar). Walking speed was 1 m s^{-1} (3.6 km h^{-1}). The nozzles used were "Teejet 110 02 vp" with a interspacing of 500 mm. Spraying height was adjusted at each site, since the mentioned nozzles were designed to be used at 500 mm above the target area. At each growth stage, the application concentrations were done in an ascending order from 0.5R to 2R. Care was taken to make sure the higher concentration treatment was circulated through the knapsack sprayer system before application commenced. All equipment and containers were washed with hydrogen peroxide to ensure that all equipment was clean for subsequent treatments.

All applications were done on rain- and wind-free days during the late morning when the dew had evaporated from the leaves. An investigation prior to application was done each time, to confirm that the target weeds were not under any form of stress, hence most applications were done two to three days after precipitation. Temperatures did not exceed 18 °C at the time of application.

4.2.2.1 Trial 1 (Glen Peter, 2013)

Treatment applications were done on 30 June 2013, 25 July 2013 and 10 August 2013 for growth stages [12-13], [14-15] and [16-17] respectively. The trial site was laid out along a contour embankment where a high wild oats population was present. Due to the nature of wild oats distribution it was decided to lay out a random plot design around a central control-strip.

4.2.2.2 Trial 2 (Pools, 2013)

Treatment applications were done on 28 June 2013, 18 July 2013 and 5 August 2013 for growth stages [12-13], [14-15] and [16-17] respectively. At the Pools trial site there was a high and even wild oats population present, and therefore a random block design could be laid out.

4.2.2.3 Trial 3 (Doringdam, 2014)

Treatment applications were done on 29 June 2014, 15 July 2014 and 4 August 2014 for growth stages [12-13], [14-15] and [16-17] respectively. At the Doringdam trial site there was a high and even wild oats population present, hence a random block design could be laid out.

The level of wild oats control was calculated by counting the number of plants in a 0.25 m^2 subplot and then extrapolating the result to plants m^{-2}.

4.2.2.4 Trial 4 (Spectrum study, 2014)

The treatment application on ryegrass was done on 29 June 2014 at a [13-15] growth stage and the treatment application on canary grass was done on 7 July 2014 at a [13-15] growth stage. For this trial only a visual estimation of weed control was done. Treatment plots were compared to the untreated plots, and expressed as a percentage.

4.2.3 Statistical analysis

All values were compared to the untreated control and evaluated statistically. Analysis of variance (ANOVA) was used for evaluating data (STATISTICA 11.0, Statsoft Inc., Tulsa, Oklahoma, USA) and the statistical significance of the results was analysed by the t-test ($p < 0.05$).

4.3 Results

4.3.1 Trial 1 (Glen Peter, 2013)

From a visual inspection of the trial site it was clear that flucarbazone-sodium treatments did have an effect on the wild oats (*Avena* spp.) population. At the end of the growing season the number of plants m^{-2} were calculated and the data was statistically analysed. In this 4x3 factorial experiment there were no interaction between the main factors flucarbazone-sodium treatments and wild oats growth stage (Table 4.1). Significant ($p < 0.05$) differences in wild oats plants m^{-2} between the different growth stages were observed in the data. As illustrated in Figure 4.1, all of the flucarbazone-sodium treatments (0.5R, R and 2R) applied at growth stage [12-13] gave 100% control, which significantly ($p < 0.05$) differs from all the levels of control observed in the growth stages [14-15] and [16-17]. This indicates that the wild oats biotype present at the Glen Peter site is extremely sensitive to flucarbazone-sodium at the [12-13] growth stage, but became more tolerant at later growth stages [14-15] and [16-17]. The data showed no significant ($p < 0.05$) differences between the flucarbazone-sodium treatments, thus indicating that growth stage rather than application rate contributes more to the success of wild oats control by flucarbazone-sodium.

Table 4.1: Interaction between main factors treatment and growth stage with regards to wild oats plants m-2 as a result of four different flucarbazone-sodium application rates (0.5R, R and 2R) at the Glen Peter site. R = recommended flucarbazone-sodium application rate (70 mℓ ha^{-1})

	F-value	Pr > F	**Significance**
Treatment	0,444	0,646	ns
Growth stage	81,527	0,000	**
Treatment * Growth stage	0,972	0,439	ns

* $p < 0.05$; ** $p < 0.01$; ns, not significant at $p = 0.05$

During the visual inspection of the trial site at Glen Peter it was noted that the wild oats treated with flucarbazone-sodium at the [14-15] growth stage appeared to be stunted and plant growth appeared to be inhibited. Even though the wild oats plants appeared stunted the majority survived till harvest, and produced seeds. The stunted appearance of the wild oats at the Glen Peter site might either be attributed to higher metabolic break down levels by the wild oats at the [14-15] growth stage or the higher level of canopy cover, by both crop and weed, at the [14-15] growth stage that could have caused less of the herbicide to reach the soil surface. The latter means that there was less of the herbicide present in the soil and thus caused a lower level of residual effect, since ALS inhibitors are known for their residual effect in soils (Beckie and Tardif 2011). When the means of the treatments, 0.5R [14-15], R [14-15] and 2R [14-15], were calculated as a percentage of the control mean, only 29%, 34% and 22% control was achieved respectively. None of the treatments at growth stage [14-15] differed significantly from each other (Figure 4.2). Even though treatments at the [14-15] growth stage differed significantly from the control, the level of control was still inadequate.

Treatments 0.5R [16-17] and R [16-17] did not differ significantly from the control, thus indicating that flucarbazone-sodium is less effective in controlling the wild oats biotype at the Glen Peter site at later growth stages. Applying flucarbazone-sodium at twice the recommended rate at growth stage [16-17] did however result in a significant reduction in the number of plants m^{-2} (Figure 4.2). However, when the mean of the treatment 2R [16-17] was calculated as a percentage of the control mean it showed that the 2R treatment only gave 31% control (Figure 4.2).

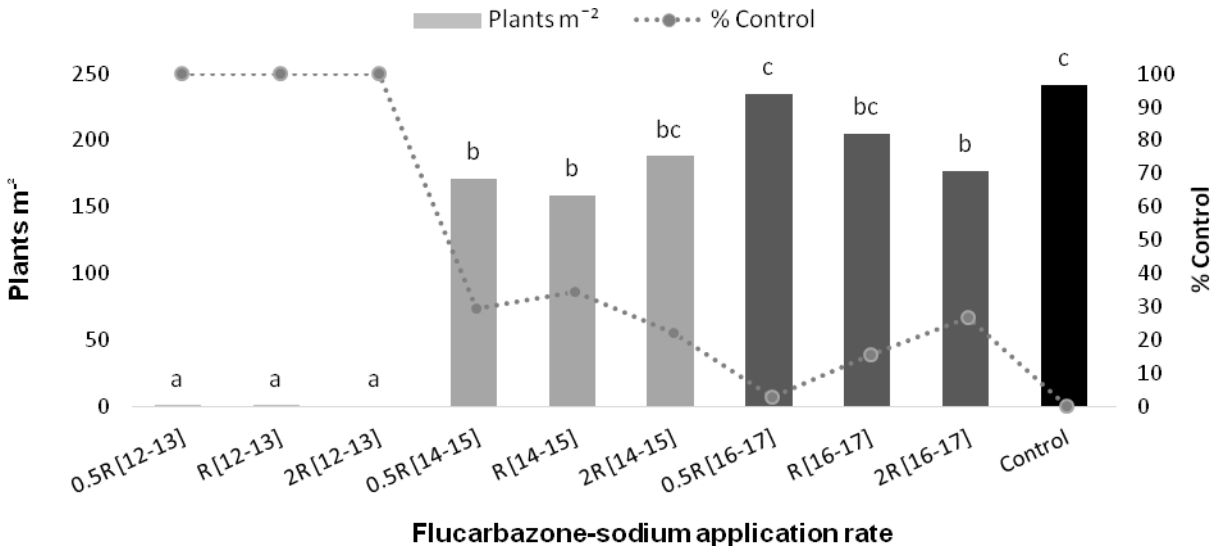

Figure 4.1: The mean number of wild oats (*Avena* spp.) plants m^{-2}, as a result of different rates of flucarbazone-sodium applied at different growth stages ([12-13], [14-15] and [16-17]) at the Glen Peter site ($LSD_{0.05}$=61.36). The percentage of control, compared to the control, is indicated by the line. Treatment combinations with different letter symbols differ significantly ($p < 0.05$)

4.3.2 Trial 2 (Pools, 2013)

During visual inspections of the trial site it was unclear whether flucarbazone-sodium showed any form of wild oats control at the Pools site. The number of plants m^{-2} was calculated at the end of the growing season and the data was statistically analysed. In this 4x3 factorial experiment there was no interaction between the main factors, flucarbazone-sodium treatments and wild oats growth stage (Table 4.2). However, a significant ($p < 0.05$) difference was observed when the [12-13] growth stage was compared to the [14-15] and [16-17] growth stages, indicating that even though control was inadequate, there is still a certain sensitivity of the wild oats population at the Pools area to flucarbazone-sodium at earlier growth stages.

Table 4.2: Interaction between main factors treatment and growth stage with regards to wild oats plants m-2 as a result of four different flucarbazone-sodium application rates (0.5R, R and 2R) at the Pools site. R = recommended flucarbazone-sodium application rate (70 mℓ ha^{-1})

	F-value	Pr > F	**Significance**
Treatment	0,813	0,454	ns
Growth stage	7,492	0,0026	**
Treatment * Growth stage	0,170	0,952	ns

* p < 0.05; ** p < 0.01; ns, not significant at p = 0.05

After establishing the level of control by calculating the treatment means as a percentage of the control (C) mean, certain trends became evident. It appeared that flucarbazone-sodium did have a slight effect on the wild oats population at Pools. The analysis of the data set showed that treatments R [12-13] and 2R [12-13] differed significantly from the control but the level of control was still inadequate since only 61% and 51% control was achieved respectively. Apart from the treatments mentioned no other treatments showed any significant difference from the control (Figure 4.2). Wild oats resistance to flucarbazone-sodium is suspected at the Pools site, however, it is not confirmed.

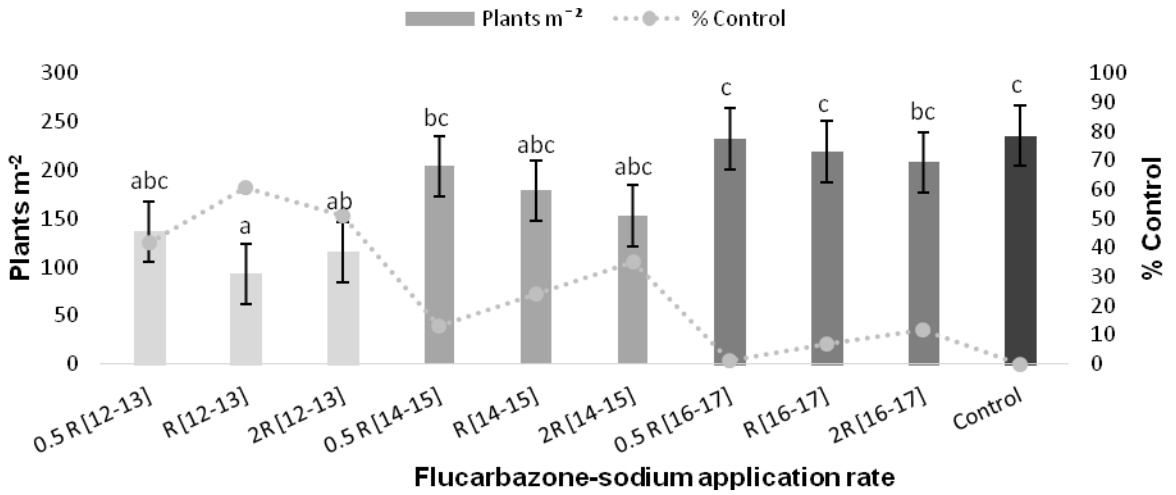

Figure 4.2: The average number of wild oats plants per square meter, when different rates of flucarbazone-sodium were applied at different growth stages ([12-13], [14-15] and [16-17]) at the Pools site. ($LSD_{0.05}$=96.757). The percentage of control, compared to the control, is indicated by the line. Treatment combinations with different letter symbols differ significantly (p < 0.05)

4.3.3 Trial 3 (Doringdam, 2014)

The visual inspection of the trial site showed clear indications that flucarbazone-sodium treatments did have an effect on the wild oats population at Doringdam. The number of plants m^{-2} were calculated and the data was statistically analysed. In this 4x3 factorial experiment there was a significant ($p < 0.05$) interaction between the main factors flucarbazone-sodium treatments and wild oats growth stage (Table 4.3). Thus, no clear statistical conclusions can be made regarding the main factors separately.

Table 4.3: Interaction between main factors treatment and growth stage with regards to wild oats plants m^{-2} as a result of four different flucarbazone-sodium application rates (0.5R, R and 2R) at the Doringdam site. R = recommended flucarbazone-sodium application rate (70 mℓ ha^{-1})

	F-value	Pr > F	**Significance**
Treatment	9,754	0,00065	**
Growth stage	350,932	0,00000	**
Treatment * Growth stage	4,529	0,00627	**

* $p < 0.05$; ** $p < 0.01$; ns, not significant at $p = 0.05$

From Figure 4.3 it appears that treatments at earlier growth stages of wild oats appeared more effective than applications at later growth stages. Treatments of flucarbazone-sodium at the growth stage [12-13] gave 99% control over the wild oats population at the Doringdam trial site. In direct contrast with results observed at the Glen Peter site the year before, adequate control was achieved at the [14-15] growth stage. Apart from the 0.5R [14-15] treatment the R [14-15] and the 2R [14-15] treatment did not differ significantly from any of the applications done at the [12-13] growth stage (Figure 4.3). This suggests that the wild oats biotype at the Doringdam site was more susceptible to flucarbazone-sodium than the population at the Glen Peter site.

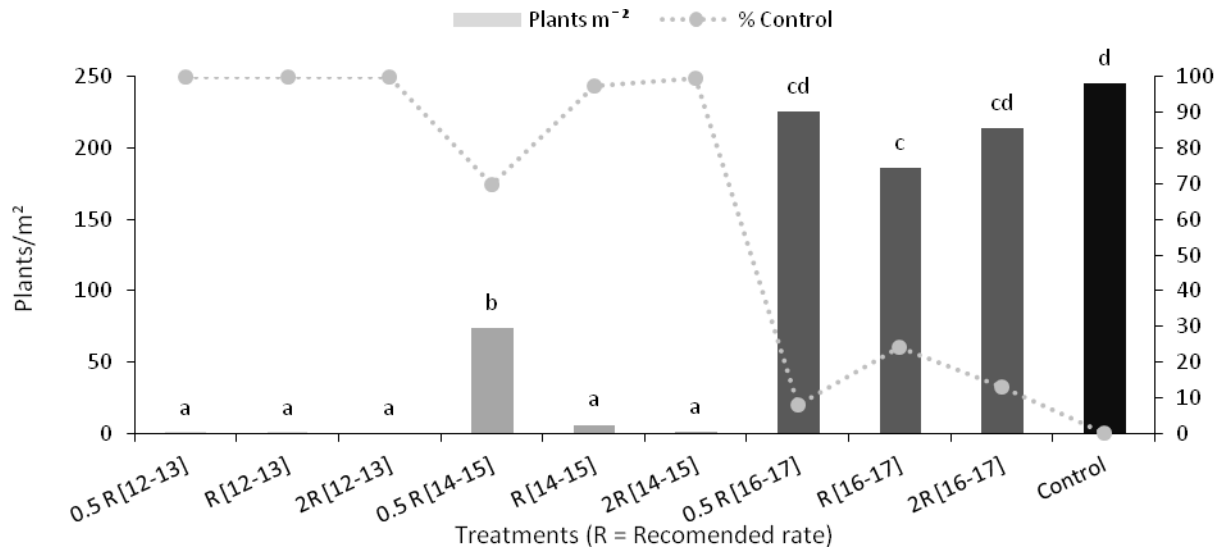

Figure 4.3: The average number of wild oats plants per square meter, when different rates of flucarbazone-sodium were applied at different growth stages ([12-13], [14-15] and [16-17]) at the Doringdam site. ($LSD_{0.01}$=30.414). Treatment combinations with different letter symbols differ significantly ($p < 0.05$)

4.3.4 Trial 4 (Spectrum study, 2014)

A visual estimation of weed control was made at Baronskop (ryegrass) and Klein Tweevlei (small-seeded canary grass) by comparing the untreated control to the various flucarbazone-treatments. None of the treatments at the Baronskop trial site showed any significant ($p < 0.05$) control or suppression of ryegrass. The sight appeared as if it was untreated. At the Klein Tweevlei trial site only the 2R treatment showed a significant ($p < 0.05$) reduction in small-seeded canary grass, although only an average 25% control was recorded (Table 4.4). The spectrum study was conducted over only one growing season and thus does not prove that flucarbazone-sodium will not control all ryegrass and small-seeded canary grass biotypes in the Western Cape. It does prove however that flucarbazone-sodium will not provide acceptable control of ryegrass and small-seeded canary grass in all areas of the Western Cape.

Table 4.4: The percentage control as a result of different flucarbazone-sodium application rate (0.5 R, R and 2R) on ryegrass (*Lolium* spp.) and small-seeded canary grass (*Phalaris* spp.) at two different sites in the Swartland area of the Western Cape. R = recommended rate (70 mℓ ha^{-1})

	Flucarbazone-sodium treatments		
	0.5 R	R	2 R
Weed species	----------% Control----------		
Ryegrass (*Lolium* spp.)	0	0	0
Small-seeded canary grass (*Phalaris* spp.)	0	3	25

4.4 General discussion

At the Glen Peter and the Doringdam sites a very high level of wild oats control was achieved when flucarbazone-sodium was applied at early growth stages [12-13]. Controlling wild oats with flucarbazone-sodium tends to be more effective at the three-leaf growth stage compared with the four- to five-leaf stages (Lockhart and Howatt 2004). The level of control differed at these two sites when flucarbazone-sodium was applied at the [14-15] growth stage, where a high level of control was achieved at the Doringdam site and a low level of control at the Glen Peter site. Other studies also reported a reduction in efficacy of graminicides on wild oats at later growth stages. Holm et al. (2000) found reduced wild oats control at delayed applications of imazamethabenz compared to early applications. Similar findings were made by Spandl et al. (1997) and Stougaard et al. (1997). There could be various reasons for the difference in wild oats control between the Glen Peter site and the Doringdam site at the [14-15] growth stage. For instance, there might be a difference in susceptibility to flucarbasozone-sodium between these two wild oat biotypes. Mengistu et al. (2003) reported variation in susceptibility within different wild oats biotypes to various aryloxyphenoxypropionate (APP) cyclohexanedione (CHD) and acetyl-coenzyme A carboxylase (ACCase) herbicides. Another possibility

is that the climatic conditions favoured the effectiveness of the product more at the Doringdam site at the time of application. The presence and persistence of ALS-inhibiting herbicides can be affected by both temperature or soil moisture content (Beckie and McKercher 1989). Soil properties such as clay content and organic matter can all have an effect of the dissipation of flucarbazone-sodium in soils (Eliason et al. 2004).

The results observed at the Pools site showed that flucarbazone-sodium was ineffective in controlling wild oats to an acceptable level. The results obtained at the Pools site is in stark contrast to the results from the Glen Peter and Doringdam sites. Mengistu et al. (2003) reported variation in herbicide response in wild oat populations in the Red River Valley of Minnesota and North Dakota. The results suggest a metabolic resistance to flucarbazone-sodium. Mengistu et al. (2003) suggested that wild oats resistance to various herbicides is often a result of enhanced metabolism through induction of P450 or GST enzyme systems. ALS-inhibiting resistance in weeds from enhanced metabolism is more often because of cross-resistance due to selection by herbicides with a different site of action (Beckie and Tardif 2011). A further study should be conducted to investigate this notion.

4.5 Conclusion

All three trial sites in this study delivered uniquely different results. The results indicate that the effectiveness of flucarbazone-sodium as a measure to control wild oats can greatly be affected by the biotype present and at what growth stage it is applied. Flucarbazone-sodium can be an effective tool to control wild oats in certain wheat production areas of the Western Cape. However, it is unclear for how long

because of increasing reports of ALS resistance suggest that flucarbazone-sodium might only be a short term solution for wild oats control.

4.6 References

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Chapter 5

Resistance of wild oats (*Avena* spp.) to flucarbazone-sodium, a new ALS-inhibiting herbicide on the South African market

5.1 Introduction

Since the introduction of synthetic herbicides, the landscape of weed control has been revolutionised. However, the evolution of herbicide-resistant weed populations has accelerated, due to the repeated use of herbicides with the same mode of action (www.passel.unl.edu). The greatest selection pressure came from the most efficient herbicides, causing a high selection gradient to resistant biotypes. Herbicide resistance can be defined as the inherited ability of a certain weed biotype to not only survive, but reproduce after exposure to the recommended rate of a certain herbicide, which in normal circumstances will cause the death of the wild biotype (www.passel.unl.edu).

In the past 20 years no new site-of-action herbicides were developed and marketing of glyphosate and glyphosate-resistant crops ultimately led to the discouragement of herbicide discovery efforts worldwide (Duke 2011). According to the *International Survey of Herbicide Resistant Weeds*, there are currently 460 unique cases of herbicide resistant weeds globally (www.weedscience.org). In general, these cases involve target-site mutations producing weeds that are resistant to either a single herbicide or related herbicides with the same mode of action (Lehnhoff 2013).

First commercialised in the early 1980s, herbicides that target the enzyme acetolactate synthase (ALS) are some of the most widely used herbicides in the world (Bellinder et al. 1994). Sulfonylurea (SU), triazolopyrimidine (TP),

imidazolinone (IMI), pyrimidinylthiobenzoate (PTB) and sulfonylaminocarbonyltriazolinones (SCT) are all herbicides that act as inhibitors of the enzyme acetolactate synthase (ALS), also known as acetohydroxyacid synthase (AHAS), an important enzyme that acts as a catalyst in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine (Cobb and Reade 2010). According to Beckie (2006) herbicides with this mode of action can select for resistance in weed biotypes in less than 10 applications. Devine and Eberlein (1997) found that there is a general trend of cross-resistance between sulfonylurea (SU) and triazolopyrimidine (TB) herbicides and between imidazolinone (IMI) and pyrimidinylthiobenzoate (PTB) herbicides. All the classes of ALS inhibitors appear to have the same level of risk regarding resistance build-up. Choosing among ALS inhibitor classes to manage ALS resistance can be considered as a risky short-term proposition (Beckie and Tardif 2011). The main reason why growers keep choosing ALS inhibitors are because of their relatively long residual activity, thus helping to control weed species with multiple flushes per growing season. However, this can exert intense selection pressure on these multiple-flush weed populations.

With the rise of modern molecular technology such as the discovery of the polymerase chain reaction (PCR), it became possible to identify and test for specific mutations that cause herbicide resistance in weed species. There are currently 26 resistance substitutions at 8 sites on the ALS enzyme (www.weedscience.org). Mutations such as Ala-122-Thr, Ala-205-Val, Gly-654 and Ser-653 have been found to cause target-site resistance to IMI herbicides. Arg-377-His and Pro-197 mutations account for resistance to SU herbicides. Pro-197 also causes resistance to TP herbicides but to a lesser extent. Trp-574 and Asp-376-Glu mutations result in resistance to all Group B herbicides (Beckie and Tardif 2011).

Currently, there are more species resistant to ALS-inhibiting herbicides than any other herbicide group (Tranel and Wright 2002). To date there are 156 weed species with confirmed resistance to ALS inhibitors worldwide, of which South Africa has five, namely ryegrass (*Lolium rigidum*), small-seeded canary grass (*Phalaris minor*), wild radish (*Raphanus raphanistrum*), common chickweed (*Stellaria media*) and wild oats (*Avena fatua*) (www.weedscience.org).

Heap (1999) compiled a list of the top ten most resistant weed species in the world on which wild oats ranked second. In South Africa, multiple resistances in wild oats to ACCase inhibitors (Group A) and ALS inhibitors (Group B) was reported back in 1986 (Cairns and Laubscher 1986). However, the specific mechanism for this resistant biotype was not reported and only glasshouse trials comparing a known susceptible wild oats biotype with the suspected resistant wild oats biotype, have been used to confirm resistance. Due to the increasing pressure by South African producers on the chemical industry to provide them with chemically alternative herbicides to control wild oats in cultivated grain fields in the Western Cape, Arysta LifeScience South Africa imported Everest® 2.0 (flucarbazone-sodium). Flucarbazone-sodium has shown excellent activity on wild oats when applied as a post-emergence herbicide to wheat (*Triticum aestivum*) in field experiments conducted in Canada and the USA (Santel et al. 1999).

As mentioned in Chapter 4, a series of field trials were conducted in 2013 where flucarbazone-sodium was applied to three different wild oats biotypes in the Western Cape. The results obtained at one of the sites (Pools, Piketberg, Western Cape, South Africa) varied significantly from the other sites with regards to total wild oats control. Resistance to flucarbazone-sodium at Pools was suspected but not confirmed. In an unrelated trial conducted by Pieterse in 2013 (personal

communication, P.J. Pieterse, 2014, University of Stellenbosch) three different wild oats biotypes were found to be resistant to the recommended rates of flucarbazone-sodium. The specific mechanism for this resistance was not reported but it appeared to be rate-sensitive to flucarbazone-sodium since all three biotypes appeared to be controlled at eight times the recommended rate. The aim of this study was to confirm resistance of the wild oats biotype found at Pools to flucarbazone-sodium, and also to investigate the possible mechanism responsible for this resistance.

5.2 Material and Methods

5.2.1 Glasshouse trials

Glasshouse trials were conducted to study the efficacy of flucarbazone-sodium on a possible resistant biotype of wild oats (*Avena* spp.) from the Pools area in Piketberg, Western Cape, South Africa. The trials were designed to determine if the wild oats biotype is resistant to flucarbazone-sodium and if so, to what extent. Hence, wild oats seeds were collected from the trial site in Pools (2013) and germinated on 16th October 2014.

Due to the fact that wild oats seeds tend to have various levels of dormancy, a germination technique, developed by Cairns (personal communication, A. Cairns, 2014, University of Stellenbosch), was used to increase the germination percentage. Firstly, all wild oats seeds were de-hulled before being placed in a desiccator. Afterwards, 1 g of sodium hydroxide (NaOH) and 1 g of ammonium chloride (NH_4Cl) were mixed together and placed separately with the de-hulled wild oats seeds in the desiccator. Next, 1 mℓ of water was added to the NaOH and NH_4Cl mixture to initiate the fumigation process. Fumigation of the wild oats seeds lasted for 20 minutes after which the seeds were taken out of the desiccator and aerated for 48 hours. After

aeration a needle was used to make small lesions in the seed coat to allow water to access the endosperm of the seed. The seeds were then placed under running water for 24 hours before placing them in a seed germination incubator at a constant temperature set at 20 ˚C with no light.

Shortly after germination, seeds were transplanted into 4 cm x 4 cm square plastic containers filled with loam soil (18% clay). Each container received four wild oats seedlings. Plants were grown under a controlled environment with day and night temperatures ranging between 20 ºC and 12 ºC respectively. The wild oats plants were monitored until they reached the appropriate growth stage. Growth stages were determined by using the BBCH scale (Lancashire et al. 1991). Plants were grown until they reached the [12-13], [14-15] and [16-17] growth stages respectively. At each growth stage there were six treatments, namely 0.5R, R, 2R, 4R and 8R, where R is the recommended application rate (70 mℓ ha^{-1}) of flucarbazone-sodium. An adjuvant (Wet-all®) was added to the treatment mixtures at the recommended application rate (50 mℓ ha^{-1}). Each treatment was replicated six times. Thirty days after herbicide application the above-ground material was harvested and oven dried for four days at 80 ºC. The dry mass was determined and the data analysed statistically. It should be mentioned that no wild oats mortalities were recorded and thus all the pots contained four wild oats plants at the end of the trial period.

5.2.2 Molecular study

Green material from the control plants grown during the glasshouse trial was sent to the ARC Small Grain Institute of South Africa situated in Bethlehem, Free State, for molecular analysis. The aim was to evaluate the biotype obtained at the Pools trial for any target-site mutations that might contribute to its resistance to flucarbazone-sodium.

5.2.3 DNA isolation

Bulked fresh leave material was collected within a 2.0 mℓ Eppendorf safe lock tube and stored at -80 ºC. A volume of 750 µℓ of CTAB (cetyltrimethylammonium bromide) extraction buffer was added to each sample (containing two 5 mm stainless steel ball bearings) to homogenise the leaf material for 30 s at 30 r s^{-1} with the Qiagen Tissue Lyser II (Qiagen Sciences Inc, Germantown MD, USA). A modified CTAB DNA extraction procedure was followed (Saghai-Maroof et al. 1984). DNA was re-suspended in 200 µℓ of 1x TE (*tris*[hydroxymethyl]aminomethane, (EDTA) ethylenediamine-N,N,N',N'-tetraacetic acid) buffer, DNA concentration and purity was determined with a Thermo Scientific Nano Drop 2000 (Thermo Fisher Scientific Inc, Waltham, USA) spectrometer. All DNA samples were diluted with 1 x TE buffer to 50 ngµ$ℓ^{-1}$ end concentration.

5.2.4 PCR assay

PCR reactions were set up in a final volume of 20 µℓ, containing 200 ng µ$ℓ^{-1}$ DNA template, 5 µℓ nuclease-free PCR water, 0.5 µℓ (10 nmol) of each primer and 10 µℓ of KAPA Taq 2X ReadyMix (Kapa Biosystems (Pty) Ltd, Cape Town, South Africa). All derived cleaved amplified polymorphic sequence (dCAPS) primers (olgios) used in this study were synthesised by Integrated DNA Technologies (Integrated DNA Technologies, Leuven, Belgium). The target codon primer name, sequences and annealing temperature per target-site mutation; Ala-122, Ala-205, Trp-574, Ser-653 (Delye et al. 2009) and Pro-197 (Kaundun et al. 2012) respectively were used. For target codon Trp-574, PCR conditions were optimised at a 60 ºC annealing temperature. PCR assays were performed in a 96 well Bio-Rad MyCycler Thermal Cycler (Bio-Rad Laboratories (Pty) Ltd, Hercules, USA) at the following cycle conditions for all target-site mutation primers: 94˚C for 3 min (1 Cycle); 94 ºC for 30

s, 60 ºC for 45 s, 72 ºC for 30 s (35 cycles) and a final elongation at 72 ºC for 5 minutes.

5.2.5 PCR-restriction digests

The following Thermo Scientific fast digest restriction enzymes (Thermo Fisher Scientific Inc), *Bgl* I, *Bst* XI, *Bpu*10 I (Delye et al. 2009) and *Hpa* I (Kaundun et al. 2012) were used for each respective target-site. Restriction digests were done in a final volume of 30 µℓ, consisting of 17 µℓ nuclease-free PCR water, 2 µℓ of 10X FastDigest® green buffer, 10µℓ PCR DNA template and 1µℓ of FastDigest® enzyme. All digests were performed in a heat block set at 37 ºC for 30 minutes.

5.2.6 Visualisation

After restriction digestion PCR-dCAPS products (10 µℓ of each sample) were loaded directly on a 2% standard agarose (SeaKem® LE Agarose, Lonza, Rockland, USA) gel, made up with 1x TBE (Tris, Boric acid, EDTA) running buffer containing, (5 µℓ per 50 mℓ) GrGreen (Inqaba Biotec, Pretoria, South Africa) nucleic acid stain. Samples were flanked by 5 µℓ of 100bp DNA ladder (Lonza) size marker. All gels were run at 100 v for 2-3 hours and visualised under UV light with the DNR MiniBis pro gel documentation system (DNR Bio-Imaging Systems Ltd, Jerusalem, Israel) and digital photographs taken. All CAPS fragments were sized, wild type (susceptible) and mutant (resistant) allele sizes were compared to published sizes (Delye et al. 2009; Kaundun et al. 2012) and scored as absent or present.

5.2.7 Statistical analysis

All values were then evaluated statistically and compared to the control. Analysis of variance (ANOVA) was used for evaluating data (STATISTICA 11.0, Statsoft Inc.,

Tulsa, Oklahoma, USA) and the statistical significance of the results was analysed by the t-test ($p \leq 0.05$).

5.3 Results and discussion

5.3.1 Glasshouse trial

Surprisingly, none of the wild oats plants in the glasshouse experiment became senescent after any of the flucarbazone-sodium applications, not even eight times the recommended rate resulted in plant death. Thus, the biotype sampled at Pools, near Piketberg appears to be 100% resistant to flucarbazone-sodium. However, upon visual inspection of the plants in this trial it was clear that flucarbazone-sodium did affect the growth of this wild oats biotype (Figure 5.1).

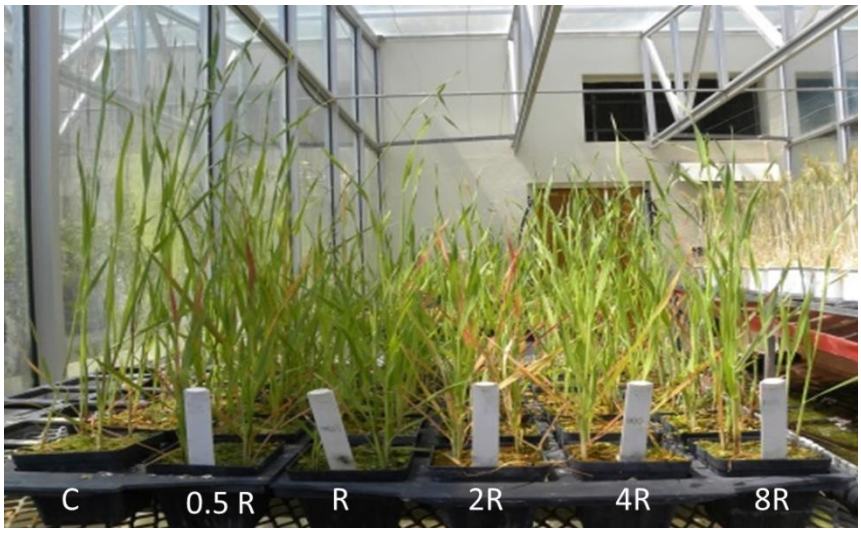

Figure 5.1: The wild oats biotype from Pools, Piketberg in the Western Cape of South Africa 30 days after being sprayed with different rates (0.5R, R, 2R, 4R, 8R) (R = Recommended dosage 70 mℓ ha-1) of flucarbazone sodium

From Table 5.1 there appears to be a clear interaction between the different treatments and the wild oats growth stages. This can be explained by the fact that the wild oats plants that were treated with flucarbazone-sodium at later growth stages had more time to grow and develop normally. This resulted in an increase in average DM at later growth stages.

Table 5.1: Interaction between main factors flucarbazone-sodium treatments and wild oats growth stage

ANOVA	*F*-value	Pr > *F*	Significance
Treatment*growth stage	16,262	<0.0000	**

* p < 0.05; ** p < 0.01; ns, not significant at p = 0.05

At all the different growth stage applications ([12-13], [14-15] and [16-17]), all rates of flucarbazone-sodium (0.5R, R, 2R, 4R and 8R) caused a significant ($p < 0.05$) reduction in DM when compared with the control (Figure 5.2). There appears to be a progressive reduction in DM as the concentration of flucarbazone-sodium increases, hence DM reduction and the increase in concentration of flucarbazone-sodium appears to be positively correlated. Even half the recommended dosage of flucarbazone-sodium at all the growth stage applications caused a significant decrease in DM (Figure 5.2).

For flucarbazone-sodium applications that were applied at the [12-13] growth stage the DM showed a significant decrease at each increasing application rate treatment, hence the 0.5R application was significantly ($p < 0.05$) higher from DM of the R application, and the DM of 2R were significantly ($p < 0.05$) lower than that of R. The 2R application did not differ significantly from the 4R application but it is significantly ($p < 0.05$) higher than the DM of the 8R application, indicating that there is a gradual decrease in DM from 2R to 4R although not statistically significant. There is no significant difference between the DM of the 4R and the 8R application at the [12-13] growth stage (Figure 5.2).

For flucarbazone-sodium applications that were applied at the [14-15] growth stage the DM showed a significant decrease at each increasing application rate treatment, hence the R application was significantly ($p < 0.05$) lower than the 0.5R application, the DM of the 2R application was significantly ($p < 0.05$) lower than the

DM of R application and the DM of 4R application was significantly ($p < 0.05$) lower than the DM of the 2R application. The DM of the 4R and the 8R applications also differed significantly ($p < 0.05$) at the [14-15] growth stage (Figure 5.2).

For flucarbazone-sodium applications that were applied at the [16-17] growth stage the DM of the R application did not differ significantly from the DM of the 0.5R application. The DM of the 2R was however significantly ($p < 0.05$) lower than that of the R application and the DM of the 4R application was significantly ($p < 0.05$) lower than the DM of the 2R application. The DM of the 4R and the 8R applications did not differ significantly from each other at the [16-17] growth stage (Figure 5.2).

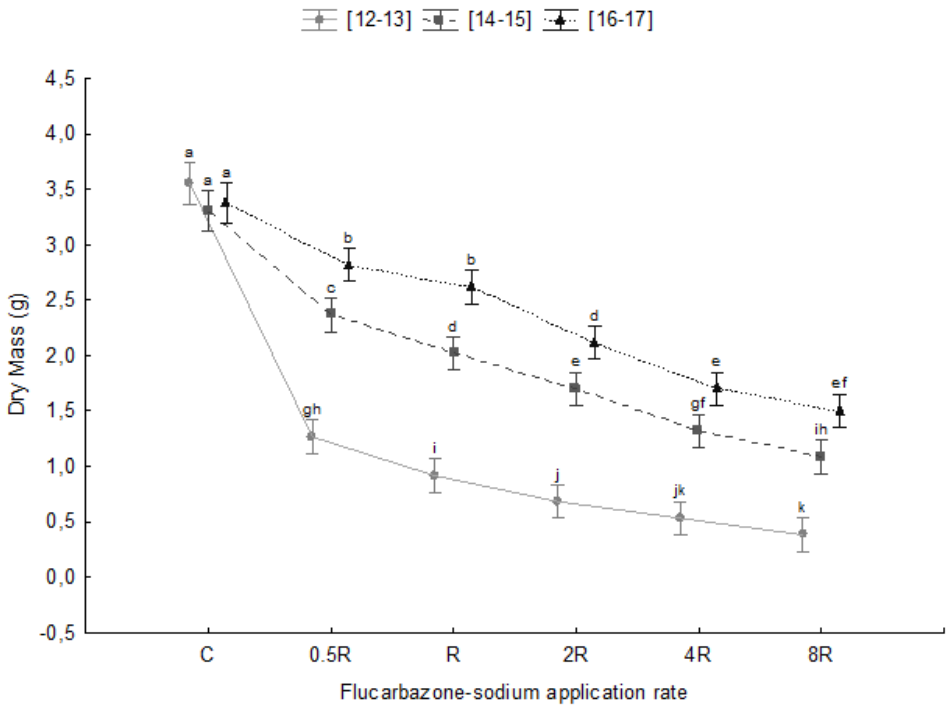

Figure 5.2: The average dry mass (DM) of wild oats biotype from Pools near Piketberg in the Western Cape after being treated with increasing rates of flucarbazone-sodium. R is the recommended rate of flucarbazone-sodium of 70 mℓ ha^{-1}. Vertical bars denote 95% confidence intervals

DM reduction appears to slow down at the 4R application as there is no significant reduction in DM between the 4R and 8R applications at most of the growth stages. A linear model, to test the relationship between DM reduction and

increasing flucarbazone-sodium application rates, did not fit the dataset well. Growth stages [12-13], [14-15] and [16-17] had R^2 values of 59%, 79% and 85% respectively. One can therefore conclude that the reduction in wild oats DM was not directly correlated with an increase in application rate of flucarbazone-sodium. An exponential model was fitted to the data set and appeared to be a more appropriate fit. Growth stages [12-13], [14-15] and [16-17] had R^2 values of 98%, 95% and 96% respectively (Figure 5.3). The exponential model of each growth stage was then statistically compared with each other (Table 5.2).

Table 5.2: Exponential model of each growth stage statistically compared to each other

Growth stages	Estimate	Std. Error	t-value	p-value	Significance
[12-13] vs [14-15]	1,468	0,377	1,24	0,22	ns
[12-13] vs [16-17]	1,473	0,359	1,32	0,19	ns
[14-15] vs [16-17]	1,003	0,207	0,01	0,99	ns

* $p < 0.05$; ** $p < 0.01$; ns, not significant at $p = 0.05$

There were no significant ($p > 0.05$) differences between any of these models. It must be noted that the control data was excluded in the calculation of these models. This was done because the DM of all the different application growth stages ([12-13], [14-15] and [16-17]) differs significantly from each other (Figure 5.2). As explained, this is because the wild oats plants that received flucarbazone-sodium applications at later growth stages were allowed to grow and develop normally for a longer period of time. The longer the wild oats plants were allowed to grow before the flucarbazone-sodium application, the more time it had to accumulate DM.

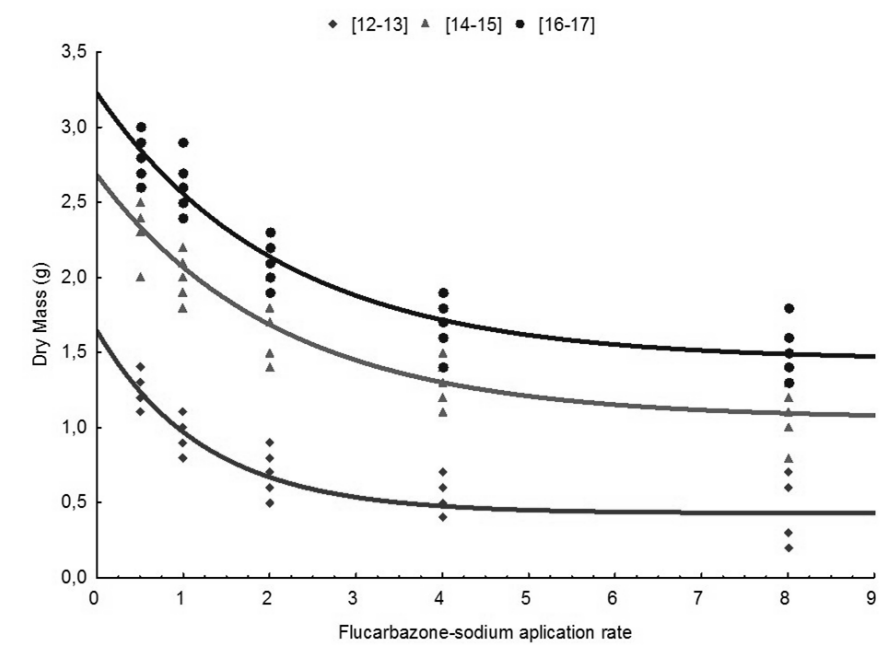

Figure 5.3: The exponential models for the three different growth stages of wild oats. The graph shows the exponential correlation between dry mass reduction as a result increasing flucarbazone-sodium application rates (0.5R, R, 2R, 4R, 8R) (R = Recommended dosage 70 mℓ ha^{-1}). X-axis is expressed as increasing multiples of R. Growth stages [12-13], [14-15] and [16-17] had R^2 values of 98%, 95% and 96% respectively

By fitting an exponential model on the data set and comparing the DM reduction of the different growth stages, one could see whether the wild oats biotype in question reacts similarly to increasing applied rates of flucarbazone-sodium, regardless of the application growth stage. It can be argued that the same mechanism is responsible for causing resistance at all the growth stages, and somewhere between 4R and 8R a threshold is reached. This might be an indication but is not conclusive of a constant metabolic breakdown potential of flucarbazone-sodium by the wild oats biotype from Pools.

5.3.2 Molecular resistance screening

As indicated in Figure 5.4, there is a clear homozygous resistance for markers Ala-205-Val and Trp-574, which means that these mutations were present on both

alleles. Homozygous resistance for the Ala-205-Val mutation indicates that the wild oats biotype sampled in the Pools area contains a target-site resistance that causes it to be resistant to imidazolinone herbicides (Beckie and Tardif 2011). Homozygous resistance for the Trp-574 mutation however means that this biotype also contains a target-site resistance that causes it to be resistant to all Group B (ALS inhibitors) herbicides (Beckie and Tardif 2011).

Heterozygous resistance was found for Pro-197 and Ser-653 mutations, meaning that the chromosome set of this particular wild oats biotype from Pools only contained these mutations on one of the alleles. Pro-197 mutations account for resistance to sulfonylurea herbicides but Pro-197 also causes resistance to triazolopyrimidine herbicides although to a lesser extent (Beckie and Tardif 2011). Ser-653 mutations account for resistance to imidazolinone herbicides. Resistant ALS alleles are dominant over susceptible ALS alleles but the degree of dominance varies among plant species or alleles (Foes et al. 1999; Hart et al. 1993; Sebastian et al. 1989). Selection for resistant ALS genes takes place even if it is only present in heterozygous form. Heterozygous resistance can therefore develop into homozygous resistance if there is a continuous selection of these mutant genes. The dominant nature of the ALS-resistant genes might partially account for the high incidence of resistance to ALS inhibitors (Tranel and Wright 2002).

The presence of Ala-205-Val, Trp-574, Pro-197 and Ser-653 mutations does not necessarily mean ALS functionality is negatively affected. ALS herbicides do not resemble the normal ALS substrate and bind at a separate binding domain from the catalytic site. Therefore, some resistance mutations may have a negligibly low effect on the functionality of ALS (Powles and Yu 2010). This particular point becomes evident when one considers the results of other studies that show varying effects of

ALS activity with regards to a particular resistant biotype. Some studies find a reduction in ALS activity (Ashigh and Tardif 2007; Eberlein et al. 1997; Eberlein et al. 1999), while others found an increase in ALS activity (Boutsalis et al. 1999; Yu et al. 2003; Yu et al. 2007) and there are a few studies that found no change in ALS activity (Boutsalis et al. 1999; Preston et al. 2006).

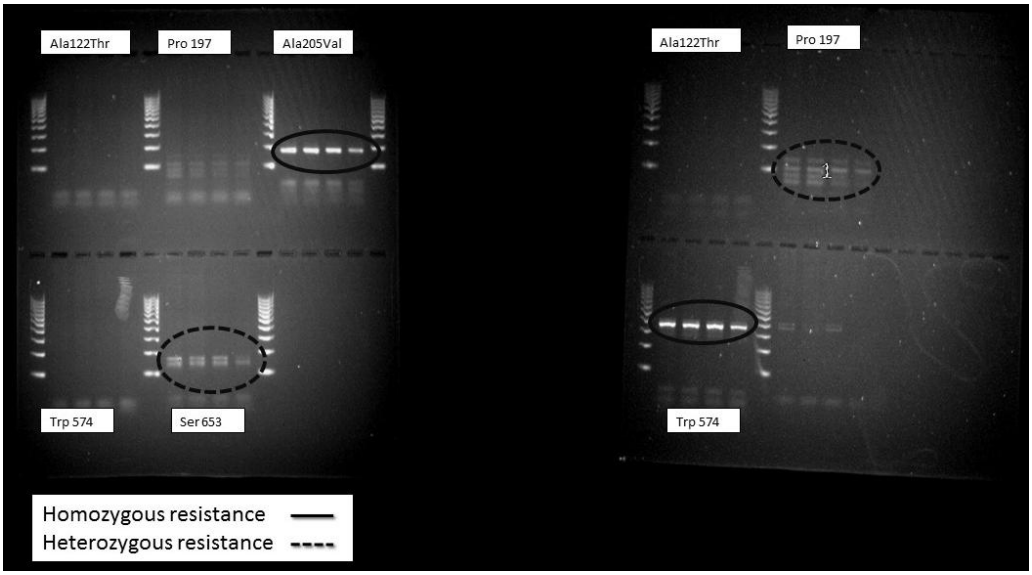

Figure 5.4: The presence of homozygous resistance mutations Ala-205-Val and Trp-574, and heterozygous resistance for mutations Pro-197 and Ser-653 in a wild oats biotype from Pools near Piketberg in the Western Cape of South Africa

5.4 Conclusion

The wild oats biotype that was sampled at Pools, Piketberg in the Western Cape for this study is clearly resistant to flucarbazone-sodium. Even eight times the recommended dosage of flucarbazone-sodium did not cause a single plant death in the glasshouse trial conducted in this study. However, this particular wild oats biotype appears to be sensitive to increasing rates of flucarbazone-sodium since the dry mass (DM) was negatively affected as the application rates for this active ingredient was increased. This might be an indication of a metabolic breakdown of flucarbazone-sodium within the wild oats plant but further studies need to be

conducted before metabolic resistance can be proved. The PCR results clearly show homozygous target-site resistance for Ala-205-Val and Trp-574 mutations. The most relevant mutation with regards to this study might be the Trp-574 mutation that is known to cause resistance to all Group B (ALS inhibitors) herbicides. No studies exist worldwide that link this mutation specifically to resistance against flucarbazone-sodium or even sulfonylaminocarbonyltriazolinone herbicides in wild oats.

If it is argued that a target-site mutation (Trp-574) is responsible for this documented case of resistance, there should not be any reduction in growth (DM). The population therefore should not differ from the control in any aspect of growth assessment. Results from the glasshouse trial clearly show that flucarbazone-sodium has a significant effect on the DM of the sampled wild oats biotype. Therefore, one cannot conclude with certainty that the Trp-574 is responsible for this documented case of resistance, or one can argue that the concept of ALS target-site resistance and its phenotypical effect needs further investigation and clarification by the weed science community.

The mechanism of resistance for the wild oats biotype present at Pools is unclear. However, this wild oats biotype does contain multiple target-site resistance mutations which indicates to the researcher that adequate control of this biotype does not lie within the parameters of the Group B (ALS inhibitors) herbicides and certainly not by using flucarbazone-sodium.

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Chapter 6

Conclusion

6.1 Summary

Wild oats (*Avena* spp.) will remain a prominent pest in cultivated wheat fields in South Africa for the foreseeable future because ryegrass (*Lolium* spp.) control currently receives the most attention from the weed science community in South Africa (Eksteen 2007; Ferreira 2011). By introducing new types of herbicides such as flucarbazone-sodium into the local market and then conducting scientific studies on its effect on the wild oats biotypes, as well its effect on locally grown wheat cultivars, is a small but effective step towards solving the problem. However, Beckie and Tardif (2011) stated that choosing among classes of ALS inhibitors to manage ALS resistance can be considered as a risky short-term proposition. It appears, however, that the days of using ALS-inhibiting herbicides for effective weed control in South Africa might be numbered. The resistance of wild oats to herbicides in South Africa appears to be confined to certain localities, most likely because this species is not known for outcrossing (Murray et al. 2009). Thus, resistance genes spread slowly from one area to the next without any intervention.

This study found that it is important to test newly available herbicides on local cultivars under local climatic conditions. For instance, all except one cultivar (SST 056) showed no real phytotoxic effect from the recommended application rate of flucarbazone-sodium (Everest® 2.0). This indicates that not all wheat cultivars react in the same way to different herbicides. It was also noted that wheat in general appeared to be affected by double the recommended application rate of flucarbazone-sodium. This fact should not deter producers from using flucarbazone-sodium to control wild oats. The alternative of not controlling wild oats will certainly

have a devastating effect on wheat production (Appendix A, Figure 1). Further studies should aim to develop financially viable methods of testing and quantifying phytotoxicity of herbicides on locally grown wheat cultivars.

Flucarbazone-sodium clearly proved to be effective in controlling local wild oats biotypes at the Glen Peter and Doringdam sites. At these sites wild oats were effectively controlled at earlier growth stages. Even half the recommended rate for flucarbazone sodium at early growth stages [12-13] proved to be more effective in controlling wild oats than double the recommended dosage at later growth stages [16-17] of production (Appendix A, Figure 4). This highlights the fact that the parameters of all newly-introduced herbicides must be tested before releasing it to producers. The Everest® 2.0 label states that the product controls resistant wild oats, controls small-seeded canary grass (*Phalaris* spp.) and suppresses ryegrass (*Lolium* spp.).

None of these claims were confirmed during this study. The resistant wilds oats at the Pools site was not controlled in field or in greenhouse trials. Even though this spectrum study was only conducted over the course of one growing season, the ryegrass and small-seeded canary grass populations used in this study were neither suppressed nor controlled by flucarbazone-sodium respectively (Appendix A, Figures & 7).

With the rise in documented herbicide resistance in South Africa, it is not surprising that wild oats resistance to flucarbazone-sodium was proved in this study. The exact mechanism that caused this documented case of resistance is not certain. However, this wild oats biotype does contain multiple target-site resistance mutations (Ala-205-Val, Trp-574, Pro-197 and Ser-653) which indicate that adequate control of

this biotype does not lie within the parameters of the Group B (ALS inhibitors) herbicides and certainly not by using flucarbazone-sodium. Further studies should be conducted to test for metabolic resistance within this wild oats biotype. Further studies should also investigate the extent of target-site resistance genes present in the wild oats populations in South Africa, as well as the phenotypical effect these mutations have on wild oats when being subjected to herbicide applications. Worldwide there does not seem to be any clear understanding on how relevant ALS resistance genes are and how these genes ultimately contribute to different levels of herbicide resistance (Powles and Yu 2010).

6.1 References

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Appendix A

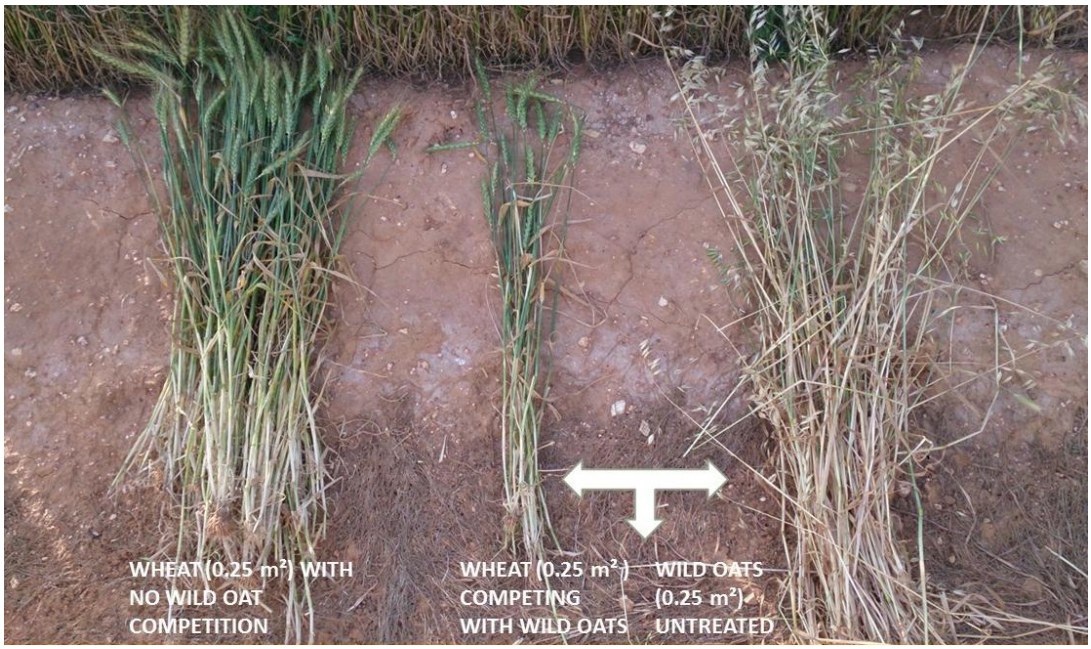

Figure 1: The effect of wild oats (*Avena fatua*) on wheat. Visual comparison between a controlled and uncontrolled 0.25m² sub-plot.

Figure 2: Trial site where the phytotoxic effect of flucarbazone-sodium was tested on five wheat cultivars.

Figure 3: Harvesting of the phytotoxicity trial.

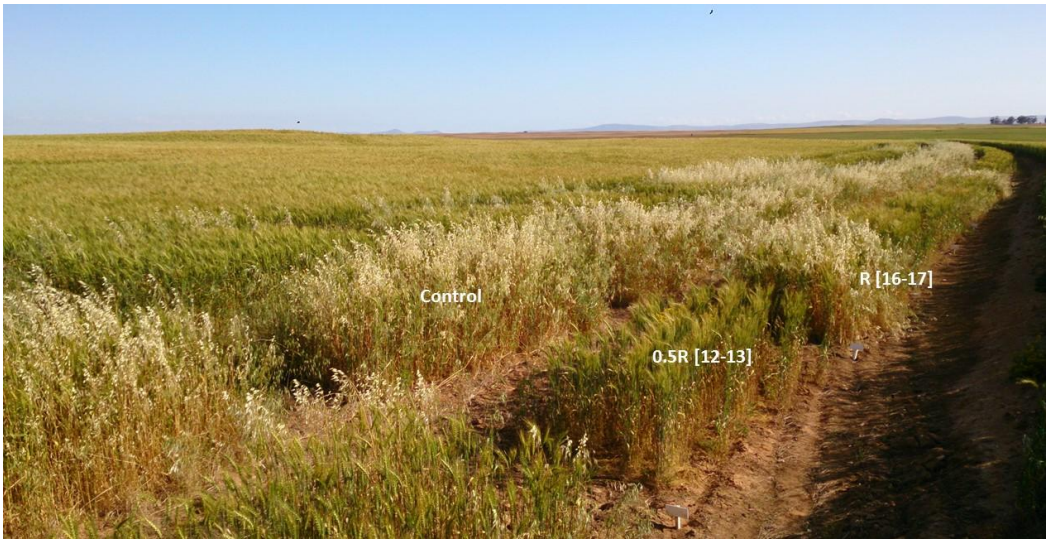

Figure 4: Trial site at Glen Peter

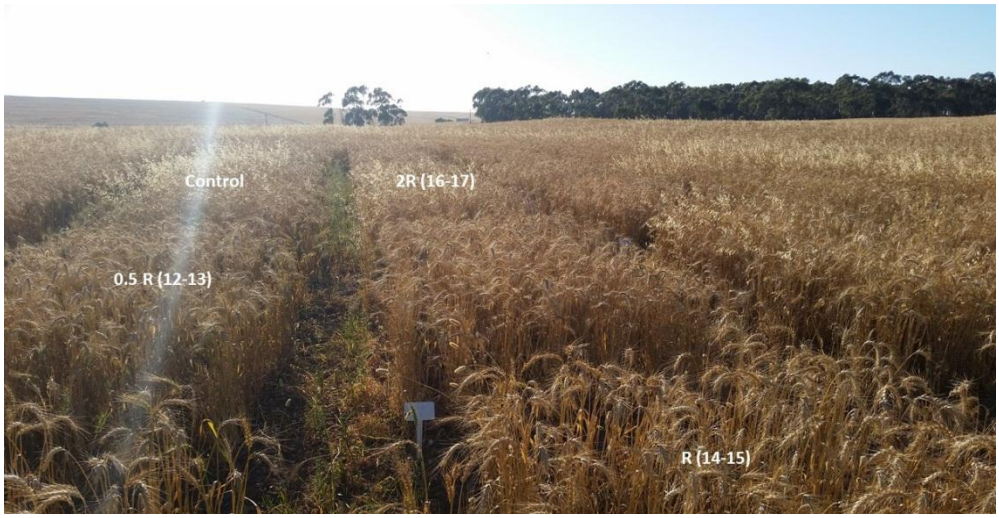

Figure 5: Trial site at Doringdam

Figure 6: Poor control of small-seeded canary grass (*Phalaris* spp.) with flucarbazone-sodium during spectrum study.

Figure 7: No suppression of ryegrass (*Lolium* spp.) by flucarbazone-sodium during spectrum study.