HERBICIDE OPTIONS FOR WEED CONTROL IN HERBICIDE RESISTANT CANOLA CULTIVARS WITH PARTICULAR REFERENCE TO GLUFOSINATE AMMONIUM

by

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner 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

Glufosinate ammonium is one of the most widely-applied broad-spectrum herbicides, controlling weeds in a huge variety of crops worldwide. Farmers rely on glufosinate ammonium because it ensures a high degree of crop safety, as it only affects the parts of the plant where it is applied. It is effective against a broad range of weeds, eliminating the need to apply several herbicides to control different weeds in a given crop. Its unique mode of action makes it ideal to be used in rotation with other herbicides to mitigate weed resistance. Despite these favourable attributes for weed control glufosinate ammonium has its shortcomings.

Glufosinate ammonium is a post emergence herbicide and its efficacy is not exempt from the effect of environmental/climatic conditions and the growth stage of weeds. The possible effect of these factors on the performance of the herbicide was investigated in a glasshouse study using ryegrass (*Lolium spp*) as the test species. The investigation consisted of four experiments. The first two experiments investigated the effect of applying different doses of glufosinate ammonium to commercial ryegrass and weedy ryegrass seedlings at different growth stages in a glasshouse. The glufosinate ammonium dosage rates were 0, 2.5, 5, 7.5 and 10 L ha^{-1}. The findings of the studies showed that neither plant age nor herbicide rate affected the efficacy of glufosinate ammonium in a commercial cultivar or weedy type ryegrass. There were no statistically significant differences between the percentage mortality caused by any of the glufosinate ammonium dosage rates between 2.5 and 10 L ha^{-1} with the percentage control being higher than 90% at all dosage rates in both experiments.

The objective of the third and fourth experiment was to determine the effect of temperature on the efficacy of ryegrass control by glufosinate ammonium. The temperatures for the third experiment were 10/15 ^{0}C (cool) and 15/20 ^{0}C (warm) night/day and for the fourth experiment 10/15 ^{0}C (cool) and 20/25 ^{0}C (warm) night/day. The glufosinate ammonium dosage rates applied were 0, 2.5, 5, 7.5 and 10 L ha^{-1}. Plants in the third experiment remained in the glasshouse throughout the study at a constant temperature regime. Under cool temperatures glufosinate ammonium controlled ryegrass plants, irrespective of the rate applied, with 100% control achieved at a dosage rate of 2.5 L ha^{-1}. This was not the case under warm temperatures where 100% control was not even achieved at the 10 L ha^{-1} dosage

rate. In the fourth experiment cool and warm temperature regimes were applied before and after spraying. The four temperature treatments applied were therefore cool (10/15 ºC), warm (20/25 ºC), cool/warm (where the plants were moved from the cool temperature to the warm one after spraying) and warm/cool (which was the opposite of cool/warm). The results observed from the fourth experiment followed the same trend as in experiment three. Ryegrass plants that were grown under warm temperature and moved to cool temperature after spraying were better controlled than under cool/warm temperatures. Dosage rates of 2.5 L ha^{-1} gave 95% control of ryegrass under cool temperatures whereas the same dosage rate only achieved about 55% control under warm temperatures.

Glasshouse and field trials were conducted at Stellenbosch University experimental farm Welgevallen (33°56'S, 18°42'E) to investigate the effect of the additive ammonium sulphate (AMS) on the efficacy of glufosinate ammonium. The glasshouse trial consisted of four ryegrass populations (one commercial cultivar (*Lolium multiflorum* cv Agri Hilton) and three suspected resistant weedy types (*Lolium* spp.) The temperature of the glasshouse was 20/25 ^{0}C night/day. The four ryegrass populations were each subjected to an experiment using a 7 x 2 factorial design with seven dosage rates (0, 0.75, 1.5, 3, 4.5, 6 and 7.5 L ha^{-1} of glufosinate ammonium) and two AMS treatments (with and without AMS) laid out in a randomized complete block design with three replicates. The AMS increased the efficacy of glufosinate ammonium on the commercial cultivar and resistant population 2 at certain critical dosage rates. The field trials were conducted in 2013 and 2014. The trials were arranged factorially in a randomised complete block design replicated four times. The treatment factors were two treatments (glufosinate ammonium alone and glufosinate ammonium plus AMS) and five glufosinate ammonium rates (0, 2.5, 5, 7.5, and 10 L ha^{-1} of glufosinate ammonium). Ammonium sulphate (10 g) was diluted in 1 L of distilled water before mixing with glufosinate ammonium. The findings of the study revealed that AMS increased the efficacy of glufosinate ammonium under field conditions in 2013 but not in 2014.

Experiments with the aim of determining the effect of propyzamide on the efficacy of four herbicides (atrazine, glufosinate ammonium, glyphosate and imazamox) was carried out in a glasshouse as well as in field studies. In the glasshouse study, ryegrass was used as a test species. The four herbicides and propyzamide were applied separately, followed by mixtures with propyzamide at the rates of 0, 0.5, 0.75

and 1x (times the recommended rate) for each of the herbicides in the mixture. Results suggest that propyzamide negatively affected atrazine efficacy on ryegrass in the glasshouse but not the efficacy of any other herbicides. Field experiments were conducted to determine the effect of adding propyzamide to the four herbicides on the efficacy and residual action of the herbicides in 2012, 2013 and 2014. Field trials were conducted at Welgevallen, Roodebloem and Langgewens experimental farms. The experimental design was a randomised complete block with nine treatments replicated four times. Propyzamide increased the efficacy of atrazine in some of the field trials as well as the efficacy of imazamox in some trials but generally the results were variable and propyzamide also did not enhance the residual action of the herbicides in most of the trials.

OPSOMMING

Glufosinaat ammonium is een van die mees algemene breëspektrum nie-selektiewe onkruiddoders wat onkruide in 'n groot verskeidenheid gewasse wêreldwyd beheer. Boere maak staat op glufosinaat ammonium omdat dit redelik veilig vir die gewas is as gevolg van die feit dat dit slegs die gedeelte van die plant waarmee dit in aanraking kom, affekteer. Dit is effektief teen 'n wye verskeidenheid van onkruide wat dit onnodig maak om verskeie onkruiddoders te gebruik om verskillende onkruide in 'n gewas te beheer. Die unieke meganisme van werking maak dit ideaal om in afwisseling met ander onkruiddoders te gebruik om onkruiddoderweerstand te bestuur. Ten spyte van al hierdie voordele het glufosinaat ammonium ook verskeie tekortkominge.

Glufosinaat ammonium is 'n na-opkoms onkruiddoder en sy effektiwiteit word beïnvloed deur omgewings- of klimaatstoestande en die groeistadia van onkruide. Die moontlike invloed van bogenoemde faktore op die effektiwiteit van glufosinaat ammonium is in 'n glashuisstudie ondersoek waar raaigras (*Lolium* spp) as toetsspesie gebruik is. Die ondersoek het uit vier eksperimente bestaan. Die eerste twee eksperimente het die effek van verskillende toedieningsdosisse van glufosinaat ammonium op raaigrassaailinge van 'n kommersiële kultivar asook 'n onkruidbiotipe op verskillende groeistadia ondersoek. Die glufosinaat ammonium dosisse was 0, 2.5, 5, 7.5 en 10 L ha^{-1}. Die resultate het getoon dat nie die toedieningsdosis of die plantgroeistadium die effektiwiteit van glufosinaat ammonium op die kommersiële raaigras kultivar of die onkruid biotipe beïnvloed het nie. Daar was nie enige statisties betekenisvolle verskille tussen die persentasie mortaliteit veroorsaak deur enige van die glufosinaat ammonium dosisse tussen 2.5 en 10 L ha^{-1} nie en die persentasie beheer was hoër as 90% by alle toedieningsdosisse in beide populasies.

Die derde en vierde eksperimente is gedoen om vas te stel of temperatuur 'n rol speel in die effektiwiteit van glufosinaat ammonium op raaigras beheer. Die temperature vir die derde glashuisproef was gestel op 10/15 ºC (koel) en 15/20 ºC (warm) nag/dag temperature en vir die vierde glashuisproef was dit 10/15 ºC (koel) en 20/25 ºC (warm). Die glufosinaat ammonium toedieningsdosisse was 0, 2.5, 5, 7.5 and 10 L ha^{-1}. Die plante in die derde eksperiment het in die onderskeie glashuise by dieselfde temperatuur gebly deur die loop van die hele eksperiment. Onder koel toestande het glufosinaat ammonium die raaigrassaailinge 100% beheer

selfs by die laagste toedieningsdosis van 2.5 L ha^{-1} en by alle dosisse bo dit. By warmer temperature egter, kon selfs die hoogste dosis van 10 L ha^{-1} nie 100% beheer behaal nie. Die vierde eksperiment was soortgelyk aan die derde eksperiment behalwe dat die koel en warmer temperature afgewissel is voor en na die plante bespuit is. Die vier temperatuurbehandelings was dus koel (10/15 ºC), warm (20/25 ºC), koel/warm (waar die plante na spuit van die koel na die warm glashuis verskuif was) en warm/koel (die teenoorgestelde van die koel/warm behandeling). Die resultate wat waargeneem is het dieselfde tendens getoon as die resultate van die derde eksperiment. Toedieningsdosisse van 2.5 L ha^{-1} het 95% beheer van raaigras wat onder koel toestande gegroei het getoon terwyl dieselfde dosis onder die warm toestande slegs 55% beheer behaal het.

Glashuis- en veldproewe is uitgevoer op die Welgevallen proefplaas van die Universiteit van Stellenbosch (33°56'S, 18°42'O) om die invloed van die byvoeging van ammoniumsulfaat (AMS) op die effektiwiteit van glufosinaat ammonium te ondersoek. In die glashuisproef was vier populasies raaigras (een kommersiële kultivar *Lolium multiflorum* cv Agri Hilton) en drie vermoedelik weerstandbiedende onkruidpopulasies (*Lolium* spp) gebruik. Die temperatuur van die glashuis was op 20/25 ^{0}C nag/dag ingestel. Die vier raaigras populasies was elk blootgestel aan 'n faktoriaal gereëlde 7 x 2 eksperiment met sewe toedieningsdosisse (0, 0.75, 1.5, 3, 4.5, 6 en 7.5 L ha^{-1} glufosinaat ammonium) en twee AMS behandelings (met en sonder AMS) wat in 'n volledige ewekansige blokontwerp met drie herhalings uitgelê is. Die AMS het die effektiwiteit van glufosinaat ammonium slegs op die kommersiële kultivar en een van die vermoedelik weerstandbiedende populasies verhoog by 'n sekere kritiese toedieningsdosis. Die veldproewe is in 2013 en 2014 uitgevoer. Die proewe is faktoriaal uitgelê in 'n volledig ewekansige blokontwerp wat vier keer herhaal is. Die behandelingsfaktore was twee behandelings (glufosinaat ammonium met en sonder AMS) en vyf glufosinaat ammonium toedieningsdosisse (0, 2.5, 5, 7.5, en 10 L ha^{-1} glufosinaat ammonium). Die AMS (10 g) is in 1 L gedistilleerde water opgelos voordat dit met die glufosinaat ammonium oplossing vermeng is. Die resultate het getoon dat die AMS die effektiwiteit van glufosinaat ammonium onder veldtoestande slegs in 2013 betekenisvol verbeter het maar nie in 2014 nie.

Proewe met die doel om die effek van propisamied op die effektiwiteit van vier onkruiddoders (atrasien, glufosinaat ammonium, glifosaat en imasamoks) te bepaal is uitgevoer in die glashuis sowel as in die veld. In die glashuisstudie is die onkruiddoders op raaigras saailinge toegedien. Die vier onkruiddoders en propisamied is alleen toegedien en dan is elk van die vier onkruiddoders ook gemeng met propisamied teen die toedieningsdosisse van 0, 0.5, 0.75 en 1.0 keer die aanbevole dosis (x) van elk van die bestanddele van die mengsel. Die resultate dui aan dat propisamied die werking van atrasien op raaigras negatief beïnvloed het maar nie die werking van enige van die ander onkruiddoders nie. Veldeksperimente is uitgevoer om die effek van propisamied op die effektiwiteit en residuele aksie van die onkruiddoders onder veldtoestande in 2012, 2013 en 2014 te bepaal. Die veldproewe is uitgevoer op die Welgevallen, Langgewens en Roodebloem proefplase. Die proefontwerp was 'n volledig ewekansige blokontwerp met nege behandelings wat vier keer herhaal is. Propisamied het die effektiwiteit van atrasien in sekere lokaliteite verbeter asook die van imasamoks in sekere proewe maar die resultate was oor die algemeen wisselvallig. Byvoeging van propisamied by die onkruiddoders het in die meeste gevalle nie die residuele werking daarvan verbeter nie.

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LIST OF ABBREVIATIONS

ai	Active ingredient
ANOVA	Analysis Of Variance
AMS	Ammonium sulphate
Cu	Copper
DAE	Days After Emergence
DAP	Days After Planting
DM_{tr}	Dry mass of treated plants
DM_c	Dry mass of control
GS	Glutamine Synthetase
HR	Herbicide Resistance
HTC	Herbicide Tolerant Canola
Fe	Iron
LL	Liberty Link
Mg	Magnesium
Na	Sodium
Ni	Nickel
ha^{-1}	per hectare
pot^{-1}	per pot
WAT	Weeks After Treatment

CHAPTER 1

INTRODUCTION

1.1 Weeds cause a substantial crop loss in any crop, and canola is no exception. In this regard some weeds seeds such as wild mustard (*Sinapis arvensis*) contaminating canola seed can lead to reduced seed quality by increasing the level of erucic acid in the extracted oil (Rose and Bell 1982). In addition Roshdy et al. (2008) reported a 35% yield loss in canola grown in Egypt as a result of weed competition. Thus, weed control in canola is very essential to obtain good yields. Chemical weed control in canola used to be relatively effective until the appearance of herbicide resistant weeds. The problems caused by these weeds and weed species closely related to canola necessitated the development of herbicide resistant canola cultivars. According to Heap (2014) the first case of herbicide resistance in South Africa was reported in 1986 by Cairns and Hugo of a wild oat (*Avena fatua)* biotype which was resistant to diclofop-methyl.

Several researchers also have confirmed the widespread resistance of weeds to herbicides such as glyphosate. Resistance of ryegrass (*Lolium rigidum* and *Lolium spp*) to ACCase and ALS inhibitors was confirmed by (Smit and De Villiers 1998; Smit et al. 1999). The resistance of ryegrass (*Lolium rigidum* and *Lolium spp*) to the non-selective herbicide glyphosate, was reported in a vineyard in the Western Cape (Eksteen et al. 2007). The resistance was later confirmed by Yu et al. (2004). In 2002, Pieterse and Kellerman also confirmed widespread resistance of ryegrass to ACCase and ALS inhibitors in annual crops such as wheat and pastures in the Western Cape. Herbicide resistant grass weeds, like *Lolium spp* in particular, adversely affects thousands of hectares in the wheat production regions of the winter rainfall area of South Africa (Pieterse and Kellerman 2002). Pieterse (2010) reported resistance to herbicides of seven modes of action groups in 15 weed species in South Africa. Without a doubt herbicide resistance is not only prevalent in the Western Cape, but it is a worldwide phenomenon. For example, resistance of ryegrass to glyphosate was reported in Australia (Pratley et al. 1996). In this instance, the resistance evolved after the continuous use of glyphosate for 15 years.

One way of managing herbicide resistance and the problems caused by weed species closely related to canola, such as wild radish (*Raphanus raphanistrum*) is the use of herbicide resistant crops. Two of these, namely the triazine resistant and imazamox resistant canola cultivars, are available in South Africa. Unfortunately, resistance to both these herbicides has developed in Australia (Norton 2003; Han et al 2012) and at least to imazamox in S.A (Pieterse 2010). This leaves glyphosate and glufosinate ammonium as the only alternatives available in controlling weeds post emergence in canola, provided that cultivars resistant to these herbicides are available, which is not currently the case in South Africa. However, plans are underway to introduce such cultivars in South Africa. Utilization of cultivars resistant to non-selective herbicide such as glyphosate and glufosinate ammonium will go a long way in managing resistant weeds in canola. It is therefore imperative to investigate the best weed control practices with regard to glyphosate and glufosinate ammonium application after emergence of canola seedlings. However, none of these herbicides have a residual action in the soil and late emerging weeds may necessitate a second application of the post-emergence herbicides at substantial cost.

Propyzamide is a pre- and post emergence herbicide known to have a long residual activity in the soil which gives a longer period of control of grass weeds. Furthermore, addition of pre-emergence herbicides such as propyzamide to post-emergence herbicides such as glyphosate and glufosinate ammonium can increase the period of weed control eliminating the need of a second application of post emergence herbicides. Glufosinate ammonium has been shown to be less effective on bigger or older weeds (Steckel et al 1997; Barnett et al. 2013) and therefore the effect of weed size on efficacy of one of the most important local weeds (ryegrass) will be assessed.

The objectives of this study were to

i. Determine the effect of applying different doses of glufosinate ammonium to ryegrass (*Lolium spp*) seedlings at different growth stages in a glasshouse study to determine an economic but effective dosage.
ii. Determine the effect of temperature on the efficacy of ryegrass control by glufosinate ammonium.
iii. Determine the effect of adding AMS on glufosinate ammonium efficacy under glasshouse and under field conditions.

iv. Determine the effect of propyzamide on the efficacy of herbicides (atrazine, glufosinate ammonium, glyphosate and imazamox) on ryegrass control in a glasshouse study.

v. Determine the effect of adding propyzamide to four herbicides mentioned above on the efficacy and residual action of the herbicides under field conditions.

Thesis outline

This thesis will be presented as scientific publications, with chapter 1 being a general introduction and objectives of the research carried out. Chapter 2 reviews the literature of weed control on herbicide resistant canola and glufosinate ammonium. Chapters 3-5 were in sequence of objectives outlined above and were written with their own abstracts, introductions, methodology, results and discussions, and conclusions. Objectives 1 and 3 are part of chapter 3, objective 3 is chapter 4, objectives 4 and 5 forms part of chapter 5. Lastly, chapter 6 form general conclusions and recommendations based on all the work done. Considering the outline here, the duplication of methodology can be seen in chapters 3, 4, and 5.

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CHAPTER 2

LITERATURE REVIEW

2.1 Weed control in canola

Young canola seedlings are very sensitive to early weed competition. Once established, canola is a good competitor with most weeds. Wild mustard (*Sinapis arvensis*) is a serious seed contaminant in canola and can cause price discounts or rejection in the market (Rose and Bell 1982). In addition, Roshdy et al. (2008) reported a total yield loss of 35 % in canola. Weed control in canola is very crucial to obtain good yields.

In order to produce a profitable canola crop proper timing at weed control is critical. Canola becomes more competitive when it grows beyond the 4 to 6 leaf canola growth stage (Canol@fact 2006). According to Canol@fact (2006) yield loss in Canada due to weed competition was 3% higher when weed control was applied at the three leaf stage compared to the one leaf stage and 12 % higher when applied at 5 leaf stage. Martin et al. (2001) indicated that canola must be kept weed free until the fourth-leaf stage to prevent > 10% yield loss.

Weed control, especially in the early stages of the crop is a major limiting factor to canola growth and eventual yield (Oilseeds WA 2006). In contrast, Harker et al. (2006) found that it was not cost effective to apply glyphosate twice at the two to four and five to six leaf stages of canola in glyphosate resistant canola. Clayton et al. (2002) found that glyphosate application resulted in higher canola yields in some parts of Western Canada. The herbicide tolerant canola may provide more effective and consistent weed control and higher canola yields.

2.2 Antagonism, additivity and synergism

The joint action of herbicides in combination is described as antagonistic if the actual control is less than the predicted control. Synergism is when the actual control is greater than the predicted control and lastly additivity is when the tank-mix combination gives equivalent weed control to the predicted control (Chuah et al. 2008b). The common practice of applying two or more herbicides sequentially or as a tank mixture in crop production systems purely focuses on improving the spectrum of weed control, reducing production costs and preventing evolution of herbicide resistant weeds (Zhang et al. 1995).

Application of clethodim in a mixture with glufosinate ammonium showed an antagonistic action resulting in a reduction in the control of goosegrass (*Eleusine indica* L.) as compared to when clethodim was applied alone (Burke et al. 2005). Control of red rice (*Oryza sativa*) increased when acifluorfen was combined with haloxyfop; this was also true for pitted morningglory (*Ipomoea lacunose* L.) and johnsongrass (*Sorghum halapense*) when imazethapyr was combined with imazaquin (Hydrick and Shaw 1994). Glufosinate ammonium gave an additive response when it was mixed with various herbicides in the control of sicklepod (*Senna Obtusifolia*) and pitted morningglory (*Ipomoea lacunose* L.). The tank mixes resulted in better control than when only glufosinate ammonium was applied (Lanclos et al. 2002).

2.3 Types of antagonism

Generally, factors such as low application rates and herbicide type influence the interaction of herbicides when tank mixed (Green 1989). However, glufosinate ammonium showed diverse effects when combined with other herbicides. Lanclos et al. (2002) observed an antagonistic response in controlling broadleaf and grass weed species as a result of low herbicide rates. Hydrick and Shaw (1994) also reported similar results when selective and non-selective herbicides were mixed; however the antagonistic effect was overcome when the non-selective rate was increased. The following types of antagonism are recognised.

2.3.1 Chemical

Chemical interaction between an antagonist and a herbicide leads to the formation of an inactive complex which affects the activity of the herbicide present at the site of action (Green 1989; Rao 2000). The inactivation of the herbicide by a given antagonist is directly proportional to the number and amount of the components in a mixture. An example of a metal complex formation is glufosinate ammonium forming a metal complex with metal ions such as Mg^{2+}, Cu^{2+}, Ni^{2+} and Fe^{2+} (Ambrose and Hoggard 1989).

2.3.2 Physiological

The characteristics of physiological antagonism can be seen when two herbicides have opposite biological effects and counteract each other (Ncedana 2011). Each component has the capacity to react with its receptor site and bring out a characteristic response, but when combined one opposes the effect of the other on the same physiological process (Green 1989). Antagonism of glufosinate ammonium by a glyphosate mixture used to control goosegrass (*Eleusine indica* L.) is an example of physiological antagonism; glufosinate ammonium destroyed the leaf tissue before glyphosate could be translocated to the roots and stem of the plant (Chuah et al. 2008b).

2.3.3 Biochemical

Biochemical antagonism occurs when one chemical (antagonist) decreases the amount of a given herbicide available at the site of action. The occurrence might be in one of the following ways: reduced rate of herbicide penetration or absorption into the plant, or reduced herbicide transportation to the site of action within the plant. It can also increase the biotransformation rate in certain plants as well as enhancing metabolic inactivation (Green 1989; Rao 2000; Ncedana 2011). An example of antagonism causing reduced herbicide absorption is that of Na-bentazon on sethoxydim absorption (Green 1989).

2.3.4 Competitive

The antagonist acts at the same site of action as the herbicide intended for destroying the weed. In some cases, the antagonist, although capable of reacting with a receptor site within the cell, may lack essential activity (Green 1989; Rao 2000). Antagonism of 2, 4-D by 2, 4, 5-T is an example of competitive antagonism. The antagonism of paraquat activity by the organic polyamine putrescine in the susceptible biotype of *Conyza bonariensis* is another example of competitive antagonism, through competitive inhibition of paraquat uptake by the plasmalemma (Penner 1989).

2.4 Glufosinate ammonium

Glufosinate ammonium (Figure 2.5) is a broad-spectrum, non-selective, post emergence herbicide normally used to control weeds in vineyards, orchards and genetically modified crops. (Maschoff et al. 2000; Avila-Garcia et al. 2012). Glufosinate [ammonium-DL-homoalanin-4-yl-(methyl)] phosphinate, the active phytotoxic metabolite of bialaphos [L-2-amino-2-4-(hydroxyl)(methyl) phosphinoyl)-butryl-L-alanyl-L-alanine], is produced by *Streptomyces viridochromogenes* or *S hygroscopicus* (Mersey et al. 1990; Krausz et al. 1999; Ramsey et al. 2002), and used as a desiccant for certain crops (Ramsey et al, 2002; Tsai et al. 2006; Carpenter and Boutin 2010).

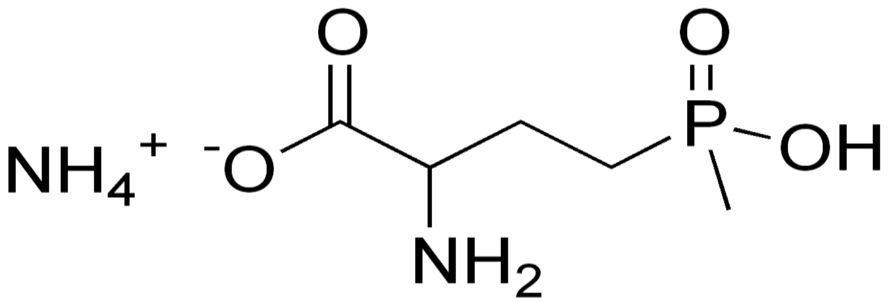

Figure 2.4 Structure of glufosinate ammonium (Faber et al.1998).

2.5 Glufosinate ammonium mechanism of action

Glufosinate ammonium kills susceptible weed species by inhibiting the enzyme glutamine synthetase, which plays a major role in the pathway that assimilates inorganic nitrogen into organic compounds and ammonia assimilation derived from nitrate reduction and photorespiration (Avila-Garcia and Mallory-Smith 2011; Avila-Garcia et al. 2012). Permanent inhibition of glutamine synthetase (GS) by glufosinate ammonium leads to a rapid increase of high levels of ammonia due to a lack of nitrogen metabolism (Jansen et al. 2000; Everman et al. 2009a; Avila-Garcia and Mallory-Smith 2011; Avila-Garcia et al. 2012). Excessive ammonia within the plant often leads to reduced photosynthetic activities due to disruption of the chloroplast structure causing inhibition of RuBisCo and carbon fixation (Jansen et al. 2000; Ramsey et al. 2002; Avila-Garcia and Mallory-Smith 2011).

2.5.1 Translocation

It is the transfer of herbicides from one part to another in plants. The translocation of shoot active herbicides is conducted through the phloem tissues in plants along with the food material. Therefore, for active translocation of such herbicides to the underground parts of the treated plant, sunlight and other conditions favourable for the process of photosynthesis by plants are essential. Previously, glufosinate ammonium controlled annual and broadleaf weed species effectively, however not all weed species presented the same degree of susceptibility (Mersey et al. 1990; Steckel et al. 1997). For instance there was a significant level of glufosinate ammonium translocation in glufosinate resistant corn compared to goosegrass (*Eleusine indica* L.), large crabgrass (*Digitaria sanguinalis*) and sicklepod (*Senna obtusifolia*) (Everman et al. 2009a*).* Translocation of herbicide within the plant is specie dependent. Subsequently, Everman et al. (2009b) observed a significant translocation in Palmer amaranth (*Amaranthus palmeri*) compared to pitted morningglory (*Ipomoea lacunosa*) and non transgenic cotton. Therefore, high translocation rates enhance the movement of glufosinate ammonium within the plant resulting in better performance. Kumaratilake et al. (2002) reported a difference in translocation pathway of glufosinate ammonium in sterile oats (*Avena sterillis*) and rigid ryegrass (*Lolium rigidum*).

2.5.2 Absorption

It is the process whereby the herbicide penetrates into the plant tissue. Herbicides are applied to the plant foliage. Hence, absorption of herbicides depends on the method of application and the plant with which the chemical comes into contact. In general, the foliage applied herbicide has to meet five major barriers before reaching the interior of individual cells for action. These barriers are surface waxes and hair, cuticle, periderm, cell wall and plasmalemma. The thickness and chemical quality of each barrier varies with the plant species and environmental conditions under which a plant is grown. Herbicide absorption is also time dependent as 59 and 83% absorption of glufosinate ammonium was observed in amaranth species at 6 and 24 hours after treatment (Coetzer et al. 2001). Palmer amaranth (*Amaranthus palmeri*) absorbed 85% of glufosinate ammonium - higher than transgenic, non-transgenic cotton and pitted morningglory (*Ipomoea lacunosa*). Everman et al. (2009b) also observed greater than 85% absorption of ^{14}C glufosinate ammonium in palmer amaranth (*Amaranthus palmeri)* 24 hours after treatment. The absorption differed according to species.

2.6 Environmental factors affecting glufosinate ammonium efficacy

Environmental conditions such as temperature, humidity, rainfall, light and wind before, during and after herbicide application influence the efficacy of foliage-applied herbicide, as well as herbicide metabolism within the plant (Rao 2000).

2.6.1 Temperature

Environmental conditions such as extremely high temperatures above 30^0C before and after herbicide application affects herbicide efficacy by reducing plant metabolic processes such as absorption and translocation within the leaf. Kumaratilake and Preston (2005) reported a significant increase in glufosinate ammonium efficacy on plants that were kept in warm growth room than under cold growth room. Similarly, efficacy of glufosinate ammonium on wild radish (*Raphanus raphanistrum* L.) was improved when it was applied under 20^0 C to 25^0 C. Temperature variations of 20^0C

to 25^{0}C influenced the efficacy of glufosinate ammonium on wild radish (*Raphanus raphanistrum* L.) control. Even though temperature is known to influence glufosinate ammonium efficacy, in some instances it can cause damage by slowing metabolic processes taking place within the plant. According to Pline et al (1999) low temperatures of 15 ^{0}C negatively affected glufosinate ammonium activity on Liberty Link soybean (LL).

2.6.2 Humidity

Relative humidity affects herbicidal penetration both physically and physiologically. The physiological effects observed in affected plants include water stress, stomatal opening and cuticular permeability (Hammerton 1967; Rao 2000). Low relative humidity prior to, during and after treatment causes cuticle dehydration thus possible reduction in absorption of water soluble herbicides such as glufosinate ammonium. Low relative humidity decreased the control of palmer amaranth, redroot pigweed and common waterhemp by glufosinate ammonium (Coetzer et al. 2001). Similarly, Mersey et al. (1990) reported high tolerance of green foxtail and barley to glufosinate ammonium at a relative humidity of 40% as compared to 90%. High relative humidity increases the translocation of the herbicide as well as the persistence of liquid deposits on the leaves. Exposure of wild oat to 95% relative humidity increased glufosinate ammonium efficacy when compared to 40% relative humidity (Ramsey et al. 2002).

2.6.3 Rainfall

Rainfall can greatly affect the efficacy of post emergence herbicides. The response of weeds to a herbicide depends on the quantity and the intensity of rain and its incidence in time. Rain before spraying increases leaf wettability and herbicidal susceptibility, by damaging the wax structure of the leaf surface. In most cases rainfall during or following herbicide spraying washes off the intercepted spray from leaves, reducing herbicide effectiveness (Hammerton 1967). The quantity of leaf-washing depends not only on the amount of rain but its intensity and also on the structure of the crop-weed stand. Occurrence of rain within the first 6 hrs of herbicide application can wash off water soluble herbicide and spray deposits on the leaves hampering penetration on the leaf surface (Hammerton 1967; Rao 2000). Generally, rainfall affects oil formulated herbicides less than aqueous solutions. Simulated rainfall shortly after application, reduced glufosinate ammonium efficacy on barley and green foxtail (Anderson et al 1993).

2.6.4 Light

Light is an important component in herbicide penetration by stimulating stomata opening and also photosynthesis activation, leading to greater movement of organic solute and herbicides from the leaf to other parts of the plant. However, the effects vary according to duration and intensity. Low light intensity and low relative humidity under controlled climatic conditions, reduces the accumulation of ammonia in plants and also affects the efficacy of glufosinate ammonium (Petersen and Hurle 2001). This could be because of the intensity under field condition usually exceeds the intensity applied under controlled environment hence the variation in glufosinate ammonium efficacy.

2.6.5 Wind

Wind has a direct effect on evapotranspiration and causes rapid drying of spray solution on the foliage, resulting in reduced herbicide absorption (Kudsk and Kristensen 1992; Rao 2000). It can, however, increase the susceptibility of a plant to herbicide by causing spray drifting, a decrease in retention as well as damage to cuticle (Hammerton 1967). However, there is limited scientific research on the influence of wind on the activity of herbicides at the time of application.

2.7 Plant factors

2.7.1 Branching pattern

Broad leaved species with an open branching pattern and horizontally held leaves are generally more susceptible to foliar applied herbicides (Hammerton 1967). The leaf structuring facilitates retention of spray droplets and easy surface coverage. Grass species, on the other hand, have minute ridged surfaces on the leaves. Often the leaves of grass species are vertically arranged and their growing points are enclosed by sheaths which serve as a protective cover. This may be the reason why non-polar herbicides are more effective against grasses as they tend to have greater surface coverage (Rao 2000). For example, glufosinate ammonium requires thorough coverage to ensure effective broad spectrum grass and broadleaf weed control (Steckel et al. 1997; Corbett et al. 2004).

2.7.2 Plant size

Young actively growing plants usually have thinner, more permeable cuticles than older plants (Rao 2000). Thus, water soluble herbicides such as glufosinate ammonium may be more effective in penetrating the cuticle of younger plants, and less effective at later application timings. Herbicide efficacy can be influenced by factors such as rate of application and weed growth stage. Application of glufosinate ammonium at a rate of 420 g ha^{-1} resulted in greater than 80% control of giant foxtail (*Setaria faberi*) at 10 cm as compared to 5 and 15 cm weed height (Steckel et al. 1997). Poor control in small (5 cm) plants was probably due to insufficient leaf area to allow sufficient uptake of the herbicide (Steckel et al. 1997).

Herbicide movement in young plants is faster than in old plants and as a result uptake by foliage and translocation in the phloem becomes greater and it reaches actively growing meristematic tissues faster. The thickness of the cuticle changes with plant age and maturity. As plants mature, the leaf surface thickens and barriers for herbicide penetration become greater (Rao 2000). Rapid shoot and root growth also favour rapid herbicide absorption

2.7.3 Plant species and variety

Some plant species, some cultivars within the species and even some strains within a variety, show differences in absorption and translocation of herbicides. Kumaratilake et al. (2002) reported the difference in distribution of glufosinate ammonium in sterile oat (*Avena sterillis*) and rigid ryegrass (*Lolium rigidum*) by glufosinate ammonium. It is more likely that the difference in distribution was due to sterile oat (*Avena sterillis)* being controlled more easily by glufosinate ammonium than rigid ryegrass.

2.8 Glufosinate ammonium antagonism

Glufosinate ammonium antagonised clethodim in the control of goosegrass. Clethodim rates of 105 and 104 g ai ha^{-1} controlled goosegrass when applied at two to four leafstage as well as at the one to four tiller, whereas glufosinate ammonium alone at 290 or 410 g ai/ha controlled goosegrass (*Eleusine indica* L.) when applied at the two to four leafstage (Burke et al. 2005). Tharp and Kells (2002) reported an increase in weed control after applying glufosinate ammonium, glyphosate and residual herbicides as tank mixtures rather than in sequence. In contrast to the findings of Tharp and Kells (2002), Gardner et al. (2006) proved that sequential application of clethodim, fluazifop-P, Quizalofop-P and sethoxydim is effective in controlling annual grasses and johnsongrass (*Sorghum halapense*).

Tank-mix combinations do not always achieve the predicted weed control, because at times it is not effective at all. For example, the interaction of glufosinate ammonium and MSMA in a tank-mix did not control broadleaf and grass weeds as it was supposed to, instead MSMA antagonised glufosinate ammonium (Koger et al. 2007). Therefore, it is important to have a clear understanding of both herbicides' mode of actions before mixing them in a tank. However, the negative the interaction between glyphosate and glufosinate ammonium observed in some species, was commonly attributed to the rapid action of glufosinate ammonium on the photosynthetic system, which resulted in reduced translocation of glyphosate within the plant (Kudsk and Mathiassen 2004, Chuah et al. 2008b, Bethke et al. 2013).

2.9 Glufosinate ammonium resistance

The overuse of herbicides has led to the rapid evolution of herbicide resistant (HR) weeds (Beckie 2006; Powles and Yu 2010) According to Moss (2002) herbicide resistance is defined as the inherited ability of a weed to survive a rate of herbicide which would normally result in effective control. Chuah et al. (2008a) described herbicide resistance as the result of repetitive use of herbicides with the same site of action in the same growing season on the same area. According to Heap (2014) globally there are 434 HR weed biotypes among 237 HR weed species (138 dicots and 99 monocots).

Goosegrass (*Eleusine indica* L.) and Italian ryegrass (*Lolium multiflorum*) were reported to have developed resistance to GA - both populations exhibited twofold and eightfold resistance towards it, respectively (Jalaludin et al. 2010). In addition, Seng et al. (2010) reported the evolution of glufosinate ammonium and paraquat multiple resistance in a biotype of goosegrass (*Eleusine indica* L.). The cause of this was the induced resistance of glufosinate ammonium and paraquat.

Avila-Garcia and Mallory-Smith (2011), reported the development of resistance to glufosinate ammonium by glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) populations with no history of glufosinate ammonium use previously in the controlled area. The glufosinate ammonium resistance levels of these populations were 2–3-fold compared with susceptible populations. Consequently, Avila-Garcia et al. (2012)

found target-site mutation as the cause of resistance to glufosinate ammonium in an Italian ryegrass (*Lolium multiflorum*) population.

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CHAPTER 3

GLUFOSINATE AMMONIUM EFFICIENCY ON RYEGRASS AS AFFECTED BY GROWTH STAGE AND TEMPERATURE

Abstract

Two glasshouse experiments were conducted to evaluate the effect of growth stage and temperature on glufosinate ammonium efficacy. Glufosinate ammonium is known to be rate and growth stage dependent. The first experiment evaluated growth stage and rates on commercial and weedy ryegrass control. Glufosinate ammonium rates applied were 0, 2.5, 5, 7.5, and 10 L ha^{-1}. Temperature as an environmental factor which affects herbicide efficiency prompted the investigation on its effect on glufosinate ammonium efficacy. The second experiment examined the effect of temperature on glufosinate ammonium efficacy on ryegrass control. Glasshouses were set to run under 10/15 ^{0}C, 15/20 ^{0}C and 20/25 ^{0}C night/day temperatures. Glufosinate ammonium rates used were 2.5, 5, 7.5, and 10 L ha^{-1}.The findings of the first experiment indicated that application of glufosinate ammonium at 2.5 L ha^{-1} controlled 90% of the 10 week commercial population, and weedy ryegrass followed the same trend. In the second experiment application of glufosinate ammonium in cool temperatures resulted in 90% control, the same trend was observed on plants that were transferred from warm to cool glasshouse after spraying. Low glufosinate ammonium rates of 2.5 L ha^{-1} controlled 90% of ryegrass plants under cool temperatures.

Keywords: commercial ryegrass, dosage, glufosinate ammonium, plant age, temperature

3.1 Introduction

Lolium rigidum (commonly known as annual or rigid ryegrass) is a Mediterranean species initially introduced as pasture crop which has developed into a major weed species world-wide (Goggin et al. 2012). In South Africa however, according to (Botha 2001; Eksteen et al. 2005; Ferreira 2011), all *Lolium* species hybridise freely with one another and therefore it is difficult to make a distinction between different ryegrass species. These hybrids pose a serious threat to winter cereals in the Western Cape (Eksteen et al. 2005). With its origin in Europe it is found in Gauteng, North West Province, Free State and KwaZulu-Natal, usually along roadsides and in other disturbed areas (Botha 2001). It also occurs as a weed in crop fields, deciduous fruit orchards as well as in vineyards in the south west Cape.

Glufosinate ammonium is a non-selective post emergent herbicide used effectively as an alternative for glyphosate and paraquat on annual and perennial grasses, as well as broadleaf weed control in vineyards and orchards (Coetzer et al. 2001). Krausz et al. (1999) reported the effective control of annual weeds by glufosinate ammonium in glufosinate-resistant corn (*Zea mays*). Previous research indicates that the efficacies of some herbicides are influenced by plant age (Rao 2002, Kumaratilake and Preston 2005). As weeds increase in size, they may become less susceptible to herbicides (Klingaman et al. 1991; Faccini and Puricelli 2007).

Weeds are most sensitive to herbicides at a young age of less than 4-6 weeks (Barros et al. 2007), but more developed weeds can also be controlled satisfactorily by glyphosate and glufosinate ammonium (Tharp et al. 1999). Kieloch and Domaradzki (2011) however, showed that Stellaria *media L*., control was affected by herbicide rate and growth stage. The efficiency of glufosinate ammonium is known to be rate, growth and species dependent (Steckel et al. 1997; Kumaratilake et al. 2002a). Steckel et al. (1997) reported better control percentages by glufosinate ammonium on giant foxtail than on common lambsquarter, common cocklebur and Pennsylvania smartweed regardless of rate or plant size.

Temperature has a profound influence on the growth and development of plants before spraying. Besides affecting cuticle development (Hull et al 1975; Baker 1974; Whitecross and Armstrong 1972), temperature may also change plant morphology and physiological processes. At 40 ^{0}C, flumiclorac was more effective in controlling *Chenopodium album* and *Amaranthus retroflexus* than was the case at 10 ^{0}C (Matzenbacher et al. 2014). Cool temperatures may not affect the phytotoxicity, but may delay the rate of the development of injury caused by a decreased rate of herbicide penetration through the plant cuticle (Kudsk and Kristensen 1992). For example, wild radish (*Raphanus raphanistrum* L.) plants grown at low temperatures (5/10 ^{0}C) were less susceptible to glufosinate ammonium than plants grown at high temperatures (Kumaratilake et al. 2002a).

Previously, there have been reports of an inconsistent response of various weed species to glufosinate ammonium under different temperatures (Anderson et al. 1993; Coetzer et al. 2001; Kumaratilake et al. 2002b; Kumaratilake and Preston

2005). The objectives of this study were to determine the effect of applying different doses of glufosinate ammonium to ryegrass seedlings at different growth stages in order to investigate the possible use of more economic but effective dosage rates and to determine the effect of temperature on the efficacy of glufosinate ammonium on ryegrass.

3.2 Materials and methods

3.2.1 Plant age study

Trial 1: Commercial annual ryegrass (L. multiflorum cv. Agriton)

A glasshouse study was conducted to determine the optimum time of application of glufosinate ammonium on annual ryegrass seedlings. Ten ryegrass (*L. multiflorum* cv. Agriton) seeds were sown directly into 8 X 8 cm pots filled with sand and thinned to four plants per pot 7 days after planting (DAP). The commercial ryegrass cultivar was used because it is closely related to the weedy ryegrass plants in the field and the seeds germinate readily without the problems caused by seed dormancy. Planting of ryegrass seeds in the pots were repeated every two weeks for 10 weeks. Plants were irrigated with a nutrient solution three times a week to supply enough nutrients using a 4 L watering can. The composition of the nutrient solution is given in Table 3.1. The glasshouse was set to run at 20-30 ^{0}C night/day.

All herbicide treatments were applied on the same day to the 2, 4, 6, 8, and 10 week old ryegrass plants. Herbicide treatments were applied by means of a pneumatic pot spraying apparatus operating at a pressure of 2 bars and delivering 400 L of water ha^{-1}. The design was a 5 X 5 factorial arranged in a randomised complete block with four replicates. The experimental factors were plant age (2, 4, 6, 8 and 10 weeks) and herbicide rate (0, 2.5, 5, 7.5 and 10 L ha^{-1} of glufosinate ammonium 200 g ai L^{-1}). Visual estimates of the percentage control were based on the percentage of senescent plants and were carried out at 6 weeks after treatment (WAT). Dry mass production was measured 6 WAT by removing the above ground parts of the plants and drying it in an oven at a constant temperature of 80 ^{0}C for two days before the mass was determined. Percentage dry mass reduction was calculated by the following formula: DM_c - DM_{tr}/DM_c X 100 where DM_{tr} is the dry mass pot^{-1} of the treated plants and DM_c is the dry mass pot^{-1} of the untreated control. Data was subjected to analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Fischer's $LSD_{0.05.}$

Trial 2: Weedy ryegrass (Lolium spp.) accession from crop fields

Seeds from a weedy population of ryegrass were used to repeat the experiment described above to investigate if the response from weedy ryegrass would be similar to the response of the commercial annual ryegrass cultivar. Apart from the different seed source the procedure was similar to the one used in the commercial annual ryegrass trial.

3.2.2 Temperature study

Trial 1: Continuous temperature regimes (10/15 and 15/20 ^{0}C)

This study was conducted in two glasshouses at 10/15 ^{0}C and 15/20 ^{0}C night/day temperatures respectively, in order to assess the effect of temperature on glufosinate ammonium efficacy. Ten ryegrass (*L. multiflorum* cv. Agriton) seeds were sown directly into 8 X 8 cm pots filled with sand and thinned to four plants per pot 7 days after planting (DAP). Plants were irrigated with the same nutrient solution described in Table 3.1. Herbicide treatments were applied on the same day to plants grown under cool and warm temperatures. Plants were kept at the same temperatures after spraying for the entirety of the study.

Herbicide treatments were applied by means of a pneumatic pot spraying apparatus operating at a pressure of 2 bars and delivering 400 L of water ha^{-1}. The design was a 2 X 4 factorial arranged in a randomised complete block with four replicates. The experimental factors were growth temperature (10/15 ^{0}C and 15/20 ^{0}C night/day) and herbicide rate (0, 2.5, 5, 7.5 and 10 L ha^{-1} of glufosinate ammonium 200 g ai L^{-1}). Visual estimates of the percentage control were based on the percentage of senescent plants and were carried out at 6 WAT. Dry mass production was measured 6 WAT by weighing the above ground plant material after drying at 80 ^{0}C for 2 days. Data was subjected to analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Fischer's $LSD_{0.05.}$

The results obtained from the study necessitated a follow up trial on the influence of temperature before and after spraying on glufosinate ammonium efficiency.

Trial 2: Varying temperature regimes (10/15 and 20/25 ^{0}C)

A glasshouse study was conducted in two glasshouses running at different temperature regimes. Glasshouse temperatures were set at 10/15 ^{0}C (cool) and 20/25 ^{0}C (warm) night/day. The same procedures as in the previous temperature study were followed. All herbicide treatments were applied on the same day to ryegrass plants grown under both cool and warm temperatures. However, after spraying, half of the plants that were growing in cool temperatures were transferred to a warm glasshouse labelled (cool/warm) and the other half remained under the same temperature settings. Plants from the warm glasshouse were moved to the cool glasshouse in a similar manner after spraying (warm/cool).

Herbicide application was as described above. The design was a 5 X 4 factorial arranged in a randomised complete block with four replicates. The experimental factors were herbicide rate (0, 2.5, 5, 7.5 and 10 L ha^{-1} of glufosinate ammonium 200 g ai L^{-1}) and 4 temperature regimes (cool, warm, cool/warm and warm/cool). The rest of the procedures were as described above.

Table 3.1: Composition of nutrient solution used throughout the study

EC = 2.0			
Element (Macro)	Concentration mg L^{-1}	Fertiliser	Concentration g 1000L^{-1}
K^{+}	237.7	$KN0_3$	303
Ca^{++}	180	K_2S0_4	261
Mg^{++}	48.6	$Ca(N0_3)_2.2H_20$	900
$N0_3^{-}$	661.33	$MgS0_4.7H_20$	492
H_2P0_4	116.4	KH_2P0_4	136
$S0_4$	390.4		
(Micro)	mg L^{-1}		
Fe: Libfer (Fe EDTA)	0.85		6.54
Mn: Manganese sulphate	0.55		2.23
Zn: Zinc sulphate	0.30		1.33
B: Solubor	0.30		1.46
Cu: Copper Sulphate	0.05		0.20
Mo: Sodium Molibdate	0.02		0.13

3.3 Results

3.3.1 Plant age study

Analysis of variance (Tables 3.2 and 3.3 refer to the commercial ryegrass cultivar (Trial 1) and the weedy accession (Trial 2) respectively) shows the significant effect of herbicide rate (*P* < 0.000) on percentage control. There was no significant interaction of main effects (plant age x herbicide rate) on percentage control in Trials 1 and 2 (Tables 3.2 and 3.3).

In the commercial cultivar (Trial 1) significant differences were detected between the lowest herbicide rate of 2.5 L ha^{-1} and higher rates of 5, 7.5 and 10 L ha^{-1} (Table 3.4) respectively. The percentage control at 2.5 L ha^{-1} was 92%, lower than the rest of the treatments, but it was still above 90% (Table 3.4) which is satisfactory under field conditions. Similar results were observed with the weedy ryegrass where the lowest herbicide rate of 2.5 L ha^{-1} controlled 95% of the ryegrass plants (Table 3.4).

Table 3.2: Analysis of variance for percentage control of commercial ryegrass seedlings (*Lolium multiflorum* cv Agriton) (Trial 1) with glufosinate ammonium

Effect	SS	Degr. of Freedom	MS	F	p
Plant age (weeks)	150.0	4	37.5	0.90	0.468415
Herbicide rate L ha^{-1}	154900.0	4	38725.0	929.40	0.000000
Plant age*Herbicide rate L ha^{-1}	600.0	16	37.5	0.90	0.571814
Error	3125.0	75	41.7		

Table 3.3: Analysis of variance for percentage control of weedy ryegrass seedlings (*Lolium* spp) (Trial 2) with glufosinate ammonium

Effect	SS	Degr. of Freedom	MS	F	p
Plant age (weeks)	212.5	4	53.1	0.850	0.498064
Herbicide rate L ha^{-1}	154337.5	4	38584.4	617.350	0.000000
Plant age*Herbicide rate L ha^{-1}	787.5	16	49.2	0.788	0.694551
Error	4687.5	75	62.5		

Table 3.4: Effect of glufosinate ammonium herbicide rates on percentage control of ryegrass seedlings 6 weeks after treatment

Herbicide rate(L ha^{-1})	Commercial ryegrass Trial 1	Weedy ryegrass Trial 2
0	0a*	0a
2.5	92b	95b
5	100c	100b
7.5	100c	98b
10	100c	98b

* Values followed by the same letters in a column do not differ significantly from each other at P = 0.05.

Analyses of variance (Table 3.5) show a significant interaction between plant age and herbicide rate on the percentage dry mass reduction of commercial ryegrass. Percentage dry mass reduction for 2 and 4 weeks old plants followed the same trend but was different from 6, 8 and 10 weeks old plants (Figure 3.1). Dry mass for the 2 and 4 weeks old plants were reduced with about 5 and 15% respectively. The dry mass reduction of the older plants were reduced more than the 2 and 4 weeks old plants but dosage rate had contrasting effects on the different aged plants, hence the significant interaction (Figure 3.1).

The weedy ryegrass results presented in Table 3.6 showed a significant interaction of plant age and herbicide rates on the percentage dry mass reduction. Percentage dry mass was reduced significantly in the 8 week old plants while the percentage reduction in the 2 and 4 week old plants were small – in fact, at the 5 and 7.5 L ha-1 dosage rates there was an increase of dry mass of the treated plants compared to the unsprayed control. (Figure 3.2).

Table 3.5: Analysis of variance for the effect of age and herbicide rate on % dry mass reduction of commercial ryegrass (*Lolium multiflorum* cv Agriton) seedlings treated with varying doses of glufosinate ammonium (Trial 1)

Effect	SS	Degr. of Freedom	MS	F	p
Plant age (weeks)	4696.73	4	1174.18	34.5729	0.000000
Herbicide rate L ha^{-1}	6092.05	4	1523.01	44.8440	0.000000
Age*Herbicide rate L ha^{-1}	1461.39	16	91.34	2.6893	0.002094
Error	2547.18	75	33.96		

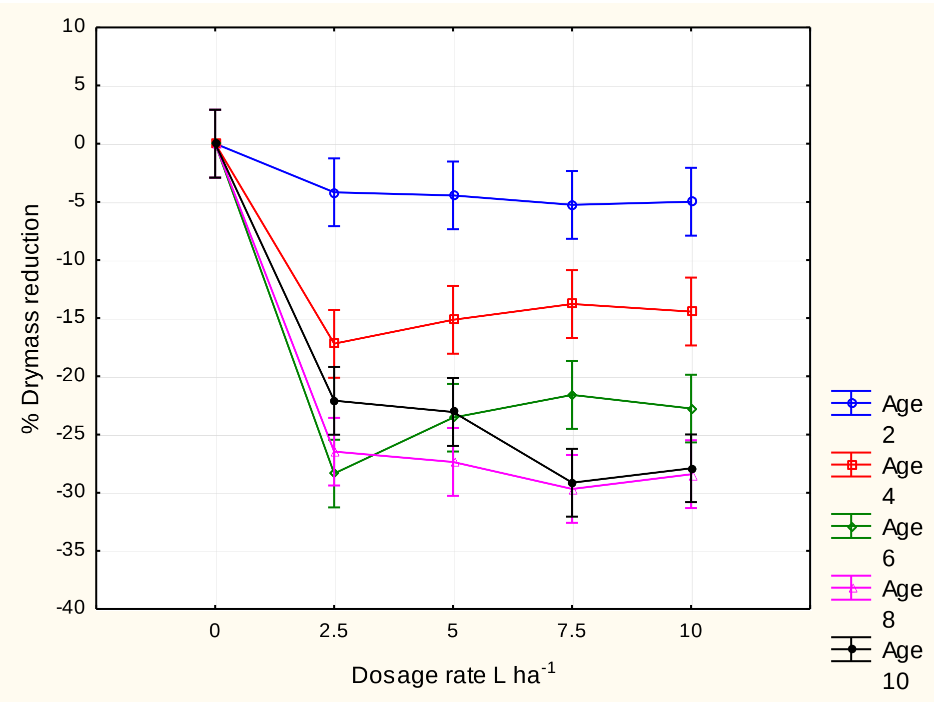

Figure 3.1: Interaction of glufosinate ammonium rates and plant age on percentage plant dry mass reduction of commercial ryegrass seedlings 6 weeks after treatment. Vertical bars on the graph indicate least significant different means.

Table 3.6: Analysis of variance for the effect of age and herbicide rate on % dry mass reduction of weedy ryegrass (*Lolium* spp) seedlings treated with varying doses of glufosinate ammonium

Effect	SS	Degr. of Freedom	MS	F	p
Plant age (weeks)	32885.46	4	8221.36	67.7847	0.000000
Herbicide rate L ha^{-1}	6425.12	4	1606.28	13.2437	0.000000
Plant age*Herbicide rate L ha^{-1}	10390.89	16	649.43	5.3545	0.000000
Error	9096.48	75	121.29		

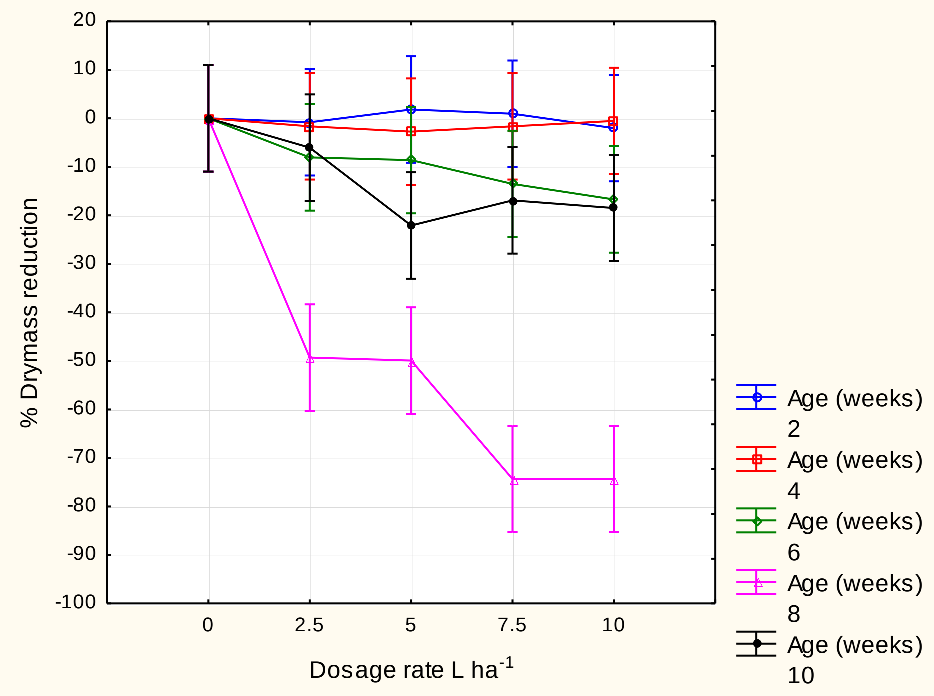

Figure 3.2: Interaction of glufosinate ammonium rates and plant age on percentage plant dry mass reduction of weedy ryegrass seedlings 6 weeks after treatment. Vertical bars on the graph indicate least significant different means.

3.3.2 Temperature study

Trial 1: Continuous temperature regimes (10/15 and 15/20 ^{0}C)

The analysis of variance Table 3.3 shows significant interaction *(P* = 0.000) of temperature and glufosinate ammonium dosage rates on the control of ryegrass in Trial 1 of the temperature study. Glufosinate ammonium applied to plants grown under cool temperature conditions gave 100% control including the lowest rate of 2.5 L ha^{-1}, whereas in plants that were growing under warm temperatures the control increased with an increase in the rate applied (Figure 3.3). The dose response graph illustrates an exponential increase in percentage control with an increase in glufosinate ammonium concentration under warm temperatures.

No significant interaction was detected with regard to temperature and glufosinate ammonium dosage rate on the percentage dry mass reduction of ryegrass 6 WAT (Table 3.8). Temperature and rates showed a significant effect (P = 0.006 and 0.000 respectively). Dry mass reduction of about 50% was observed in plants that were growing under warmer temperatures compared to only 28% of plants growing under cool temperatures' (Table 3.9). Percent dry mass reduction for all the rates was the same.

Table 3.7: Analysis of variance on percentage control of ryegrass plants 6 weeks after treatment

Effect	SS	Degr. of Freedom	MS	F	p
Temperature ^{0}C	9000.0	1	9000.0	57.600	0.000000
Rates L ha^{-1}	51781.3	4	12945.3	82.850	0.000000
Temperature*Rates	11781.3	4	2945.3	18.850	0.000000
Error	4687.5	30	156.2		

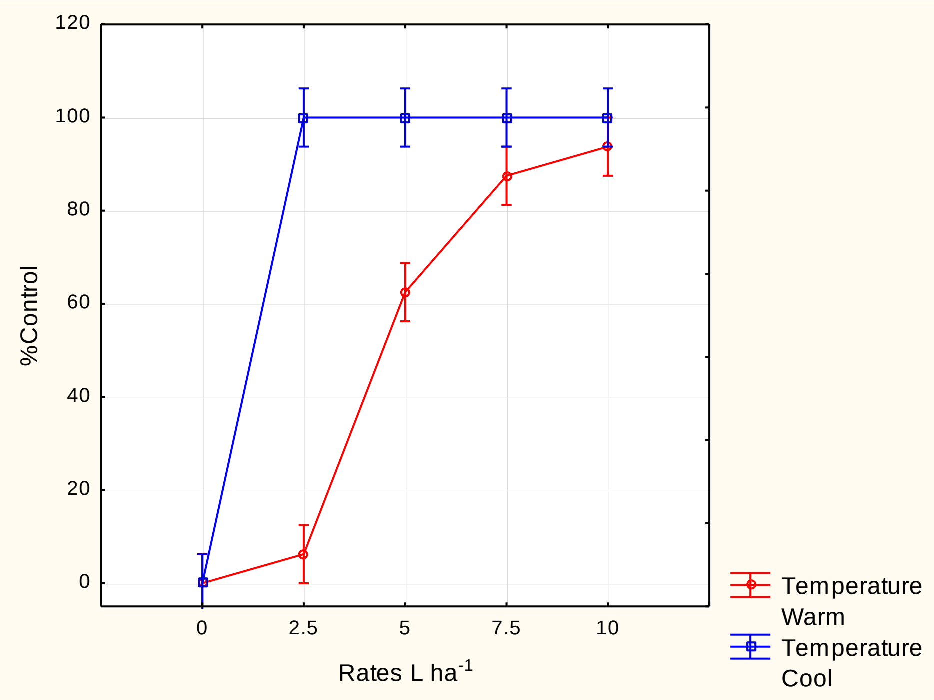

Figure 3.3: Effect of temperature and glufosinate ammonium rates on the control of ryegrass plants 6 weeks after treatment. Vertical bars on the graph indicate least significant different means.

Table 3.8: Analysis of variance for the effect of temperature and herbicide rate on percentage dry mass reduction of commercial ryegrass (*Lolium multiflorum* cv Agriton) seedlings treated with varying doses of glufosinate ammonium

Effect	SS	Degr. of Freedom	MS	F	p
Temperature ^{0}C	5242.57	1	5242.57	8.5088	0.006634
Rates L ha^{-1}	18671.20	4	4667.80	7.5759	0.000242
Temperature*Rates	1820.61	4	455.15	0.7387	0.573004
Error	18484.06	30	616.14		

Table 3.9: Effect of temperature and glufosinate ammonium rates on the percentage dry mass reduction of commercial ryegrass 6 weeks after treatment

Temperature 0 ^{0}C	% Dry mass reduction
Warm	-50a
Cool	-28b
Rates L ha^{-1}	
0	0a
2.5	-56b
5	-59b
7.5	-34b
10	-47b

Values followed by the same letters for a specific parameter do not differ significantly from each other at $P = 0.05$.

Trial 2: Varying temperature regimes (10/15 and 20/25 ^{0}C)

Analysis of variance for percentage control showed a significant interaction between glufosinate ammonium dosage rate and temperature (P = 0.003) on ryegrass grown under 10/15 ^{0}C and 20/25 ^{0}C temperatures in Trial 2 of the temperature study (Table 3.10). The lowest glufosinate ammonium concentration of 2.5 L ha^{-1} gave 94% control under cool temperatures (Figure 3.4) compared to the lowest percentage control of 58% under warm temperatures. Plants that were moved from warm to cool temperatures after spraying gave significantly higher percentage control (82%) at 2.5 L ha^{-1} compared to those that remained in the warm glasshouse. Plants that were moved from cool temperatures to warm temperatures gave significantly lower (72%) control than the plants that remained under cool temperatures after treatment and also plants that were moved from warm to cool temperatures after spraying. All dosage rates higher than 2.5 L ha^{-1} resulted in 100% control irrespective of the temperature regime under which the plants grew.

Percentage dry mass reduction of plants grown in Trial 2, showed a significant interaction of temperature and rates on ryegrass plants 6 WAT (Table 3.11). According to Figure 3.5, percentage dry mass reduction for the cool, warm and cool/warm temperature treatments were similar but differed significantly from the warm/cool temperature treatment. Generally, plants that were grown under warm temperatures and moved to cool temperatures after spraying gave the highest percentage dry mass reduction (Figure 3.5).

Table 3.10: Analysis of variance for the effect of temperature and herbicide rate on percentage control of commercial ryegrass (*Lolium multiflorum* cv Agriton) seedlings treated with varying doses of glufosinate ammonium

Effect	SS	Degr. of Freedom	MS	F	p
Temperature ^{0}C	625.0	3	208.3	2.857	0.04443[illegible]
Rates L ha^{-1}	120000.[illegible]	4	30000.[illegible]	411.42[illegible]	0.00000[illegible]
Temperature*Rates	2500.[illegible]	12	208.3	2.857	0.00361[illegible]
Error	4375.[illegible]	60	72.9		

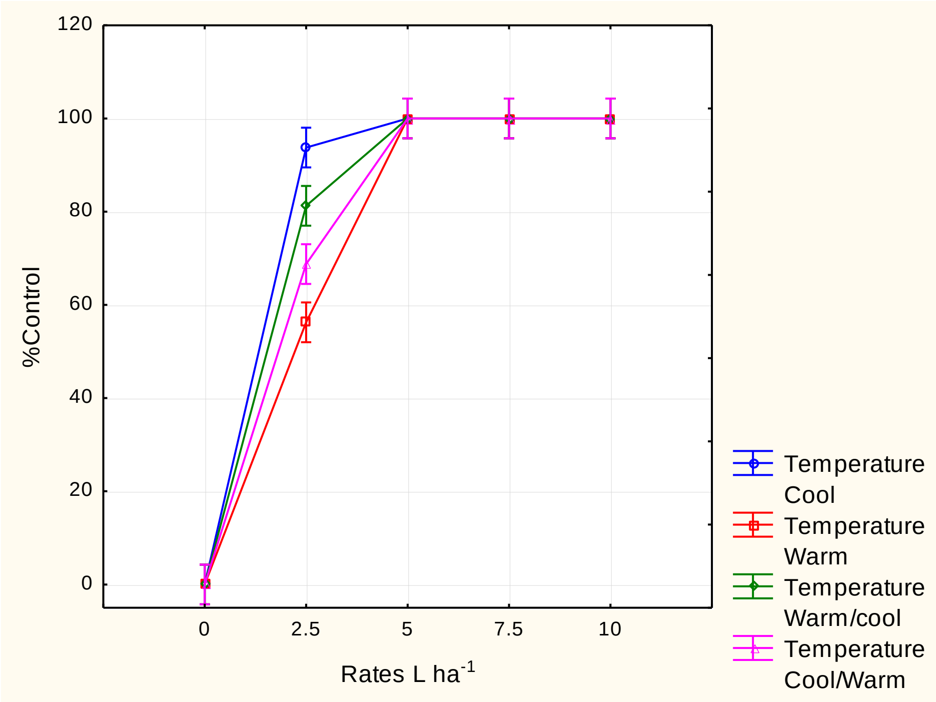

Figure 3.4: Effect of temperature and glufosinate ammonium rates on the percentage control of ryegrass plants 6 weeks after treatment. Vertical bars on the graph indicate least significant different means.

Table 3.11: Analysis of variance for the effect of age and herbicide rate on percentage dry mass reduction of commercial ryegrass (*Lolium multiflorum* cv Agriton) seedlings treated with varying doses of glufosinate ammonium

Effect	SS	Degr. of Freedom	MS	F	p
Temperature ^{0}C	33015.3	3	11005.1	47.4931	0.000000
Rates L ha^{-1}	34904.5	4	8726.1	37.6580	0.000000
Temperature*Rates	10187.4	12	848.9	3.6637	0.000382
Error	13903.2	60	231.7		

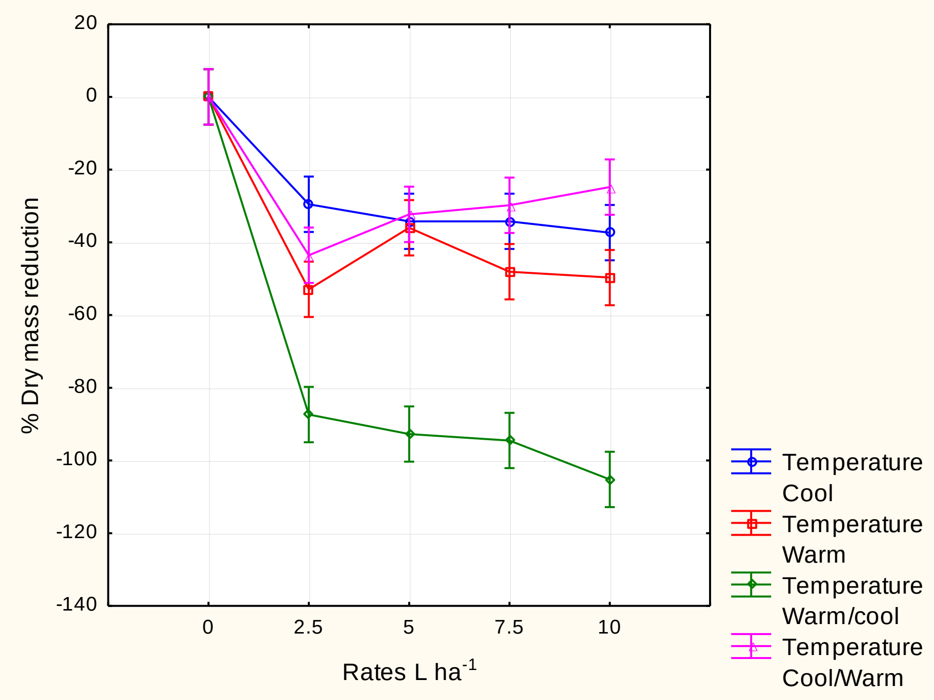

Figure 3.5: Effect of temperature and glufosinate ammonium rates on the percentage dry mass reduction of ryegrass 6 weeks after treatment. Vertical bars on the graph indicate least significant different means.

3.4 Discussion

3.4.1 Plant age study

Glufosinate ammonium is an important herbicide in non-selective weed control. In this study, high control rates were achieved on ryegrass plants irrespective of dosage rate and plant age. Neither plant age nor herbicide rate influenced the percentage control of ryegrass seedlings in the commercial annual ryegrass and weedy ryegrass samples tested. Generally, herbicides control young plants better than older plants simply because they have not developed a natural barrier to chemical entry (Kumaratilake et al.2002a). In this case, glufosinate ammonium rates as low as 2.5 L ha^{-1} effectively controlled the ten week old ryegrass plants (Table 3.4).

The increased control of larger plants could be the result of a better herbicide absorption rate by the plant. Adequate soil moisture and good growing conditions prior to treatment are often found to enhance herbicide efficacy, as stressed plants are usually difficult to control. Similarly, Ahmadi et al. (1980) reported a reduced efficacy of glyphosate due to water stress.

Nevertheless, according to the labels of various glufosinate ammonium formulations worldwide, efficiency decreases when the size of weeds to be controlled increase. This was not evident from the current study as all dosage rates higher than 2.5 L ha^{-1} controlled all ryegrass plants irrespective of size and even at 2.5 L ha^{-1} there was no indication that smaller plants were more sensitive, hence the lack of significant interactions. This work was done on a commercial ryegrass cultivar because of ease of germination of such seeds.

The percent dry mass reduction observed in Figure 3.1 generally shows that higher percentage reductions were associated with older plants except in the case of the 10 week old plants. The lower reduction of the 10 week old plants compared to the 8 week plants could be due to the roots becoming pot bound as the plants grew larger earlier and at some stage experienced moisture stress. The general increase in percentage reduction with increasing plant size could be due to the fact that the plants were at different ages (therefore different sizes) at the time of herbicide application. After application the control plants developed unaffected and because of the larger size the growth rate would have been higher. This could have resulted in much greater percentage reductions in dry mass where the plants were older and larger at the time of treatment.

Weedy ryegrass

The results obtained from the weedy type of ryegrass in Trial 2 did not differ significantly from the commercial population in Trial 1 in terms of the percentage control of the ryegrass 6 WAT (Table 3.4). Glufosinate ammonium rates as low as 2.5 L ha^{-1} also controlled the ten week old weedy ryegrass plants. Therefore, theoretically, under similar conditions as in this glasshouse, a dosage rate that controls small ryegrass seedlings should also control ten week old ryegrass plants. In contrast Steckel et al (1997) reported difference in weed control due to age and herbicide rate. Similarly, Jordan et al. (1997) reported application rate and timing as critical factors on glyphosate efficacy in weed control, which was not the case with the findings of this study.

Results from the weedy type experiment demonstrated a significant interaction of main effects; dosage rate and plant size on the percentage dry mass reduction. The same trends displayed by the commercial ryegrass were observed with weedy ryegrass except in this case the discrepancy in data recorded at 8 weeks and 10 weeks respectively, was much more substantial. Although the trials with commercial ryegrass and weedy ryegrass were conducted in the cool season and warm season respectively, the temperature in the greenhouse remained constant throughout the experiments. However, probably due to longer daylengths, the weedy ryegrass produced much more dry material than the commercial ryegrass.

3.4.2 Temperature study

Trial 1: Continuous temperature regimes (10/15 and 15/20 ^{0}C)

Glufosinate ammonium efficacy was tested on ryegrass in the glasshouse under 10/15 ^{0}C and 15/20 ^{0}C temperature regimes respectively. Cool temperatures affected the response of glufosinate ammonium on ryegrass. The results obtained from this study demonstrated that ryegrass plants grown under 10/15 ^{0}C responded effectively (100%) to varying concentrations of glufosinate ammonium as compared to those that grew in a 15/20 ^{0}C temperature regime (Fig 3.3).

Previous studies have indicated that although glufosinate ammonium is considered to be non-selective, environmental conditions and other factors such as weed species and herbicide rate influences its efficacy (Mersey et al. 1990; Anderson et al. 1993; Steckel et al. 1997; Coetzer 2001). Kumaratilake and Preston (2005) reported poor control of *Raphanus raphanistrum* L under 5/10 ^{0}C temperature because of the reduced accumulation of glufosinate ammonium in the meristem regions of the plant. Their findings are contrary to the results found in this study with ryegrass.

Temperature not only affects herbicide efficacy on the plant but it also affect plant metabolism. Temperature influences herbicidal activity due to its direct association with the chemical reaction rate. Therefore, photosynthesis, plant growth and plant development are dependent on temperature. This was evident on plants that were grown under 10/15 ^{0}C which were smaller in size than those grown under 15/20 ^{0}C temperatures. Generally, plant species has optimum temperatures for tissue development. For example, plants of winter species *Brassica oleracea* were less sensitive to oxyfluorfen at a temperature of 20/25^{0}C, compared to temperatures of 10 to 15^{0}C (Harrison and Peterson, 1999).

There were no significant differences between dosage rates in terms of percentage dry mass reduction (Table 3.9). This is an indication that even under warm conditions, where the plants were not satisfactorily controlled by the glufosinate ammonium dosage rates used, it was more or less equally stunted by the different dosage rates. The great difference in terms of percentage dry mass reduction between the cool and warm temperatures is probably because of the faster growth rate of the control plants during the four weeks after spraying until the plants were harvested.

Trial 2: Varying temperature regimes (10/15 and 20/25 ^{0}C)

The second trial was conducted under 10/15 ^{0}C and 20/25 ^{0}C temperature regimes. Similar responses as in the previous trial were also observed in this trial only at the 2.5 L ha^{-1} dosage rate. . It is evident that plants under 10/15^{0}C were controlled more effectively than plants under 20/25^{0}C at the lowest herbicide rate of 2.5 L ha^{-1} (Figure 3.4). The glufosinate ammonium efficacy was reduced on plants that were grown under 10/15 ^{0}C temperature conditions and subsequently transferred to 20/25 ^{0}C temperature conditions after spraying as compared to plants that were kept under 10/15 ^{0}C temperatures throughout the experiment. This suggests that the temperature following glufosinate ammonium application was crucial in obtaining better herbicide efficacy. Similarly, plants that were grown under 20/25 ^{0}C temperature conditions and transferred to 10/15 ^{0}C after spraying were better controlled at 2.5 L ha^{-1} than plants which remained at 20/25 ^{0}C, again indicating that cooler temperatures after spraying enhanced efficacy of glufosinate ammonium. Coetzer et al. (2001) reported that at 26/21 ^{0}C, glufosinate ammonium controlled redroot pigweed less effectively than Palmer amaranth and common waterhemp. Similarly, Kumaratilake et al. (2002b) also reported more effective control of *Sisymbrium orientale L* than *Raphanus raphanistrum* L with glufosinate ammonium under cool temperatures of 10/15 ^{0}C. The control was species dependent, because the same glufosinate ammonium concentration gave 100% control on *Raphanus raphanistrum* L under warm temperatures of 20/25 ^{0}C (Kumaratilake and Preston 2005). The findings of this study revealed that low temperatures have influenced the efficacy of glufosinate ammonium on the control of ryegrass.

3.5 Conclusions

This study shows that application of glufosinate ammonium rates as low as 2.5 L ha^{-1} controlled commercial and weedy ryegrass effectively irrespective of plant age. Therefore, a dosage rate that controls small ryegrass seedlings should also control ten week old ryegrass plants under a given set of climatic conditions similar to those in the glasshouse where the trial was carried out.

Application of 2.5 L ha^{-1} of glufosinate ammonium caused 100% mortality to ryegrass plants grown under cool temperatures but not under warm temperatures. It therefore appears as if glufosinate ammonium would be more efficient to control ryegrass when applied in cooler seasons (e.g. late autumn, winter and early spring) than in warmer seasons. It is however imperative to further investigate the full range of temperatures from 5 to 30 ºC to determine what the real optimal temperature for the best efficacy of glufosinate ammonium is for ryegrass.

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CHAPTER 4

EFFECT OF AMMONIUM SULPHATE (AMS) ON THE EFFICACY OF GLUFOSINATE AMMONIUM

Abstract

Ammonium sulphate is used as adjuvant in improving herbicide efficacy. Glasshouse and field experiments were conducted to investigate the effect of AMS on the efficacy of glufosinate ammonium. Glufosinate ammonium was applied in mixtures with AMS and without AMS. The glufosinate ammonium rates used in the glasshouse and in the field were 0, 2.5, 5, 7.5, and 10 L ha^{-1}. Four ryegrass populations (1 Commercial ryegrass and 3 suspected resistant populations) were used in the glasshouse study. Glufosinate ammonium gave more effective control on the commercial population and resistant population 2 when it was mixed with AMS than when it was applied alone. Therefore, addition of AMS to glufosinate ammonium increased the efficacy on the control of commercial and resistant population 2 but not on the suspected resistant population 1 and 3. Five glufosinate ammonium rates were applied with AMS and without AMS on a broad spectrum of weeds in the field. Although AMS improved glufosinate ammonium efficacy on commercial and resistant population 2 in the glasshouse, it did not give the same results in the field. Addition of AMS improved efficay of glufosinate ammonium in the field in 2013 but not in 2014. It therefore appears as if AMS will only improve the efficacy of glufosinate ammonium under particular conditions.

Keywords: adjuvant, ammonium efficacy, fertilizer, ryegrass, sulphate

4.1 Introduction

In view of the techniques and methods of weed management around the world, chemical control is largely adopted (Han and Wang 2002; Zhang 2003). Therefore, ways to increase herbicide activity, as well as to achieve effective control with the herbicide at low rates, are very important. Glufosinate ammonium is a non-selective post emergence herbicide used for weed control since the mid 1980’s in over 50 different countries (Maschhoff et al. 2000; Coetzer et al. 2001; Kumaratilake et al. 2002; Kumaratilake and Preston 2005).

Addition of adjuvants increases herbicidal activity (Nandula et al. 2007; McCullough and Hart 2008; Nurse et al. 2008). Maschhoff et al. (2000) reported that adding 20 g L^{-1} of ammonium sulphate (AMS) increased the efficacy of glufosinate ammonium on barnyard grass (*Echinochloa crus-galli),* giant foxtail *(Setaria faberi)* and velvetleaf *(Abutilon theophrasti)*. Ammonium sulphate is a fertiliser commonly used as an adjuvant in crop protection. An adjuvant is any substance that modifies the herbicidal activity or spray characteristics when added to a spray tank (Zawierucha and Penner 2001; Ramsey et al. 2005; Pacanoski 2010). Addition of AMS to spray tanks is known to overcome interactions of salts present in water (Pratt et al. 2003)**.** Therefore, the quality of water added to spray tanks greatly impacts herbicide effectiveness.

Apart from the ability to overcome large amounts of antagonistic salts, AMS is also known to improve the activity of weak herbicides (herbicides which split into two negative ions when mixed in water) such as glyphosate and glufosinate ammonium (Pratt et al. 2003; Singh et al. 2011). The addition of AMS can increase the herbicide uptake by preventing the formation of cation salts with weak-acid herbicides (Wanamarta and Penner 1989; Nalewaja and Matysiak 1992).

Several researchers have hypothesised that AMS promotes foliar penetration of glyphosate by reducing the rate of herbicide crystallisation on the leaf surface (Pline et al. 1999, Singh and Sharma 2001; Pratt et al. 2003; Singh et al. 2011). Addition of AMS enhanced the activity of glufosinate ammonium and glyphosate on common milkweed (*Asclepias sinaca*) and horsenettle (*Solanum carolinense*) (Pline et al 2000).

Several hypotheses regarding the effect of AMS on glyphosate and glufosinate ammonium has been proposed by scientists but most evidence point to increased herbicide absorption and translocation within the whole plant (Bradley et al. 2000; Pline et al. 2000; Pratt et al. 2003; Nalewaja et al. 2007; Singh et al. 2011; Soltani et al. 2011). For example, AMS increased absorption of glyphosate in velvetleaf (Young et al. 2003). Similarly, AMS increase glufosinate ammonium efficacy on velvetleaf and giant foxtail by increasing foliar absorption and translocation (Maschhoff et al. 2000). However, there is limited information on the effect of AMS on glufosinate ammonium efficacy. The objective of the study was to determine if AMS could increase the efficacy of glufosinate ammonium on ryegrass (*Lolium spp*.) populations as well as mixed weed populations.

4.2 Materials and methods

4.2.1 Glasshouse study

A glasshouse trial was conducted at Stellenbosch University experimental farm Welgevallen (33°56'S, 18°42'E) to investigate the effect when adding AMS to improve efficacy of glufosinate ammonium on ryegrass. Ten ryegrass seeds were sown in 8 x 8 cm pots and thinned to four plants per pot 7 days after planting (DAP). Pure river sand was used as a growth medium (See Table 4.1 for soil physical and chemical properties). Ryegrass seedlings were watered with a nutrient solution (See Table 3.1 in Chapter 3 for composition of the nutrient solution). Four ryegrass populations (one commercial cultivar (*Lolium multiflorum* cv Agri Hilton) and three suspected resistant weedy types (*Lolium* spp.) were used in the experiment and the temperature of the glasshouse was set to run at 20/25^0 C night/day. Seeds of the suspected resistant populations were collected from the following localities: Moorreesburg (33^{0}09'S 18^{0}40'E) district, Eendekuil (32^{0}41'S 18^{0}53'E) district and Hopefield (33^{0}03'56'S 18^{0}21'03'E) district.

The four ryegrass populations were each subjected to a 7 x 2 factorial trial with seven dosage rates (0, 0.75, 1.5, 3, 4.5, 6 and 7.5 L ha^{-1} of glufosinate ammonium) and two AMS treatments (with and without AMS) laid out in a randomised complete block design with 3 replicates. The data of the four ryegrass populations were analysed separately.

Herbicide treatments were applied 4 weeks after planting by means of a pneumatic pot spray apparatus operating at a pressure of two bars and delivering 400 L of water ha^{-1}. The different glufosinate ammonium concentrations were mixed with 10 g of AMS in 1 L of distilled water. Plants were left outside the spraying room for 30 minutes after spraying to allow the herbicide to dry on the leaves before the sprayed pots were returned to the glasshouse and watered daily by hand to avoid moisture stress.

Efficacy of glufosinate ammonium with and without AMS was evaluated 6 weeks after treatment (WAT) by visual assessment of percent mortality as well as dry matter production. Visual estimates of the percentage control were based on the number of senescent plants. Dry mass production was measured 6 WAT by removing the above ground parts of the plants and drying it in an oven at a constant temperature of 80 ^{0}C for two days before mass was determined. Dry mass production was expressed as dry mass pot^{-1}.

Data was subjected to analysis of variance (ANOVA) using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Fischer's $LSD_{0.05.}$

Table 4.1: Physical and chemical properties of pure river sand used in glasshouse studies

	Physical and chemical properties	
pH	5.3	
Resistance	6760	ohms
Texture	Sand	
Acidity	0.21	cmol(+) kg^{-1}
Calcium	0.41	mg kg^{-1}
Magnesium	0.09	mg kg^{-1}
Potassium	11	mg kg^{-1}
Sodium	8	mg kg^{-1}
P (citric acid)	22	mg kg^{-1}
Total cations	0.77	cmol kg^{-1}
Copper	0.10	mg kg^{-1}
Zinc	0.18	mg kg^{-1}
Manganese	5.69	mg kg^{-1}
Boron	0.02	mg kg^{-1}
Carbon	0.03	%
Sulphur	3.30	mg kg^{-1}
Iron	7.34	mg kg^{-1}

4.2.2 Field Study

AMS trial 1 (2013)

Field studies were conducted at Welgevallen experimental farm from June until August to determine the effect of AMS on glufosinate ammonium efficacy. The trials consisted of a 2 x 5 factorial in a randomised complete block design with four replicates. The treatment factors were two treatments (glufosinate ammonium only and glufosinate ammonium plus AMS) and five glufosinate ammonium rates (0, 2.5, 5, 7.5, and 10 L ha^{-1} of glufosinate ammonium). The glufosinate ammonium was added to a 1% AMS solution. Treatments were applied with a knapsack sprayer delivering 200 L of water ha^{-1}. Plot sizes were 1.5 m x 5 m.

The efficacy of the treatments was evaluated by visually assessing the percentage control (mortality) of the whole spectrum of weeds 6 weeks after treatment (WAT). Percentage mortality was measured on a scale of 0 to 100%, where 0 = no visible control and 100 = total plant mortality. Data was subjected to analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Fischer's $LSD_{0.05.}$

AMS trial 2 (2014)

The same set of procedures were followed as the above trial, except that dry weight determinations were carried out at 6 WAT by randomly throwing two squares (30 x 30 cm) into each plot and uprooting the weeds within the square. The plant roots were separated from the aboveground parts and only the latter was used for the evaluation. The same methodology as in AMS trial 1 was employed.

4.3 Results

4.3.1 Glasshouse trial

Commercial ryegrass population

There is a significant two way interaction ($P > 0.0021$) between the main effects AMS treatment and dosage rate on ryegrass control (Table 4.2). The interaction is illustrated in Figure 4.1. At the dosage rate of 4.5 L ha^{-1} AMS significantly increased the control of ryegrass by glufosinate ammonium to 100%. It is evident that there are no differences between the efficacy of only glufosinate ammonium treatments and the treatments with glufosinate ammonium plus AMS at the other dosage rates (Figure 4.1).

The variable, dry mass, of the commercial population did not show any significant interaction (Table 4.3). However, the rates applied differed from each other at P = 0.0000. All glufosinate ammonium dosage rates significantly decreased dry matter production. Dry mass production at low rates of 0.75 and 1.5 L ha^{-1} was significantly higher when compared to the high dosage rates of 3, 4.5, 6 and 7, 5 L ha^{-1} (Table 4.4). However, the 0.75 and 1.5 L ha^{-1} dosage rates resulted in zero mortality (Figure

4.1) Addition of AMS did not provide any advantage as far as dry mass reduction of the commercial ryegrass population is concerned.

Table 4.2: Effect of AMS treatment (with or without ammonium sulphate) and glufosinate ammonium dosage rate on the percentage control of a commercial ryegrass (*Lolium multiflorum* cv Agri Hilton) population 6 weeks after treatment

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	238.10	1	238.10	1.7778	0.193169
Rates	68839.29	6	11473.21	85.6667	0.000000
Treatment*Rates	3720.24	6	620.04	4.6296	0.002195
Error	3750.00	28	133.93		

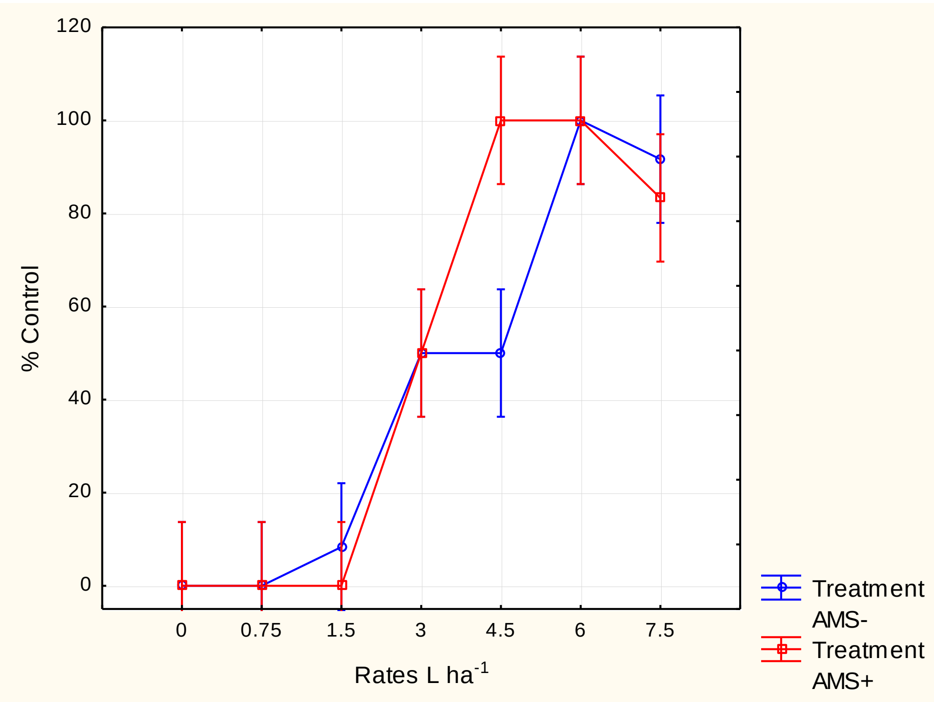

Figure 4.1: Interaction of AMS treatments and glufosinate ammonium dosage rates on the percentage control of a commercial ryegrass population (*Lolium multiflorum* (cv Agri Hilton) 6 weeks after treatment. Vertical bars indicates least significant different means

Table 4.3: Effect of AMS treatment (with or without ammonium sulphate) and glufosinate ammonium dosage rate on the dry mass production of a commercial ryegrass (*Lolium multiflorum* cv Agri Hilton) population 6 weeks after treatment

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	0.026752	1	0.026752	3.7781	0.062041
Rates	0.182714	6	0.030452	4.3006	0.003405
Treatment*Rates	0.048714	6	0.008119	1.1466	0.362131
Error	0.198267	28	0.007081		

Table 4.4: Effect of glufosinate ammonium dosage rates on dry mass reduction of a commercial ryegrass (*Lolium multiflorum* cv Agri Hilton) population 6 weeks after treatment

Rates L ha^{-1}	Dry mass g pot^{-1}
0	0.33a
0.75	0.26a
1.5	0.26a
3	0.17b
4.5	0.11b
6	0.15b
7.5	0.15b

*Values followed by the same letter does not differ significantly at *P* = 0.05

Moorreesburg (Suspected resistant population 1)

There was no significant interaction between AMS treatments and glufosinate ammonium dosage rate for the Moorreesburg population (Tables 4.5 and 4.6) on percent control and dry mass. A significant (P=0.000) effect was observed on herbicide rates applied (Tables 4.5 and 4.6) on the percentage control and dry mass production of the Moorreesburg population. As far as the treatments were concerned, there were no significant differences in percentage control between glufosinate ammonium without AMS and glufosinate ammonium with AMS applied to this population (data not shown). Percentage control increased with an increase in herbicide concentration (Table 4.7). Dry mass production of dosage rates applied did not differ from each other (Table 4.7).

Table 4.5: Effect of AMS treatment (with or without ammonium sulphate) and glufosinate ammonium dosage rate on the percentage control of a weedy ryegrass (*Lolium* spp.) population from Moorreesburg 6 weeks after treatment

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	1205.36	1	1205.36	1.6531	0.209075
Rates	54761.90	6	9126.98	12.5170	0.000001
Treatment*Rates	1190.48	6	198.41	0.2721	0.945344
Error	20416.67	28	729.17		

Table 4.6: Effect of AMS treatment (with or without ammonium sulphate) and glufosinate ammonium dosage rate on the dry mass production of a weedy ryegrass (*Lolium* spp.) population from Moorreesburg 6 weeks after treatment

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	0.002002	1	0.002002	1.0120	0.323031
Rates	0.115048	6	0.019175	9.6911	0.000009
Treatment*Rates	0.011248	6	0.001875	0.9475	0.477685
Error	0.055400	28	0.001979		

Table 4.7: Effect of glufosinate ammonium dosage rates on the percentage control and dry mass reduction of a weedy ryegrass (*Lolium* spp.) population from Moorreesburg 6 weeks after treatment

Dosage rates L ha^{-1}	Control (%)	Drymass (g pot^{-1})
0	0a*	0·23a
0.75	0a	0.13b
1.5	20b	0.17b
3	41bc	0.09b
4.5	62cd	0.06b
6	79cd	0.10b
7.5	100d	0.09b

*Values followed by the same letter in a column does not differ significantly at P = 0.05

Eendekuil (Suspected resistant population 2)

The significant (P = 0.038) interaction between AMS treatments and glufosinate ammonium dosage rates in terms of percentage control is shown in Table 4.8 and illustrated in Figure 4.2. Glufosinate ammonium plus AMS resulted in significantly better control (79%) of the Eendekuil population at a dosage rate of 3 L ha^{-1} compared to only glufosinate ammonium applied (18%) (Figure 4.2). However, at no other dosage rate did addition of AMS significantly improve control of the population. Addition of AMS to glufosinate ammonium had no significant effect on the dry mass reduction of the Eendekuil population (Table 4.9). More dry mass was produced at low dosage rates of 0.75, 1.5, 3 and 4.5 L ha^{-1} and vice versa for high dosage rates of 6 and 7.5 L ha^{-1} (Table 4.10).

Table 4.8: Effect of treatment (with or without ammonium sulphate) and glufosinate ammonium dosage rate on the percentage control of a weedy ryegrass (*Lolium* spp.) population from Eendekuil, 6 weeks after treatment

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	952.38	1	952.38	3.2000	0.084463
Rates	55803.57	6	9300.60	31.2500	0.000000
Treatment*Rates	4672.62	6	778.77	2.6167	0.038489
Error	8333.33	28	297.62		

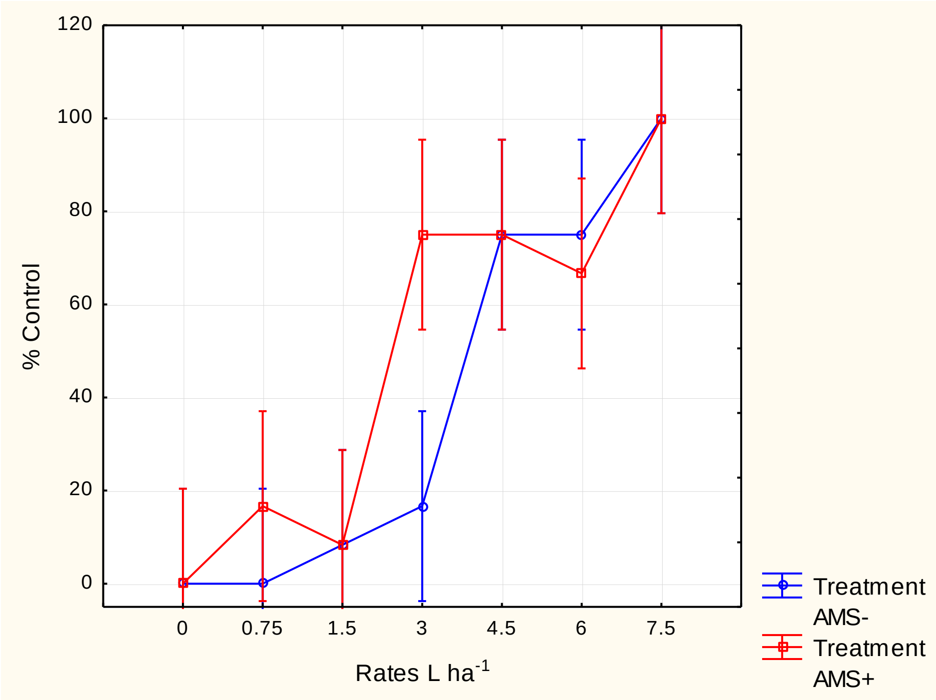

Figure 4.2: Interaction between glufosinate ammonium and dosage rates on the percentage control of a weedy ryegrass population (*Lolium* spp.) from Eendekuil, 6 weeks after spraying. Vertical bars indicates least significant different means

Table 4.9: Effect of AMS treatment (with or without ammonium sulphate) and glufosinate ammonium dosage rate on the dry mass production of a weedy ryegrass (*Lolium* spp.) population from Eendekuil, 6 weeks after treatment

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	0.003621	1	0.003621	0.5259	0.474339
Rates	0.177362	6	0.029560	4.2930	0.003441
Treatment*Rates	0.043029	6	0.007171	1.0415	0.420080
Error	0.192800	28	0.006886		

Table 4.10: The effect of glufosinate ammonium dosage rate on dry mass production of a weedy ryegrass (*Lolium* spp.) population from Eendekuil 6 weeks after treatment

Dosage rates L ha^{-1}	Dry mass(g pot^{-1})
0	0.28a*
0.75	0.20ab
1.5	0.16b
3	0.14b
4.5	0.12b
6	0.09c
7.5	0.07c

*Values followed by the same letter does not differ significantly at P = 0.05

Hopefield (Suspected resistant population 3)

Ammonium sulphate treatment and glufosinate ammonium dosage rates showed no significant interaction on percentage control and dry mass, of the Hopefield population, respectively (Tables 4.11 and 4.12). However there were significant differences (P = 0.000) on the effect of dosage rates on percentage control of the population (Table 4.11). Furthermore, treatments and dosage rates caused statistically significant differences in terms of dry mass production of the Hopefield population (Table 4.12). There were no significant differences between dosage rates of 4.5, 6 and 7.5 L ha^{-1} of glufosinate ammonium in terms of percentage control (Table 4.13). Even the highest rate of 7.5 L ha^{-1} did not result in 100% control, indicating possible resistance to glufosinate ammonium in this population of ryegrass (Table 4.13). Dry mass production after application of 6 and 7.5 L ha^{-1} of glufosinate ammonium was however significantly less than at the lower dosage rates (Table 4.13). Addition of AMS, reduced dry mass production of this population of ryegrass, significantly (Table 4.14).

Table 4.11: The effect of AMS treatment (with or without ammonium sulphate) and glufosinate ammonium dosage rate on the percentage control of a weedy ryegrass (*Lolium* spp.) population from Hopefield 6 weeks after treatment

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	372.02	1	372.02	1.0000	0.325875
Rates	57380.95	6	9563.49	25.7067	0.000000
Treatment*Rates	2440.48	6	406.75	1.0933	0.390646
Error	10416.67	28	372.02		

Table 4.12: Effect of AMS treatment (with or without ammonium sulphate) and glufosinate ammonium dosage rate on the dry mass production of a weedy ryegrass (*Lolium* spp.) population from Hopefield 6 weeks after treatment

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	0.616860	1	0.616860	23.8961	0.000038
Rates	2.317681	6	0.386280	14.9638	0.000000
Treatment*Rates	0.326157	6	0.054360	2.1058	0.084354
Error	0.722800	28	0.025814		

Table 4.13: Effect of glufosinate ammonium dosage rates on the percentage control and dry mass production of a weedy ryegrass (*Lolium* spp.) population from Hopefield 6 weeks after treatment

Dosage rates L ha^{-1}	Control (%)	Dry mass (g pot^{-1})
0	0a*	0.84a
0.75	0a	0.42b
1.5	4a	0.37b
3	50b	0.32b
4.5	75bc	0.21b
6	79bc	0.11c
7.5	87c	0.10c

*Values followed by the same letter in a column does not differ significantly at P = 0.05

Table 4.14: Effect of AMS treatments (AMS+ = with ammonium sulphate and AMS- = without ammonium sulphate)) on dry mass production of a weedy ryegrass (*Lolium* spp.) population from Hopefield

Treatment	Drymass (g pot^{-1}) mean
AMS+	0.22a*
AMS-	0.46b

*Values followed by the same letter does not differ significantly at *P* = 0.05

4.3.2 Field trial

Field trial 1 – 2013

No significant interaction between AMS treatment and glufosinate ammonium dosage rate (Table 4.15) was detected on the percentage control of the weed spectrum in the field. Nevertheless, main effects AMS treatment and dosage rates had significant effects (P = 0.001 and 0.000) on the percentage control of weeds in the field. Addition of AMS to glufosinate ammonium increased weed control (Table 4.16) in the field significantly from 30% to 46%, over all glufosinate ammonium dosage rates. The dosage rates 2.5 and 5 L ha^{-1} gave the same control ratings of 35 and 36%, but differed from 7.5 (53%) and 10 L ha^{-1} (Table 4.16). The percentage control at 10 L ha-1 (66%) however, was not acceptable.

Table 4.15: Effect of glufosinate ammonium dosage rate and ammonium sulphate treatment on the percentage control of weeds in the field 6 weeks after treatment in 2013

Effect	SS	Degr. of Freedom	MS	F	p
Treatments	2560.00	1	2560.00	12.6160	0.001286
Rates	19846.25	4	4961.56	24.4512	0.000000
Treatments*Rates	833.75	4	208.44	1.0272	0.409307
Error	6087.50	30	202.92		

Table 4.16: Effect of AMS treatments (AMS- = without ammonium sulphate and AMS+ = with ammonium sulphate) and glufosinate ammonium dosage rates on the percentage control of weeds in the field 6 weeks after treatment in 2013

Treatments	% Control
AMS-	30.25a
AMS+	46.25b
Rates L ha^{-1}	
0	0a
2.5	35b
5	36b
7.5	53c
10	66c

*Values followed by the same letter does not differ significantly at $P = 0.05$

Field trial 2 - 2014

Results in Tables 4.17 and 4.18 suggest that there was no interaction between the main factors (AMS treatments and glufosinate ammonium dosage rates) in terms of the response variables, percentage control and dry mass. Addition of AMS to glufosinate ammonium did not show any significant effect on the percentage control and dry mass production of weeds in the field (Tables 4.17 and 4.18). However, a significant effect of dosage rates was detected on the percentage control and dry mass (Tables 4.17 and 4.18). The percentage control of different rates applied, was not significantly different from each other. The dry mass production for rate 5, 7.5 and 10 L ha^{-1} did not differ from each other but differed from the low rate of 2.5 L ha^{-1} (Table 4.18).

Table 4.17: Effect of glufosinate ammonium dosage rates and ammonium sulphate treatments on the percentage control of weeds in the field 6 weeks after treatment in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatments	950.6	1	950.6	2.2600	0.143207
Rates	37983.8	4	9495.9	22.5758	0.000000
Treatments*Rates	1171.2	4	292.8	0.6961	0.600586
Error	12618.7	30	420.6		

Table 4.18: Effect of glufosinate ammonium dosage rates and ammonium sulphate treatments on the dry mass of weeds collected in the field 6 weeks after treatment in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatments	12.488	1	12.488	0.3009	0.587401
Rates	4434.473	4	1108.618	26.7089	0.000000
Treatments*Rates	62.317	4	15.579	0.3753	0.824367
Error	1245.221	30	41.507		

Table 4.19: Effect of glufosinate ammonium dosage rates on the percentage control and dry mass of weeds in the field 6 weeks after treatment in 2014

Rates L ha^{-1}	% Control	Dry mass (g m^{-2})
0	0a	344a
2.5	88b	30b
5	72b	82bc
7.5	70b	72bc
10	69b	111c

4.4 Discussion

4.4.1 Glasshouse trial

Ryegrass populations varied in their response to glufosinate ammonium only and glufosinate ammonium plus AMS. The addition of AMS had no significant effect on the percentage control of the Moorreesburg and Hopefield populations. On the contrary, addition of AMS to glufosinate ammonium at 3 L ha^{-1} on the Eendekuil and at 4.5 L ha^{-1} on the commercial population increased the control from 20% to 79% and from 45% to 100% respectively. This is to an extent similar to findings by Pline et al. (2000) who reported that AMS increased the efficacy of glyphosate in common lambsquarters and horsenettle at the 0.125 and 0.25 kg ha^{-1} glyphosate rates, common milkweed at the 0.125 kg ha^{-1} rate and giant foxtail at all rates tested. In contrast, Young et al. (2003) reported that addition of AMS did not increase the foliar absorption of glyphosate in common lambsquarters but it increased absorption in velvet leaf.

Application of AMS with the herbicide increased the herbicidal efficacy as shown in previous studies. Glyphosate at 0.43 kg ae ha^{-1} plus AMS provided greater control than glyphosate at 0.43 kg ae ha^{-1} without ammonium sulphate (Bradley et al 2000). This is in agreement with the findings of research studies conducted by Mersey et al. (1990); Steckel et al. (1997) and Maschhoff et al. (2000) on the effective control of barnyard grass*,* giant foxtail and velvetleaf*,* when AMS was added to glufosinate*.*

The efficacy of glufosinate ammonium differed slightly on the commercial ryegrass population and the Moorreesburg, Eendekuil and Hopefield populations. The registered glufosinate ammonium dosage rate (7.5 L ha^{-1}) gave 100% control of the Moorreesburg and Eendekuil ryegrass populations. The Hopefield population was poorly controlled at 5 and 7.5 L ha^{-1} glufosinate ammonium dosage rates. It is evident that the Moorreesburg and Eendekuil populations were more sensitive to glufosinate ammonium than the Hopefield population. Similarly, commercial ryegrass at 7.5 L ha^{-1} did not give 100% control, whether AMS was added or not. This could be an indication that some resistance to glufosinate ammonium is present in the commercial population because the plants from the different populations were growing under the same conditions, were sprayed on the same day and were of equal age. Ryegrass is notorious for genetic variability (Eksteen et al 2005; Ferreira 2011) and it is possible that mutations that render the plant resistant to glufosinate ammonium could have occurred in the commercial ryegrass population.

The results of the study partly confirm the findings of several researchers on the use of AMS as an adjuvant to influence the efficacy of herbicides which possesses a negative charged ion like glyphosate and glufosinate ammonium (Franz et al.1998; Bradley et al. 2000; Pratt et al. 2003; Young et al. 2003; Nurse et al. 2008).

4.4.2 Field trials

Field trial 1 – 2013

Field results did not show a significant interaction between treatment and rates on weed spectrum under field conditions. However, application of AMS with glufosinate ammonium in the field provided a mean of 46% control, whereas AMS without glufosinate ammonium gave only 30% control. Control of weeds was however not satisfactory (66%) even at the highest dosage rate of glufosinate ammonium. This could possibly be due to unfavourable climatic conditions (temperature and/or moisture stress) because the weeds were relatively small (varying from about three to eight leaf stages).

Previous studies showed the influence of AMS on glufosinate efficacy on the control of different weed species (Pline et al.1999). Other researchers have shown that the addition of AMS increases herbicide efficacy and absorption (Hart and Wax 1996; Jordan et al. 1996). Mascchoff et al. 2000 reported that addition of AMS increased the absorption of glufosinate ammonium in barnyardgrass control. Ramsdale et al. (2003) noted that glyphosate provided better control of hard red spring wheat and oats when AMS was in the spray mixture.

Field trial 2 - 2014

The results showed that AMS with and without glufosinate ammonium controlled weeds in the field equally well because there was no significant differences between glufosinate ammonium efficacy with or without AMS. In contrast, Soltani et al (2011) reported that glufosinate ammonium with AMS controlled velvetleaf, redroot pigweed (*Amaranthus retroflexus*) and common lambsquarter (*Chenopodium album*) better than glufosinate ammonium without AMS, but did not observe the same trend on greenfoxtail (*Setaria viridis*) and barnyardgrass. Absorption was found to be the reason behind the observed poor control of glufosinate ammonium with AMS on greenfoxtail and barnyardgrass. The effect of applying AMS in mixture with other herbicides therefore appeared to be species dependent.

Application of AMS enhanced the control of johnsongrass (*Sorghum halapense*) by glyphosate at 0.42 kg ha^{-1} (Salisbury et al.1991). Jordan et al (1989) observed a 6-fold increase in ^{14}C absorption when AMS was added to sethoxydim. According to the results of this study, application of glufosinate ammonium with and without AMS did not differ from each other in terms of the percentage of weeds controlled. Overall, none of the dosage rates applied with or without AMS gave 100% control. It therefore does not appear as if addition of AMS increased efficacy of glufosinate ammonium in this trial.

4.5 Conclusions

The results of these glasshouse studies demonstrated that AMS increased the efficacy of glufosinate ammonium on some, but not all ryegrass populations only at certain critical dosage rates. Under field conditions, glufosinate ammonium performed poorly in both years and the addition of AMS did not improve the efficacy of the herbicide. More work on this aspect should be carried out and environmental conditions should also be taken into consideration.

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CHAPTER 5

DOES PROPYZAMIDE INFLUENCE THE EFFICACY OF FOUR COMMONLY USED HERBICIDES IN THE WINTER RAINFALL REGION?

Abstract

A combination of two or more herbicides provides more consistent control of certain weeds, reduce the risk of herbicide resistance in weeds, and reduce costs while reducing the total amount of active ingredient applied. The aim of this study was to determine the influence of propyzamide on the efficacy of four commonly used herbicides in the Western Cape. The herbicides used were: atrazine, glufosinate ammonium, glyphosate and imazamox in a glasshouse as well as in field studies. The first glasshouse study was carried out on a weedy ryegrass population and each of the selected herbicides mentioned above was applied to the ryegrass as standalone or in a mixture with propyzamide at the recommended rates. Visual percentage control and dry mass production were evaluated. Addition of propyzamide increased control with imazamox because the ryegrass was resistant to imazamox. The propyzamide however decreased efficacy of atrazine. In the second glasshouse study the four herbicides were again mixed with propyzamide and applied as standalone or in mixture at dosage rates of 0, 0.5, 0.75 and 1 times the recommended rates on a commercial ryegrass cultivar. Percentage control and dry mass production were again evaluated and interactions were tested to determine if antagonism or synergism occurred. Propyzamide had antagonistic effects on atrazine and glufosinate ammonium but antagonism to glyphosate and imazamox only occurred at the lowest dosage rate of 0.5 times the recommended rate. Field studies were carried out at three localities (Welgevallen, Roodebloem and Langgewens experimental farms). The four herbicides mentioned above were applied to weeds in the field either as standalone or in a mixture with propyzamide. In contrast to the glasshouse studies, propyzamide increased efficacy of atrazine significantly in some of the field trials. There were also significant increases in the efficacy of glyphosate, glufosinate ammonium and imazamox in some of the trials. In some cases, propyzamide decreased efficacy of glyphosate and glufosinate ammonium significantly. Overall, it does not appear to be feasible to add

propyzamide to the four herbicides because of inconsistent results and the high economical cost of propyzamide.

Keywords: antagonism, interaction, propyzamide, resistance, synergism

5.1 Introduction

Propyzamide is a systemic herbicide used primarily for controlling annual and perennial grasses and some broad-leaved weeds (BCPC 2000; Mann et al. 2012). Propyzamide decomposes slowly below 15 ^{0}C. Persistence is greatest in sandy soils with low organic matter. Propyzamide inhibits photosynthesis and cell division. It is absorbed by the roots and is translocated in the plant. A small amount of the active ingredient is absorbed by the foliage. The resultant effect appears on the plants as distorted growth of stems and yellowing of the leaves. Propyzamide is the common name for 3, 5-dichloro N-(l,1-dimethyl-2-propynyl)-benzamide. Propyzamide is most effective when applied in cool and wet soils (BCPC 2000).

The herbicides atrazine and imazamox used to be effective in controlling weeds in canola in the Western Cape until ryegrass and wild radish developed resistance to these two herbicides. The problems associated with these weeds, particularly wild radish that are closely related to canola, necessitated the development of herbicide resistant canola cultivars. Harker et al. (2000) compared weed control in herbicide resistant canola cultivars with traditional herbicide regimes. They found that in most cases, the herbicide resistant systems (glyphosate, imazethapyr/imazamox and glufosinate ammonium) provided better yields than the traditional sethoxydim plus ethametsulfuron treatments. Harker et al. (2000) and O’Donovan et al. (2006) reported that application of glyphosate twice (at the two to four and five to six leave stages of canola) in glyphosate resistant canola is not cost effective.

Application of two or more herbicides simultaneously, either using pre-packaged mixtures or in a tank mix, is a very common approach in intensive agriculture (Blouin et al. 2004; Damalas 2004; Damalas and Eleftohorinhos 2001). This is because the application of a single herbicide, even though it may provide good control of certain weeds, is often inadequate for satisfactory and cost effective weed control. Mixtures of two or more herbicides may provide more consistent control of certain weeds, reduce the risk of herbicide resistance in weeds, and reduce costs while reducing the total amount of active ingredient applied (Harker and O’Sullivan 1991; Zhang et al.

1995). The basic assumption of a herbicide combination is that each act independently when applied in a mixture (Zhang et al. 1995). However, control from a combination of two herbicides may be greater than (synergistic), less than (antagonistic), or equal (additive) to the summed effect of the herbicides applied alone (Colby 1967; Green 1989; Hatzios and Penner 1985).

Generally, a successful weed management program depends on an understanding of how herbicides react when mixed with each other. The objective of this study was to determine the effect of adding propyzamide to the efficacy of herbicides currently used for weed control in canola in the Western Cape.

5.2 Materials and methods

5.2.1 Glasshouse study

Trial 1: Weedy ryegrass population (Lolium spp)

A glasshouse experiment was conducted in 2013 at Welgevallen experimental farm located in Stellenbosch. Ten ryegrass seeds from a weedy population were sown directly into 8 x 8 cm pots and thinned to four plants per pot, seven days after planting (DAP). The soil used was collected from a field on the farm (Welgevallen). Chemical and physical composition of the soil is given in Table 4.1. The plants were watered with a nutrient solution described in Chapter 3. Four herbicides were applied at the recommended rate and also in a mixture with propyzamide at the recommended rate for propyzamide. The herbicides applied were atrazine, glyphosate (360 g L^{-1}), glufosinate ammonium, imazamox and propyzamide. The application rates for the herbicides were 3 L ha^{-1}, 1.5 L ha^{-1}, 7.5 L ha^{-1}, 1.2 L ha^{-1} and 1.5 kg ha^{-1}, respectively. Herbicide treatments were applied 4 weeks after planting (WAP).

Herbicide treatments were applied by means of a pneumatic pot spraying apparatus operating at a pressure of 2 bars and delivering 200 L of water ha^{-1}. The experimental design was a randomised complete block design with four replicates. The treatments consisted of nine herbicide treatments (unsprayed, glyphosate, atrazine, imazamox, glufosinate ammonium, glyphosate/propyzamide, glufosinate ammonium/propyzamide, atrazine/propyzamide and imazamox/propyzamide).

Visual estimates of the percentage control were based on the percentage of senescent plants and were carried out at 6 weeks after treatment (WAT). Dry mass production was measured 6 WAT by removing the above ground parts of the plants and drying it in an oven at a constant temperature of 80 ^{0}C for two days before dry mass was determined. Dry mass production was expressed as the dry mass produced pot^{-1}.

Data was subjected to analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Fischer's $LSD_{0.05}$.

Trial 2: Commercial ryegrass (Lolium multiflorum cv. Agriton)

A follow-up trial was conducted in 2014 on commercial ryegrass. Commercial ryegrass was used because of ease of germination and the objective was to test for antagonism or synergism. In the case of weedy ryegrass, possible herbicide resistance could have interfered with the results. The experimental design was a completely randomized block design arranged factorially with 2 x 4 treatment factors replicated three times. Treatment factors were two herbicides, a herbicide applied as stand alone and also in mixture with propyzamide at four rates (0x, 0.5x, 0.75x and 1x where x is the recommended rate for each herbicide). The same herbicides used in Trial 1 (atrazine, glyphosate, glufosinate ammonium and imazamox) were used and the 1x rate for the herbicides were 3 L ha^{-1}, 1.5 L ha^{-1}, 7.5 L ha^{-1} and 1.2 L ha^{-1} respectively. The 1x rate for propyzamide was 1.5 kg ha^{-1}. The experiments with the four herbicides were analysed separately.

The application of the herbicides, evaluation of the results and analyses of the data was similar to the procedures described in Trial 1 above.

Herbicide combinations were determined to be antagonistic, synergistic, or additive by comparing the observed plant responses in terms of percentage control with the expected response when the herbicides are combined. Expected values were calculated using Colby's equation; $E = (X + Y) – (XY/100)$ (Colby 1967). In the equation, X and Y are the percent growth inhibition by herbicide A and B, respectively, and E is the expected percent growth inhibition by herbicides A and B combined. Combinations were determined as antagonistic, synergistic, or additive if the observed response was less than, greater than, or similar to the expected response, respectively. Colby's equation was used to determine the herbicide effects expected from the mixtures.

5.2.2 Field study

Field trial 1: Effect of different treatments on weed control in unplanted fields

Field trials were conducted on unplanted plots at Welgevallen (in Stellenbosch), Roodebloem (outside Caledon) and Langgewens (outside Moorreesburg) experimental farms in 2012 to determine the effect of propyzamide on efficacy of atrazine, glufosinate ammonium, glyphosate and imazamox under field conditions. The herbicide treatments were an unsprayed control, atrazine (3 L ha^{-1}), atrazine/propyzamide (3/1.5 L/Kg ha^{-1}), glufosinate ammonium (7.5 L ha^{-1}), glufosinate ammonium/propyzamide (7.5/1.5 L/Kg ha^{-1}), imazamox (1.2 L ha^{-1}), , imazamox/propyzamide (1.2/1.5 L/Kg ha^{-1}), glyphosate (360 g L^{-1}) (1.5 L ha^{-1}) and glyphosate/propyzamide (1.5/1.5 L/Kg ha^{-1}). The experimental design was a randomised complete block with nine treatments replicated four times. The plot area was 2 x 5 m.

Herbicides, except the atrazine treatments on Welgevallen, were applied when the weeds were well established. Atrazine and atrazine/propyzamide were sprayed 4 days after land cultivation at Welgevallen and the remaining treatments were sprayed 5 weeks after emergence (WAE) of weeds in the field. However, at Roodebloem and Langgewens all herbicide treatments were applied 30 days after land cultivation. All herbicide treatments at all localities were applied with a knapsack sprayer at a water delivery rate of 200 L ha^{-1}.

The efficacy of different treatments was evaluated six weeks after treatment by determining the percentage weed control per treatment compared to the unsprayed plot as well as the dry mass production in the treated plots. Percentage mortality was obtained through visual observation on a scale of 0 to 100%, where 0 = no visible injury and 100 = plant death (Burke et al. 2005). Dry mass determinations at all three localities was achieved by randomly throwing four squares (30 cm x 30 cm) into each plot,followed by cutting the weeds within the square at the soil surface. Afterwards, the weeds were dried in an oven for 48 hrs at a constant temperature of 80^0C to determine the dry mass.

Data was subjected to analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Fischer's $LSD_{0.05.}$

Field trial 2: Effect of different herbicide treatments on plots planted with GM canola cultivars

The same trial as above was repeated in 2013 and 2014 at Welgevallen, Roodebloem and Langgewens experimental farms. All trials except the imazamox treatments were carried out on plots planted with a triazine tolerant (TT) cultivar to determine the effect of the different treatments on weed population dynamics. The imazamox treatments were planted with a Clearfield (CL) cultivar.

The herbicide treatments were as follows: atrazine (3 L ha^{-1}), glyphosate (1.5 L ha^{-1}), imazamox (1.2 L ha^{-1} + 2% ammonium sulphate), glufosinate ammonium (7.5 L ha^{-1}), glyphosate/propyzamide mixture (1.5 L/1.5 kg ha^{-1}), glufosinate ammonium/propyzamide mixture (7.5 L/ 1.5 kg ha^{-1}), atrazine/propyzamide mixture (3 L/1.5 kg ha^{-1}), imazamox/propyzamide mixture (1.5 L/1.5 kg ha^{-1}), propyzamide alone (1.5 kg ha^{-1}) and trifluralin (1.5 L ha^{-1}). Treatments were laid out in a complete randomised block design replicated four times.

Trifluralin was applied at planting and a rake was used to cover the soil to avoid the decomposition of herbicide by the sun. The rest of the treatments were applied at the one to three canola leaf stages. The canola cultivars were planted with a plot planter with 30 cm row spacing. Plot sizes were 1.5 m x 7 m. The area between the different treatments was wide enough (2 m) to enable each treatment to have a control next to it. Application methods were similar to those used in 2012.

The efficacy of different treatments was evaluated six weeks after treatment and again 10 weeks after treatment. However, at Roodebloem in 2013 only the 6 WAT evaluation was done because of very low weed infestations in that particular year. Percentage control was obtained through visual observation and comparing the weed growth in the treated plots to the unsprayed plot directly next to the treated plot. The percentage control was ranked on a scale of 0 to 100%, where 0 indicated no visible effect from the herbicide on the weed population and 100 where there was total mortality of weeds. Estimates between 0 and 100 were made by combining an estimation of the proportion of senescent weeds as well as the percentage suppression of the weeds compared to the unsprayed control.

Data was subjected to analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Fischer's $LSD_{0.05.}$

Table 5.1: Physical and chemical properties of soil used in glasshouse study on herbicide mixtures on ryegrass

	Physical and chemical properties	
pH (KCl)	5.7	-
Resistance	2690	ohms
Texture	Sandy loam	-
Calcium	3.80	cmol(+)/kg
Magnesium	0.54	cmol(+)/kg
Potassium	151	mg/kg
Sodium	22	mg/kg
P (citric acid)	156	mg/kg
Total cations	4.83	cmol kg
Copper	2.67	mg/kg
Zinc	2.69	mg/kg
Manganese	65.98	mg/kg
Boron	0.14	mg/kg
Carbon	0.78	%
Sulphur	2.30	mg/kg
Iron	166.10	mg/kg

5.3 Results

5.3.1 Glasshouse results

Trial 1: Weedy ryegrass (Lolium spp. 2013)

There was a significant effect of herbicide treatments on the percentage control and dry mass reduction of a weedy ryegrass population in the glasshouse study. This was shown by a significant effect of the main effect of treatments p = 0.000 (Tables 5.2 and 5.3). All the herbicide treatments except imazamox applied as stand alone did increase percentage control compared to the untreated control plot (Figure 5.1). Propyzamide improved the efficacy of imazamox control only on ryegrass significantly from 0% to about 65%. However, atrazine efficacy was significantly reduced from about 90% control of ryegrass when applied alone to about 62% when it was mixed with propyzamide (Figure 5.1). Imazamox and imazamox plus propyzamide treatments were the only treatments with significantly more dry mass than the rest of the treatments (Figure 5.2). Herbicide treatments did not follow the same pattern of effectiveness in terms of dry mass production than in terms of the percentage control.

Table 5.2: Analysis of variance for the percentage control of a weedy ryegrass population with different herbicides in the glasshouse in 2013

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	75300.9	8	9412.6	28.4353	0.000000
Error	14895.8	45	331.0		

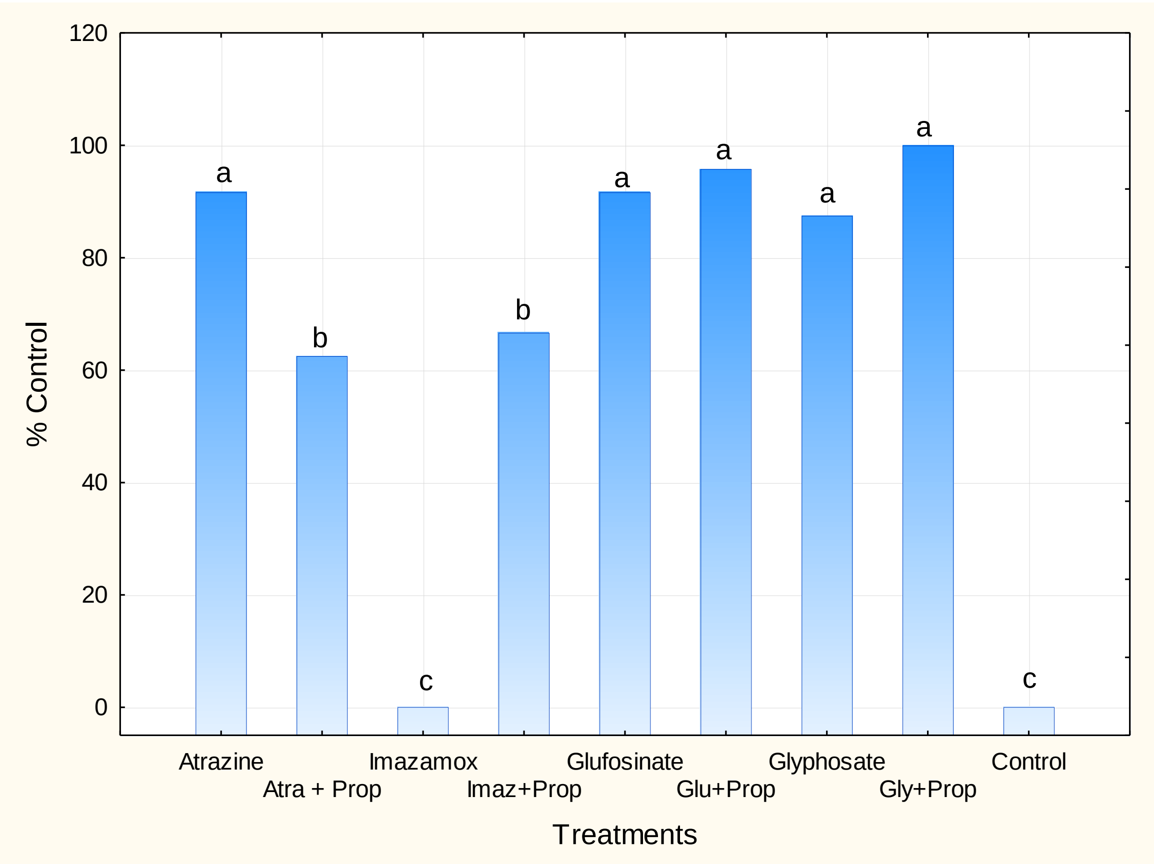

Figure 5.1: The effect of different herbicides on the percentage control of a weedy ryegrass population in in a glasshouse study in 2013. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05.

(Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

Table 5.3: Analysis of variance for the dry mass production of a weedy ryegrass population with different herbicides in the glasshouse in 2013

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	932.4376	8	116.5547	86.6496	0.00
Error	60.5307	45	1.3451		

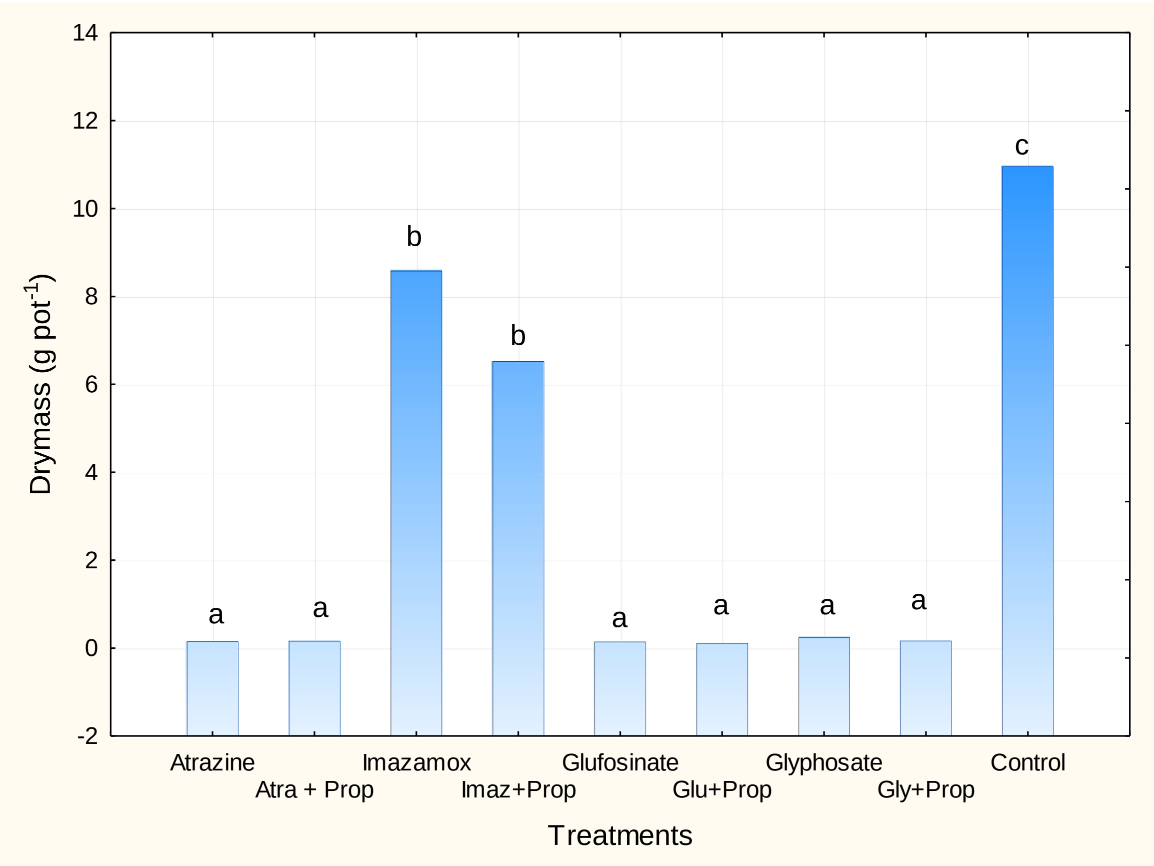

Figure 5.2: The effect of different treatments on the dry mass production of a weedy ryegrass population in the glasshouse in 2013. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

Trial 2: Commercial ryegrass (Lolium multiflorum cv. Agriton 2014)

Atrazine and propyzamide

No treatment by dosage rate interaction was detected in terms of percentage control or dry mass production when atrazine and atrazine plus propyzamide were applied on ryegrass control in the glasshouse (Table 5.4 and 5.6). In terms of percentage control there were also no differences within the main factors treatment and dosage rate. However, treatment and dosage rate had significant effects (*p* = 0.000 and 0.011 respectively) on dry mass reduction of ryegrass plants treated with atrazine alone and atrazine plus propyzamide. Antagonism occurred with propyzamide and atrazine mixtures irrespective of the dosage rates applied (Table 5.5). Higher dry mass was recorded on plants that were sprayed with atrazine plus propyzamide mixture (Figure 5.3). Generally, when atrazine or atrazine plus propyzamide was applied to the ryegrass plants, dry mass production was decreased significantly compared to the control treatment, except for the 0.75x treatments, which were not significant (Figure 5.4).

Table 5.4: Analysis of variance for the effect of atrazine and atrazine + propyzamide on the control of commercial ryegrass in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	416.667	1	416.667	1.14286	0.30091
Rates (L ha^{-1})	2291.667	3	763.889	2.09524	0.14113
Treatment*Rates (L ha^{-1})	3541.667	3	1180.556	3.23810	0.05003
Error	5833.333	16	364.583		

Table 5.5: Antagonistic interaction of atrazine and propyzamide on the percentage control of commercial ryegrass at 6 weeks after treatment (WAT) in 2014

Atrazine	Propyzamide		% control	
(L ha^{-1})	(kg ha^{-1})	WAT	Observed	Expected
1.5	0	6	16	-
0	0.75	6	16	-
1.5	0.75	6	25	29
2.25	0	6	50	-
0	1.125	6	8	-
2.25	1.125	6	25	54
3	0	6	16	-
0	1.5	6	33	-
3	1.5	6	25	43

Table 5.6: Analysis of variance on the dry mass production of commercial ryegrass after treatment with atrazine and atrazine plus propyzamide in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	14.5704	1	14.5704	20.3367	0.000356
Rates (L ha^{-1})	10.8684	3	3.6228	5.0565	0.011855
Treatment*Rates (L ha^{-1})	0.5545	3	0.1848	0.2580	0.854561
Error	11.4633	16	0.7165		

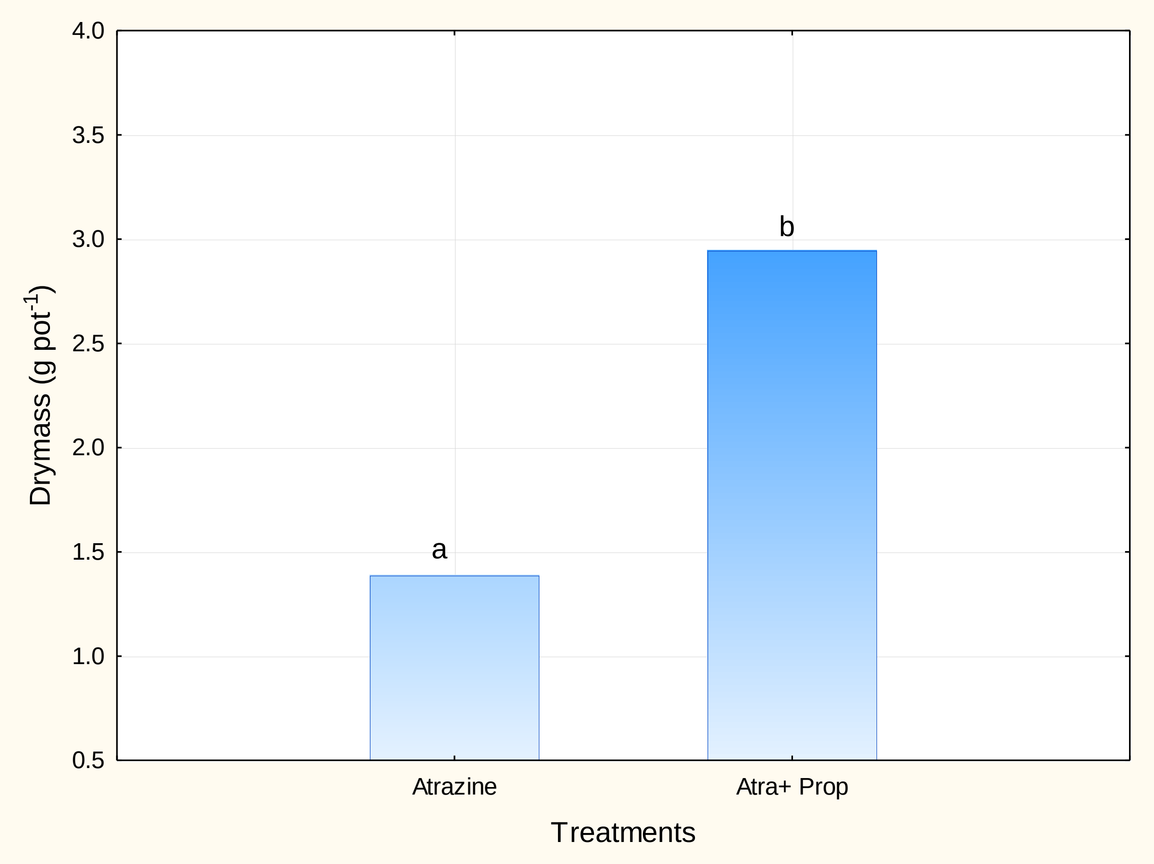

Figure 5.3: The effect of atrazine and atrazine plus propyzamide treatments on the dry mass production of commercial ryegrass in the glasshouse in 2014. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05.

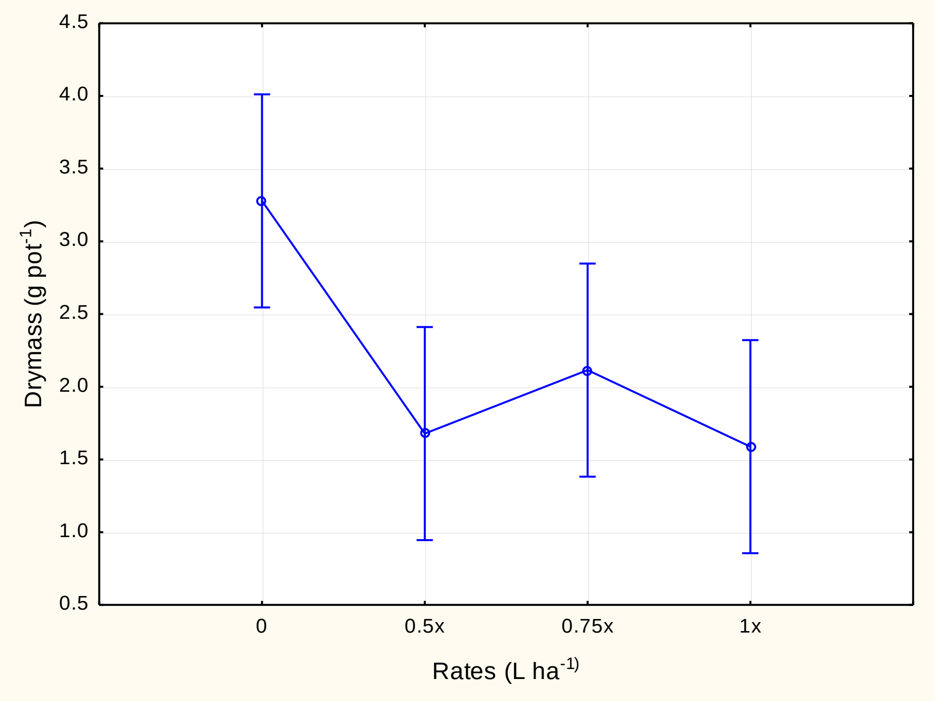

Figure 5.4: The effect of atrazine and atrazine plus propyzamide rates on the dry mass production of commercial ryegrass in the glasshouse in 2014. Vertical bars indicate least significant means.

Glufosinate ammonium and propyzamide

According to Table 5.7 there is no significant interaction between treatments and dosage rates applied in terms of the percentage control of commercial ryegrass. Nevertheless, main effects treatment and dosage rates had significant effects at p = 0.001 and 0.000. Glufosinate ammonium gave 70% control of ryegrass when only it was applied without propyzamide but only 40% when applied with propyzamide (Figure 5.5). Highest control ratings of 85 and 80% were observed on plants that were treated at 0.75 and 1 x dosage rates of glufosinate ammonium and glufosinate ammonium plus propyzamide (Figure 5.6). An antagonistic response occurred when propyzamide was added to glufosinate ammonium at all dosage rates (Table 5.8).

According to Table 5.9 there was a significant interaction between treatments and dosage rates (p = 0.028) in terms of the dry mass of ryegrass treated with glufosinate ammonium with propyzamide and without propyzamide at different concentrations. Plants that were treated with only glufosinate ammonium showed a significant reduction in dry mass compared to the untreated control at all rates applied (Figure 5.7). Propyzamide significantly reduced the efficacy of glufosinate ammonium at the 0.5x dosage rate.

Table 5.7: Analysis of variance on the percentage control of commercial ryegrass with glufosinate ammonium and glufosinate ammonium plus propyzamide in the glasshouse in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	5859.38	1	5859.38	15.0000	0.001348
Rates (L ha^{-1})	29453.13	3	9817.71	25.1333	0.000003
Treatment*Rates (L ha^{-1})	3411.46	3	1137.15	2.9111	0.066571
Error	6250.00	16	390.63		

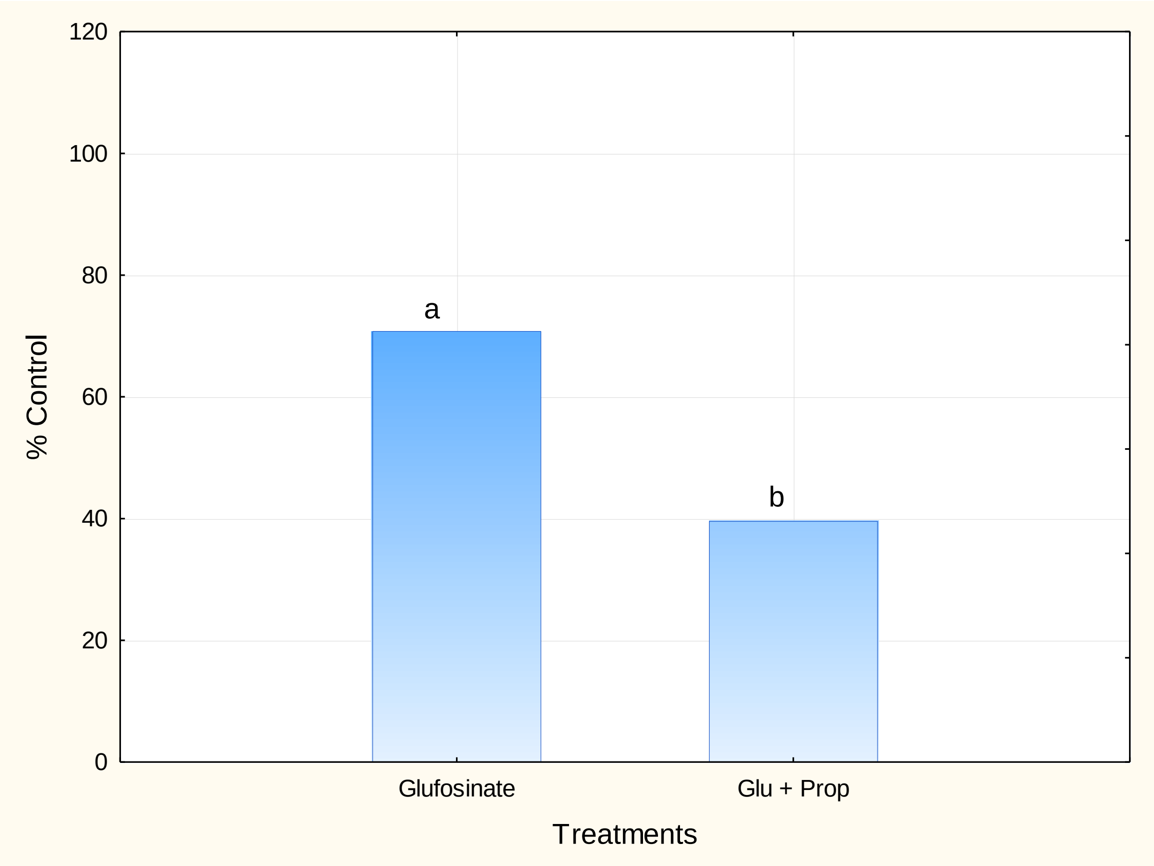

Figure 5.5: The effect of glufosinate ammonium and glufosinate ammonium plus propyzamide treatments on the percentage control of ryegrass in a glasshouse in 2014. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. Glu (Glufosinate ammonium), Prop (Propyzamide).

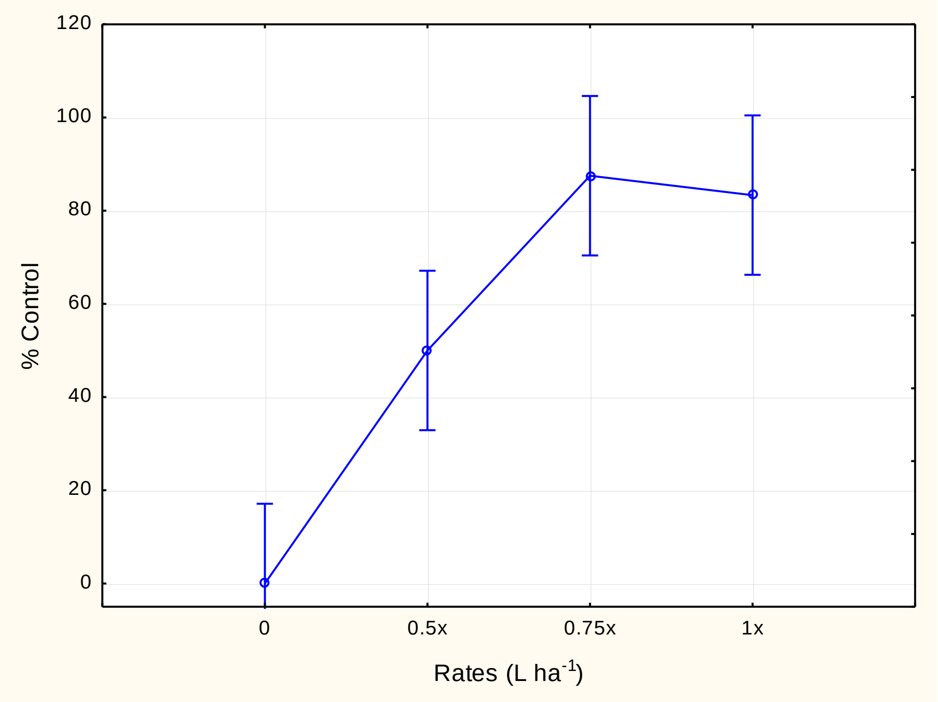

Figure 5.6: The effect of glufosinate ammonium and glufosinate ammonium plus propyzamide dosage rates on the percentage control of commercial ryegrass in the glasshouse in 2014. Vertical bars indicate least significant means.

Table 5.8: Antagonistic effects of adding propyzamide to glufosinate ammonium (Glu) 6 weeks after treatment (WAT) on commercial ryegrass control in 2014

Glu	Propyzamide		% control	
(L ha^{-1})	kg ha^{-1}	WAT	Observed	Expected
3.75	0	6	83	-
0	0.75	6	16	-
3.25	0.75	6	16	85
5.625	0	6	100	-
0	1.125	6	8	-
5.625	1.125	6	75	100
7.5	0	6	100	-
0	1.5	6	41	-
7.5	1.5	6	66	100

Table 5.9: Analysis of variance on the dry mass production of commercial ryegrass with glufosinate ammonium and glufosinate ammonium plus propyzamide in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	1.16600	1	1.16600	10.7092	0.004789
Rates (L ha^{-1})	13.68925	3	4.56308	41.9096	0.000000
Treatment*Rates (L ha^{-1})	1.28498	3	0.42833	3.9340	0.028030
Error	1.74207	16	0.10888		

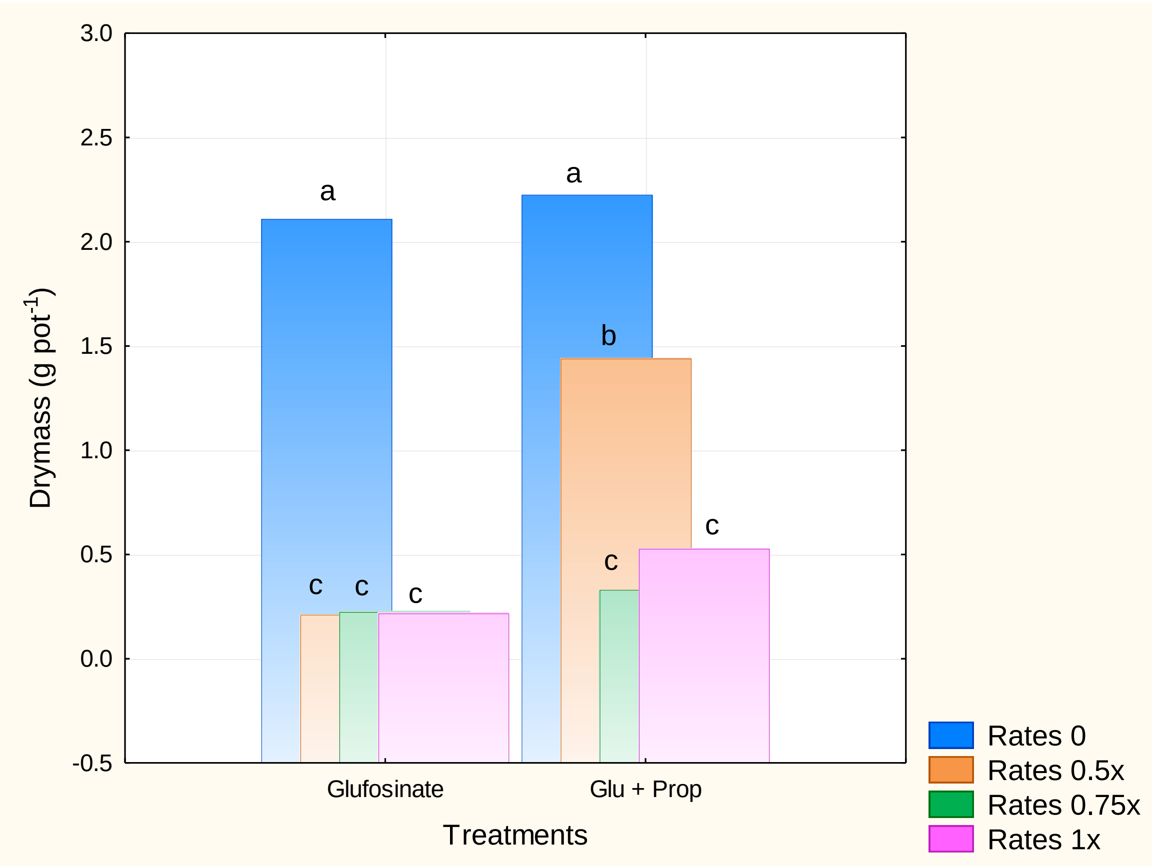

Figure 5.7: Interaction of glufosinate ammonium and glufosinate ammonium plus propyzamide on dry mass production of commercial ryegrass in the glasshouse in 2014. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. Glu (Glufosinate ammonium), Prop (Propyzamide).

Glyphosate and propyzamide

A significant (p = 0.019) interaction between treatment and rates occurred when glyphosate with and without propyzamide was applied for the control of commercial ryegrass plants (Table 5.10). Application of glyphosate without propyzamide significantly increased control of ryegrass at all rates applied compared to the untreated control (Figure 5.8). However, the mixture with propyzamide resulted in significant reduction in control at 0.5x dosage rates and lower control but not significantly so at 0.75x dosage rates. This was confirmed by the antagonistic response at low dosage rates of 0.5 and 0.75x and additive response at high rates of 1x (Table 5.11). Table 5.12 shows non-significant interaction of treatment and dosage rate on dry mass production of ryegrass treated with glyphosate and glyphosate without propyzamide. In contrast, a significant effect was detected on dosage rates applied (Figure 5.9). Dry mass production among the rates applied was significantly different from the control but not from each other.

Table 5.10: Analysis of variance on the percentage control of commercial ryegrass with glyphosate and glyphosate plus propyzamide in the glasshouse in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	2109.38	1	2109.38	8.1000	0.011676
Rates (L ha^{-1})	34453.13	3	11484.38	44.1000	0.000000
Treatment*Rates (L ha^{-1})	3411.46	3	1137.15	4.3667	0.019911
Error	4166.67	16	260.42		

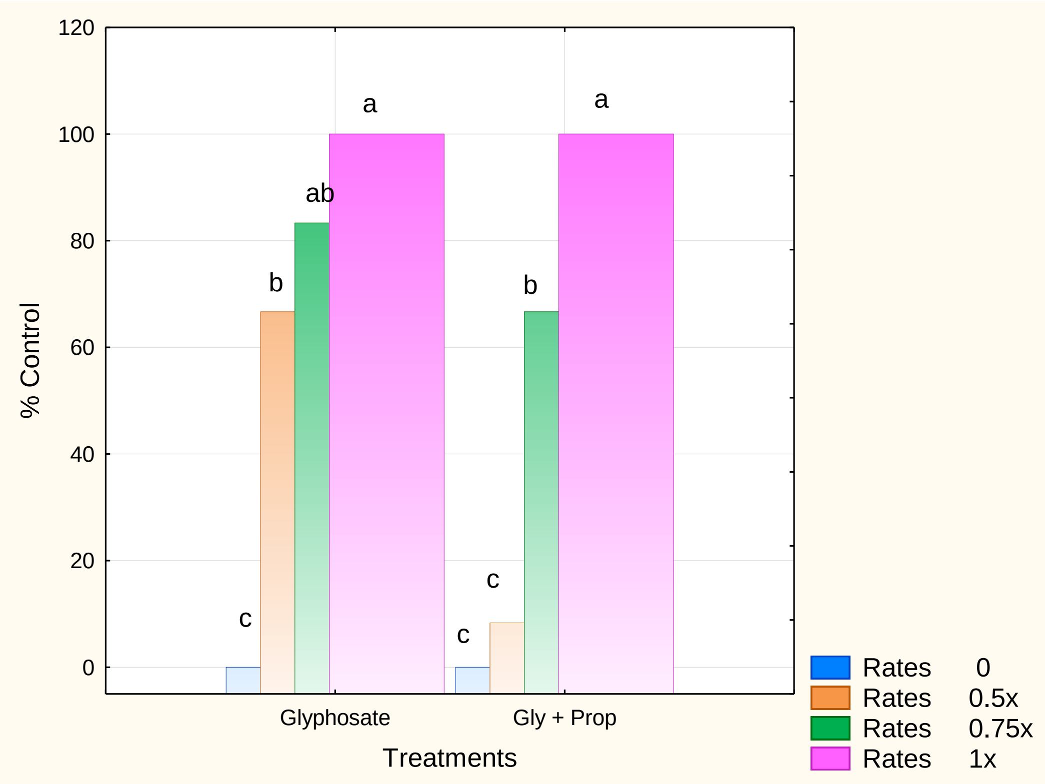

Figure 5.8: Interaction of glyphosate and glyphosate plus propyzamide on the control of commercial ryegrass in a glasshouse in 2014. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. Gly (Glyphosate), Prop (Propyzamide).

Table 5.11: Antagonistic effect of adding propyzamide with glyphosate 6 WAT on commercial ryegrass control in a glasshouse in 2014

Glyphosate	Propyzamide		% control ryegrass	
(L ha^{-1})	kg ha^{-1}	WAT	Observed	Expected
0.75	0	6	66	-
0	0.75	6	16	-
0.75	0.75	6	8	71
1.125	0	6	83	-
0	1.125	6	8	-
1.125	1.125	6	66	84
1.5	0	6	100	-
0	1.5	6	33	-
1.5	1.5	6	100	100

Table 5.12: Analysis of variance on dry mass production of commercial ryegrass with glyphosate and glyphosate plus propyzamide in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	0.08760	1	0.08760	0.7982	0.384878
Rates (L ha^{-1})	8.75225	3	2.91742	26.5814	0.000002
Treatment*Rates (L ha^{-1})	0.10315	3	0.03438	0.3133	0.815546
Error	1.75607	16	0.10975		

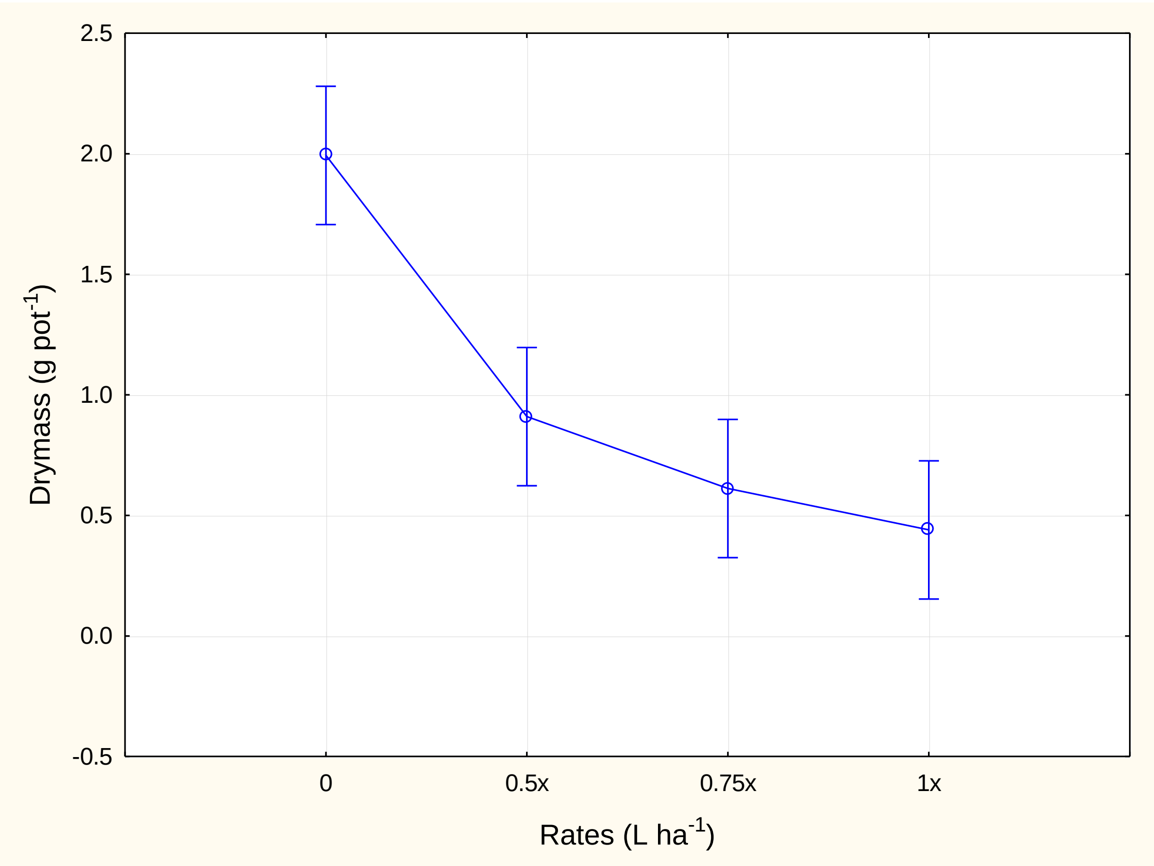

Figure 5.9: The effect of glyphosate and glyphosate plus propyzamide dosage rates on dry mass production of commercial ryegrass in the glasshouse in 2014. Vertical bars indicate least significant means.

Imazamox and propyzamide

Similar to glyphosate, a significant (p = 0.025) interaction between treatments and dosage rates occurred when propyzamide was applied to ryegrass plants (Table 5.13). Propyzamide significantly enhanced the efficacy of imazamox on the control of ryegrass at dosage rates of 0.75x and non-significantly at dosage rates of 1x.

However, at 0.5x it appears as if propyzamide had a negative effect on efficacy of imazamox on the control of commercial ryegrass (Figure 5.10). Table 5.14 confirmed an antagonistic effect between propyzamide and imazamox at low dosage rates of 0.5x but at higher dosage rates of 0.75x and 1x, it changed into synergistic effects. According to Table 5.15, there is a significant (p = 0.015) interaction in terms of the dry mass of ryegrass treated with imazamox with and without propyzamide. The trends observed in the percentage control parameter were generally echoed by the dry mass results (Figure 5.11). Addition of propyzamide to imazamox, resulted in a significant increase in dry mass production of the ryegrass at the 0.5x dosage rates, indicating antagonism. At the higher dosage rates of 0.75x and 1x, there were no significant increases or decreases in terms of dry mass production.

Table 5.13: Analysis of variance on the percentage control of commercial ryegrass with imazamox and imazamox plus propyzamide in the glasshouse in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	1276.04	1	1276.04	2.72222	0.118451
Rates (g ha^{-1})	9869.79	3	3289.93	7.01852	0.003164
Treatment*Rates (g ha^{-1})	5703.13	3	1901.04	4.05556	0.025427
Error	7500.00	16	468.75		

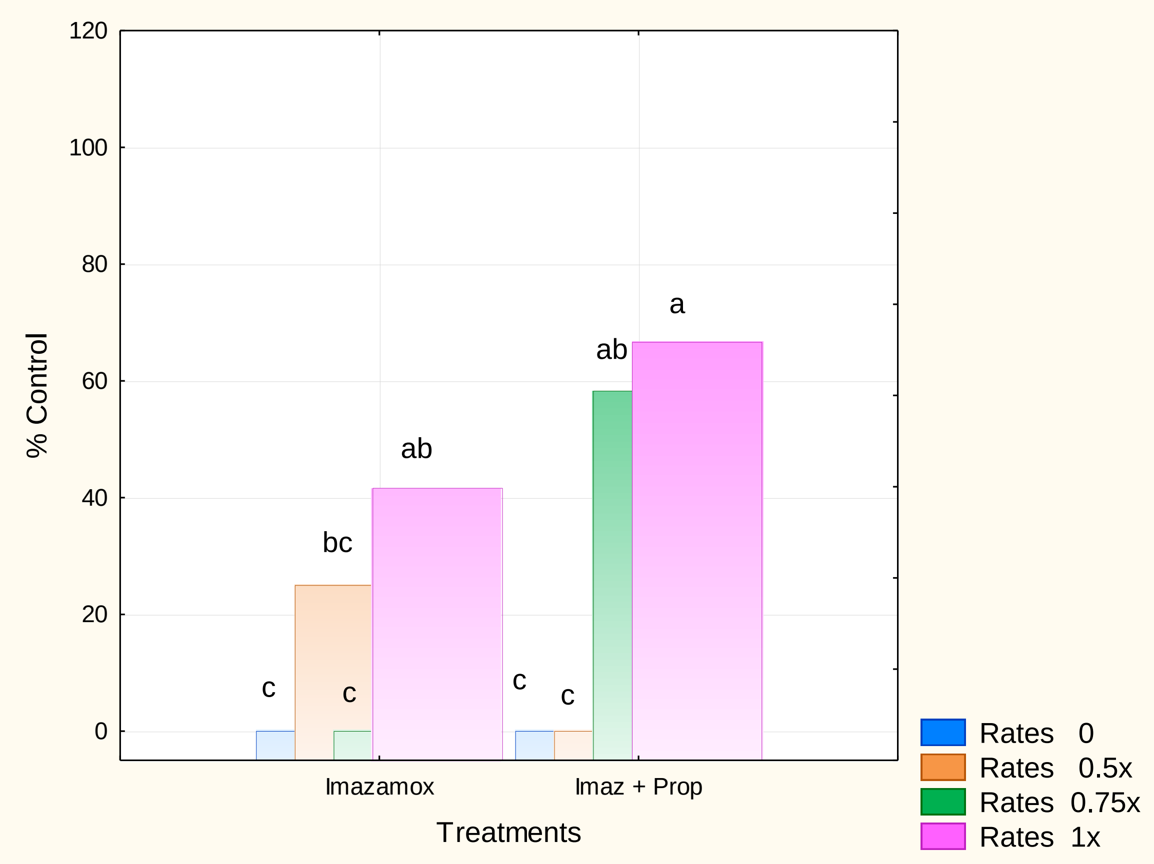

Figure 5.10: The interaction effect of imazamox and imazamox plus propyzamide on the control of commercial ryegrass in the glasshouse in 2014. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05.

Table 5.14: Antagonistic effects of adding propyzamide with imazamox 6 weeks after treatment (WAT) on commercial ryegrass control in a glasshouse in 2014

Imazamox	Propyzamide		% control ryegrass	
(L ha^{-1})	kg ha^{-1}	WAT	Observed	Expected
0.6	0	6	25	-
0	0.75	6	16	-
0.6	0.75	6	0	37
0.9	0	6	23	-
0	1.125	6	8	-
0.9	1.125	6	58	29
1.2	0	6	41	-
0	1.5	6	33	-
1.2	1.5	6	66	60

Table 5.15: Analysis of variance on the dry mass production of commercial ryegrass treated with imazamox and imazamox plus propyzamide in a glasshouse in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	14.7110	1	14.7110	11.2317	0.004056
Rates (g ha^{-1})	16.8386	3	5.6129	4.2854	0.021211
Treatment*Rates (g ha^{-1})	18.3291	3	6.1097	4.6647	0.015854
Error	20.9565	16	1.3098		

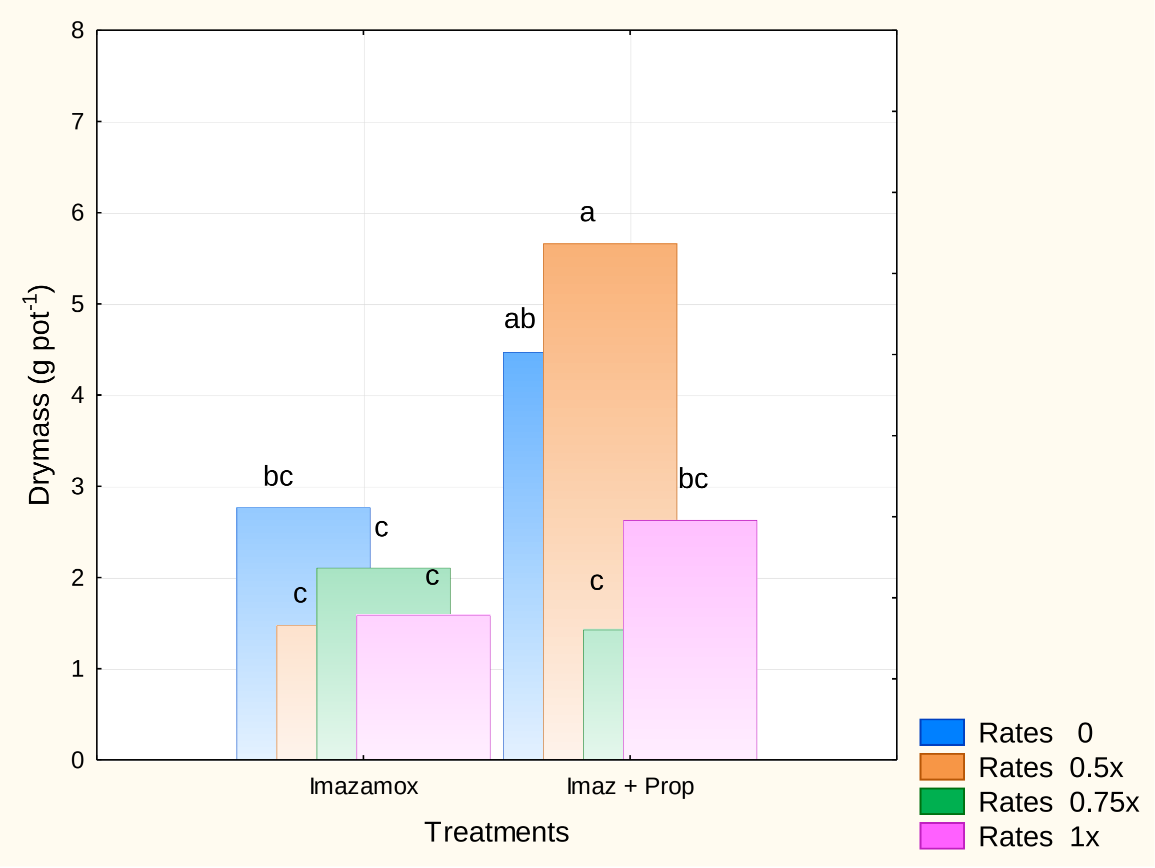

Figure 5.11: The interaction of imazamox and imazamox plus propyzamide on dry mass production of commercial ryegrass in the glasshouse in 2014. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05.

5.3.2 Field results

Trial 1: Effect of different treatments on weed population dynamics at three localities in 2012

Different herbicide treatments varied significantly in their ability to control weeds at Welgevallen. This was shown by a significant (p =0.000) effect of treatments applied on the response variable, percentage control (Table 5.16). Addition of propyzamide to atrazine increased weed control in the field slightly but significantly to 74% (Figure 5.12) than when only atrazine was applied (62%). However, control of weeds by imazamox, glyphosate and glufosinate ammonium did not improve or decrease significantly when propyzamide was added (Figure 5.12). Table 5.17 shows a non-significant effect of treatments on the dry mass production of different weeds in the field 6 WAT. However, Fischer's LSD showed some differences between treatments (Figure 5.13). Dry mass production after treatment with atrazine and glyphosate and their mixtures with propyzamide were similar, but different from glufosinate ammonium and imazamox and their mixtures with propyzamide (Figure 5.13).

Table 5.16: Analysis of variance on the percentage of weed control 6 weeks after treatment at Welgevallen in 2012

Effect	SS	Degr. of Freedom	MS	F	p
Treatments	16087.50	8	2010.94	13.9891	0.000000
Error	3881.25	27	143.75		

Table 5.17: Analysis of variance on the dry mass of weeds in the field 6 weeks after treatment at Welgevallen in 2012

Effect	SS	Degr. of Freedom	MS	F	p
Treatments	2939.60	8	367.45	1.60777	0.169114
Error	6170.77	27	228.55		

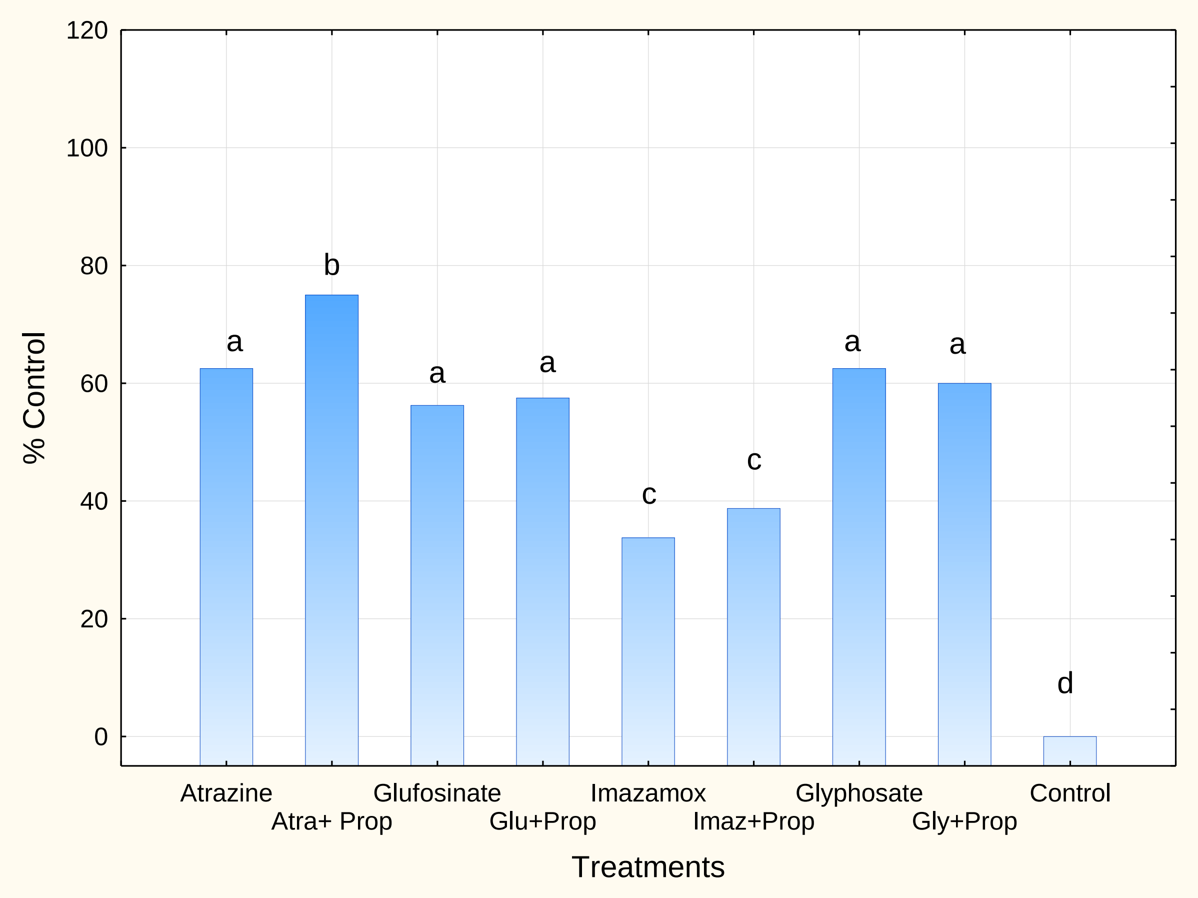

Figure 5.12: The effect of herbicide treatments on weed control in the field 6 weeks after treatment at Welgevallen in 2012. The same letters above treatment bars indicate no significant differences between treatments at $p = 0.05$. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

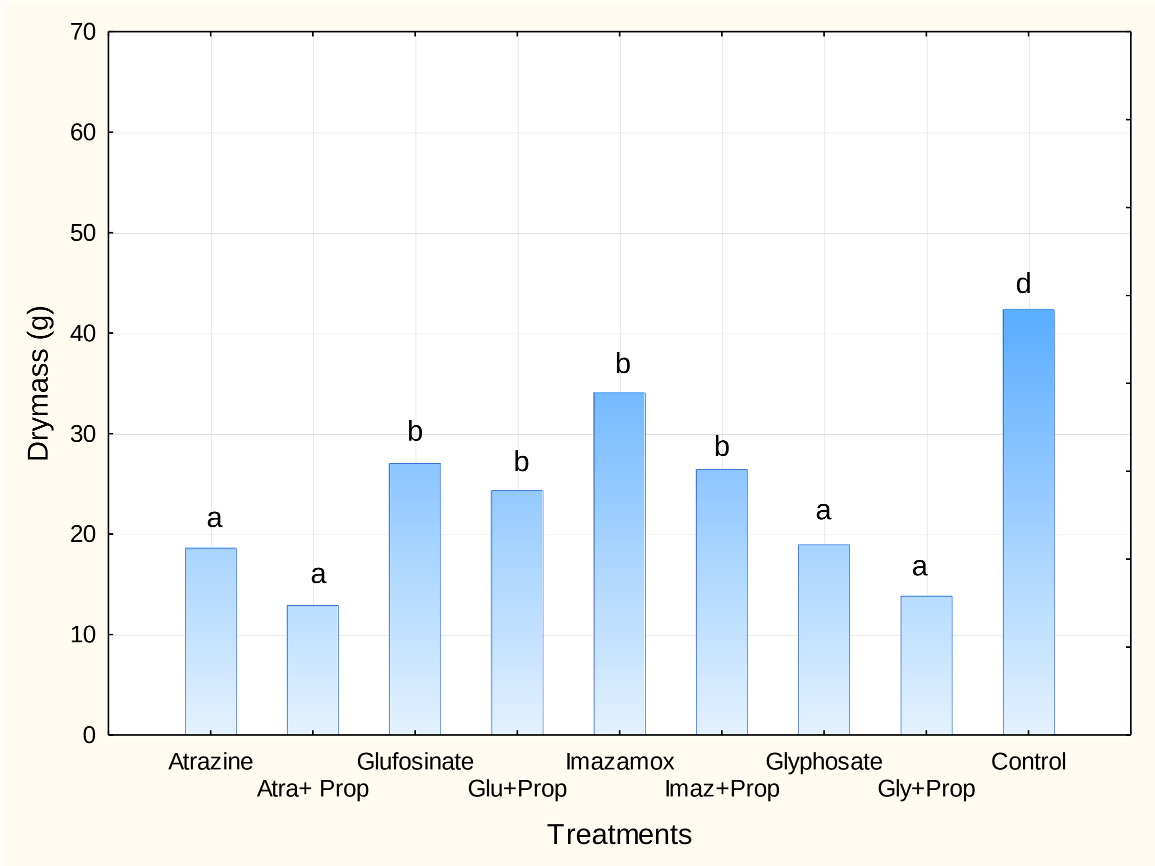

Figure 5.13: Effect of different herbicide treatments on weed dry mass in the field 6 weeks after treatment at Welgevallen 2012. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

The Roodebloem results followed the same trend as those of Welgevallen. Analysis of variance (Tables 5.18 and 5.19) shows the significant effect (p = 0.000) of treatments on the control and dry mass production of weeds in the field. Addition of propyzamide enhanced the efficacy of herbicides under field conditions but the increase was not statistically significant (Figure 5.14). Glyphosate only and in a mixture with propyzamide gave significantly better control than the other treatments. Propyzamide prolonged the residual effect of the treatment in the soil, hence the increased efficacy. Dry mass production of plots treated with imazamox and imazamox plus propyzamide was significantly higher than the other treated plots (Figure 5.15), confirming the trend of a lower percentage control shown by the two imazamox treatments in Figure 5.14. Propyzamide appeared to increase the efficacy of glyphosate in the field (Figure 5.15).

Table 5.18: Analysis of variance for percentage control of weeds in the field 6 weeks after treatment at Roodebloem in 2012

Effect	SS	Degr. of Freedom	MS	F	p
Treatments	21297.2	8	2662.2	49.571	0.000000
Error	1450.0	27	53.7		

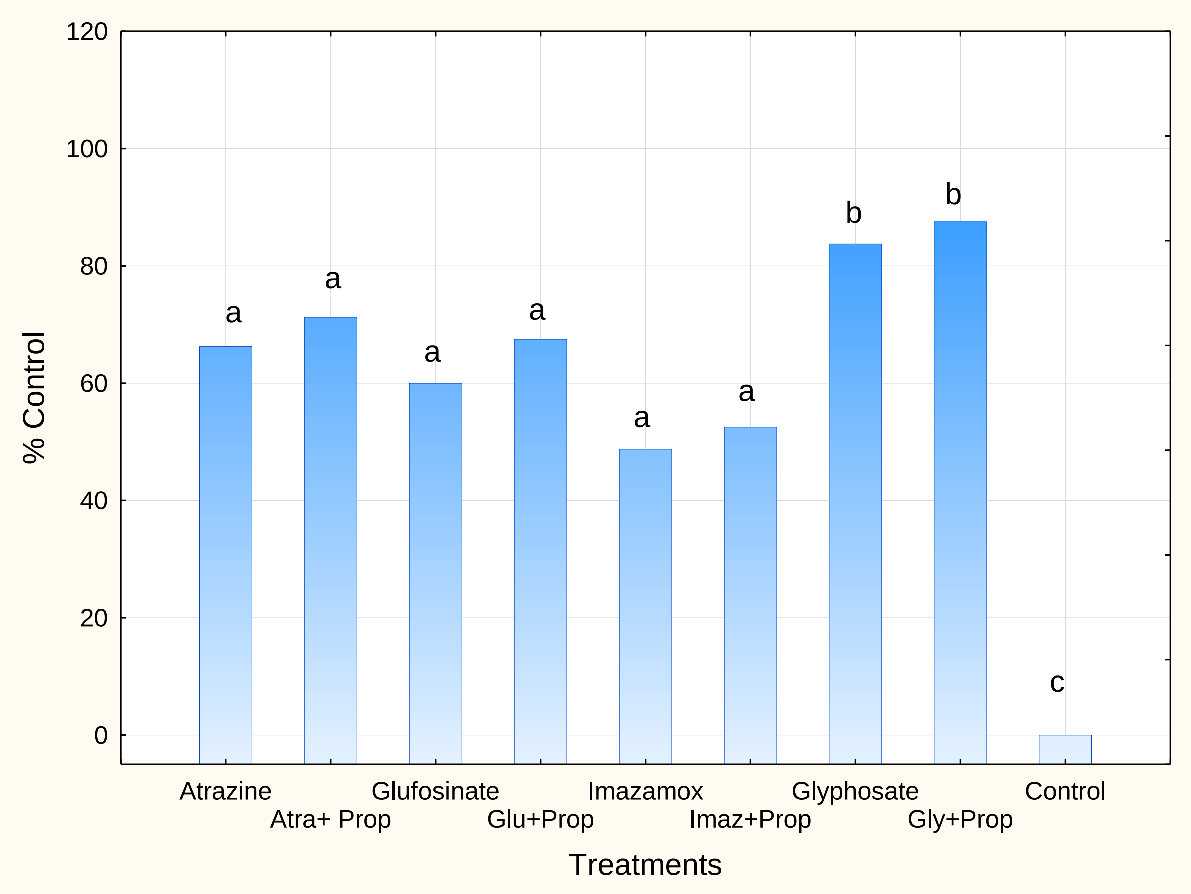

Figure 5.14: The effect of different herbicide treatments on weed control at Roodebloem 6 weeks after treatment in 2012. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

Table 5.19: Analysis of variance for the dry mass of weeds at Roodebloem 6 weeks after treatment in 2012

Effect	SS	Degr. of Freedom	MS	F	p
Treatments	3259.72	8	407.47	5.9223	0.000206
Error	1857.65	27	68.80		

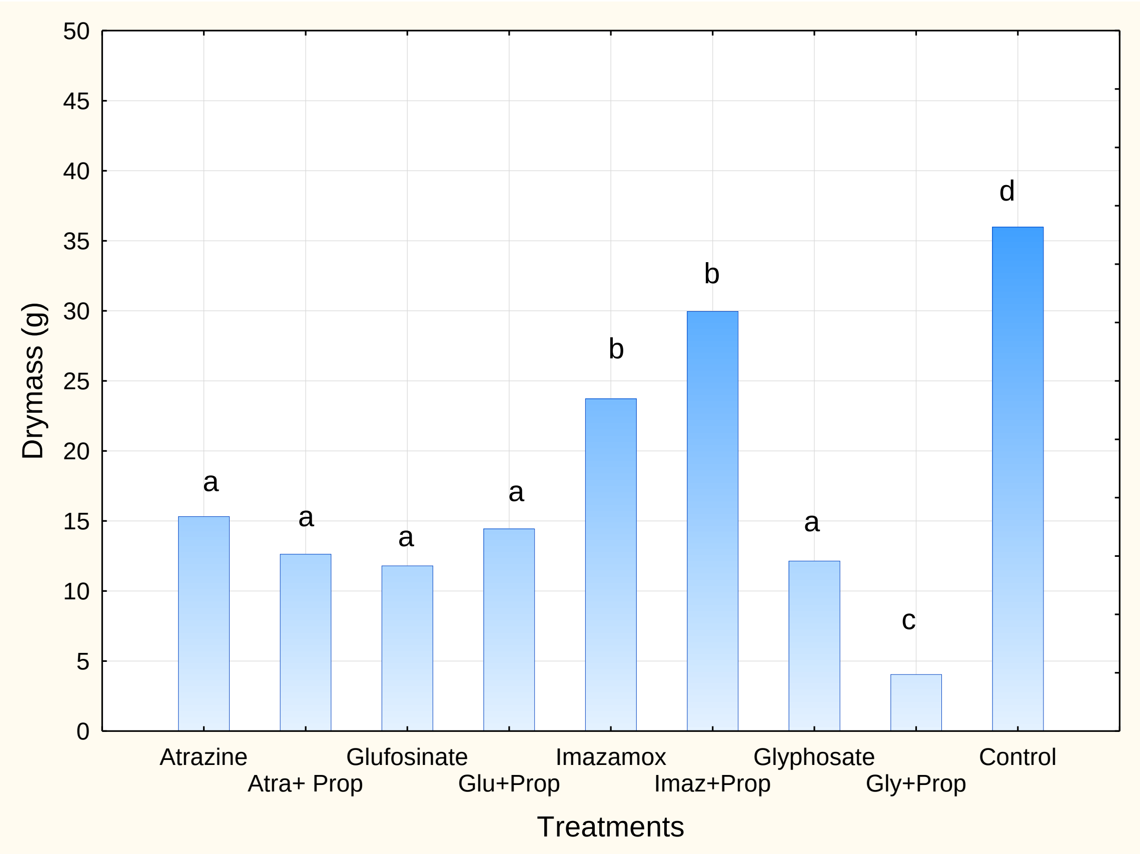

Figure 5.15: The effect of different treatments on the dry mass of weeds at Roodebloem 6 weeks after treatment in 2012. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

The results from Langgewens followed the same trend as those of Roodebloem. There was a significant effect detected on the percentage control and dry mass production of weeds in the field (Tables 5.20 and 5.21). Application of propyzamide did not significantly improve or reduce the control by any of the four herbicides tested

(Figure 5.16). Atrazine and glyphosate and their mixtures with propyzamide resulted in the best percentage control. Imazamox only and imazamox plus propyzamide plots gave the highest dry mass production of respectively (Figure 5.17).which confirms the lowest percentage control caused by these treatments (Figure 5.16).

Table 5.20: Analysis of variance on the control of weeds at Langgewens 6 weeks after treatment in 2012

Effect	SS	Degr. of Freedom	MS	F	p
Treatments	26268.1	8	3283.5	34.597	0.000000
Error	2562.5	27	94.9		

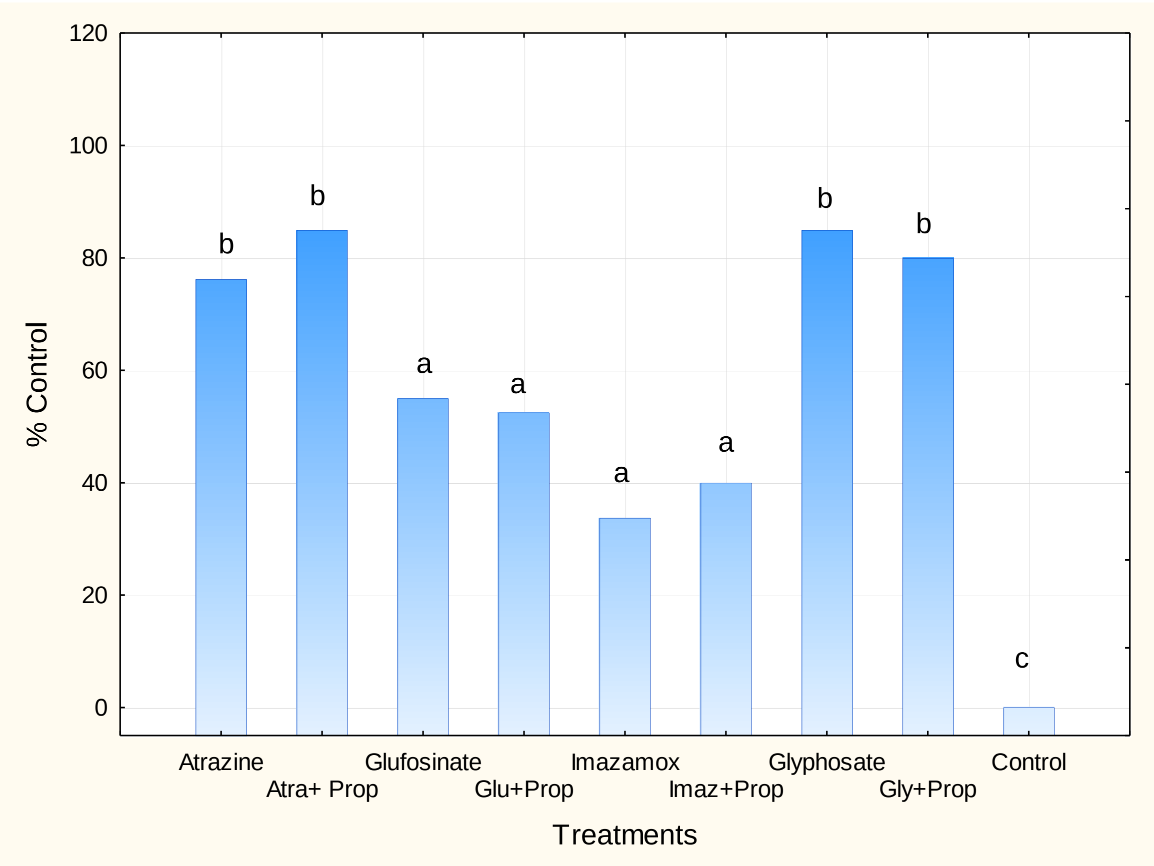

Figure 5.16: The effect of different herbicide treatments on the control of weeds at Langgewens 6 weeks after treatment in 2012. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

Table 5.21: Analysis of variance for the dry mass of weeds treated with different herbicides at Langgewens 6 weeks after treatment in 2012

Effect	SS	Degr. of Freedom	MS	F	p
Treatments	12673.41	8	1584.18	3.29970	0.009309
Error	12962.60	27	480.10		

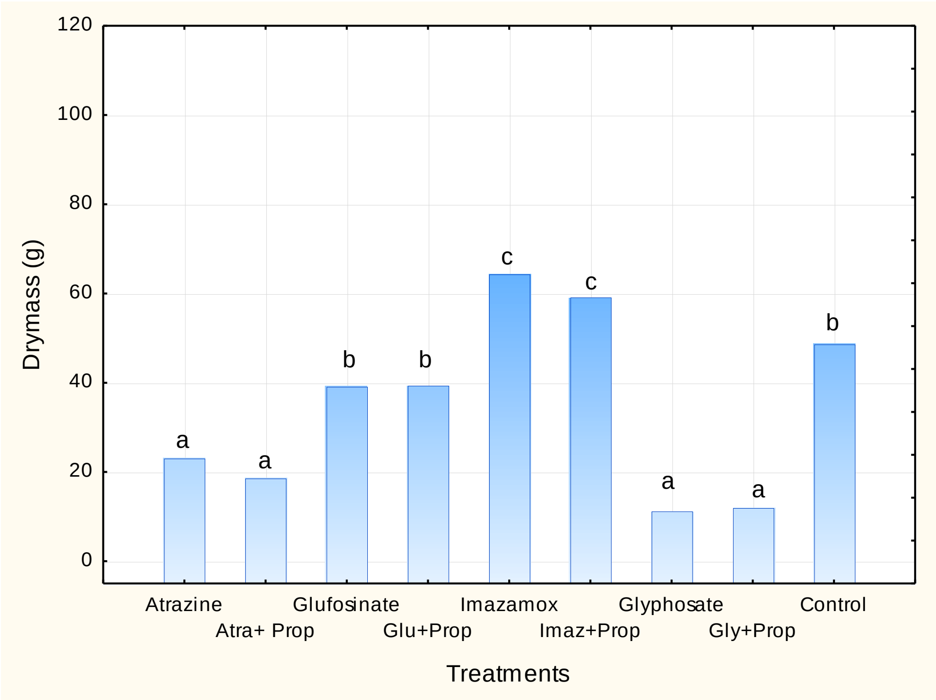

Figure 5.17: The effect of different herbicide treatments on the dry mass of weeds at Langgewens 6 weeks after treatment in 2012. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

Trial 2: Effect of different herbicide treatments on plots planted with herbicide resistant canola cultivars in 2013 and 2014

There was a significant interaction (p = 0.000) of treatment and time on the control of weeds at Welgevallen at both 6 and 10 weeks after treatment (WAT) in 2013 (Table 5.22). At 6 WAT, application of propyzamide with the other herbicides generally increased percentage control, significantly so in the case of atrazine, imazamox and glufosinate ammonium (Figure 5.18). At 10 WAT, application of propyzamide generally decreased control by the herbicides, and significantly so in the case of glufosinate ammonium and glyphosate. There were no treatments where control 10 WAT was significantly better than 6 WAT except for application of trifluralin and propyzamide alone. In two treatments viz. glufosinate ammonium with propyzamide and glyphosate with propyzamide, control 10 WAT was significantly poorer than at 6 WAT.

Table 5.22: Analysis of variance for the control of weeds at Welgevallen 6 and 10 weeks after treatment in 2013

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	51861.0	9	5762.3	81.341	0.000000
Time (weeks)	4.0	1	4.0	0.057	0.811841
Treatment*Time (weeks)	11217.7	9	1246.4	17.594	0.000000
Error	4250.5	60	70.8		

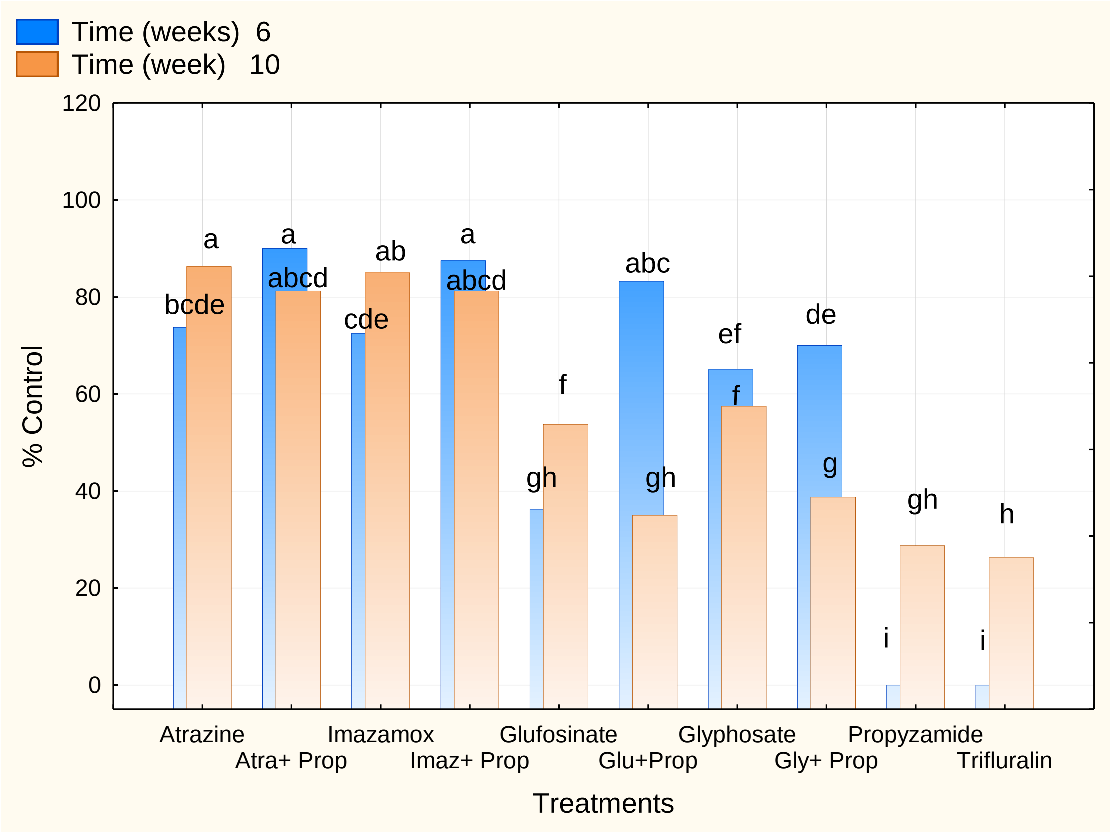

Figure 5.18: Effect of different treatments on the control of weeds at Welgevallen 6 and 10 weeks after treatment in 2013. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

There was no significant interaction of treatments and times observed in the second trial in 2014, but a significant effect was detected on main factors; treatments and time (Table 5.23). Propyzamide improved the efficacy of atrazine significantly but had no significant positive or negative effect on the efficacy of the other three herbicides (Figure 5.19). However, there was poor control of weeds when only propyzamide and trifluralin were applied. The glufosinate ammonium and glufosinate ammonium plus propyzamide treatments gave significantly poorer control than the other three herbicides but better than only trifluralin and propyzamide. Percentage control by the herbicide treatments decreased from 68% 6 WAT to 59% at 10 WAT (Figure 5.20).

Table 5.23: Analysis of variance for the control weeds at Welgevallen 6 and 10 weeks after treatment in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	82348.7	9	9149.9	49.182	0.00000C
Time (weeks)	1445.0	1	1445.0	7.767	0.007114
Treatment*Time (weeks)	642.5	9	71.4	0.384	0.93846ε
Error	11162.5	60	186.0		

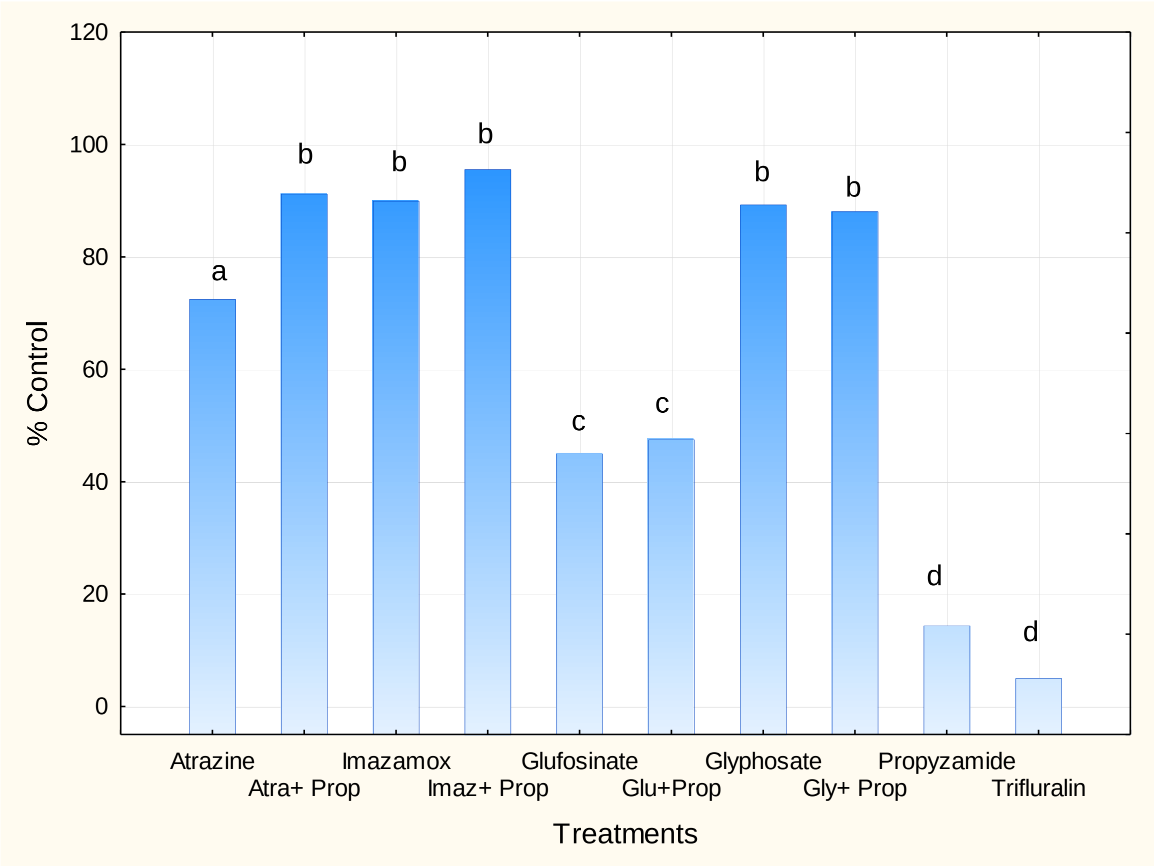

Figure 5.19: The effect of different herbicide treatments on weed control at Welgevallen (averages of 6 and 10 weeks after treatment) in 2014. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

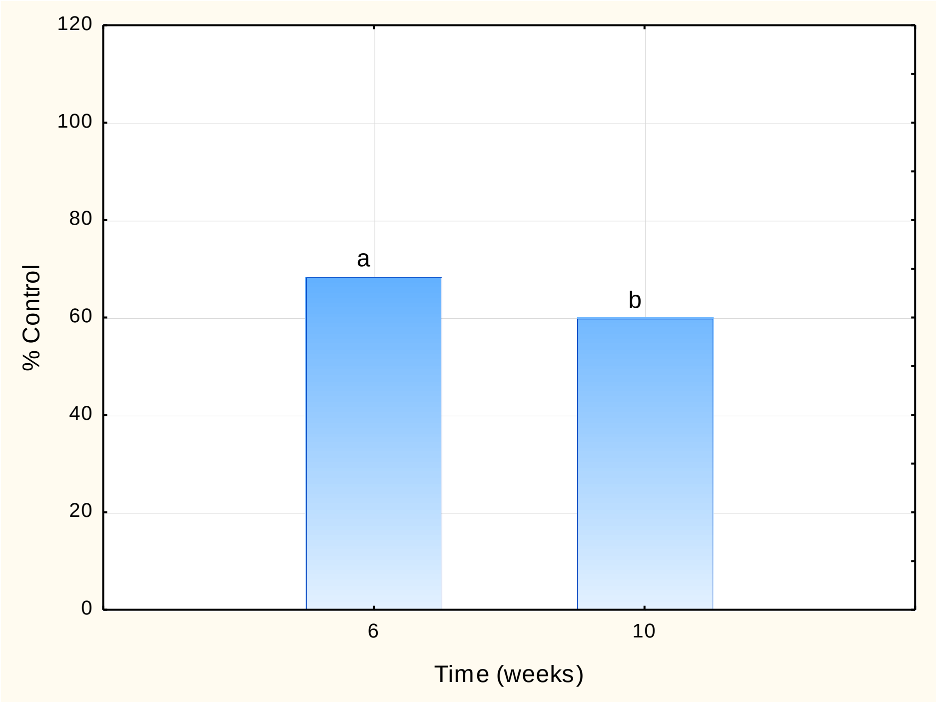

Figure 5.20: The effect of time on the efficacy of herbicides applied on weed control in the field at Welgevallen in 2014. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05.

The analysis of variance for the percentage control of weeds at Roodebloem in 2013, showed the significant effect of treatments in the field at 6 WAT (Table 5.24). Because of very low weed infestations at Roodebloem in 2013 no second evaluation at 10 WAT was made and therefore only a one-way ANOVA analysis was carried out on the data. Herbicide treatments applied with or without propyzamide were not statistically different from each other but did differ significantly from only trifluralin and propyzamide applications (Figure 5.21). Addition of propyzamide did not have any significant effect on efficacy of the four herbicides tested.

Table 5.24: Analysis of variance on the control of weeds at Roodebloem 6 weeks after treatment in 2013

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	2164.2	9	240.5	3.205	0.007767
Error	2250.8	30	75.0		

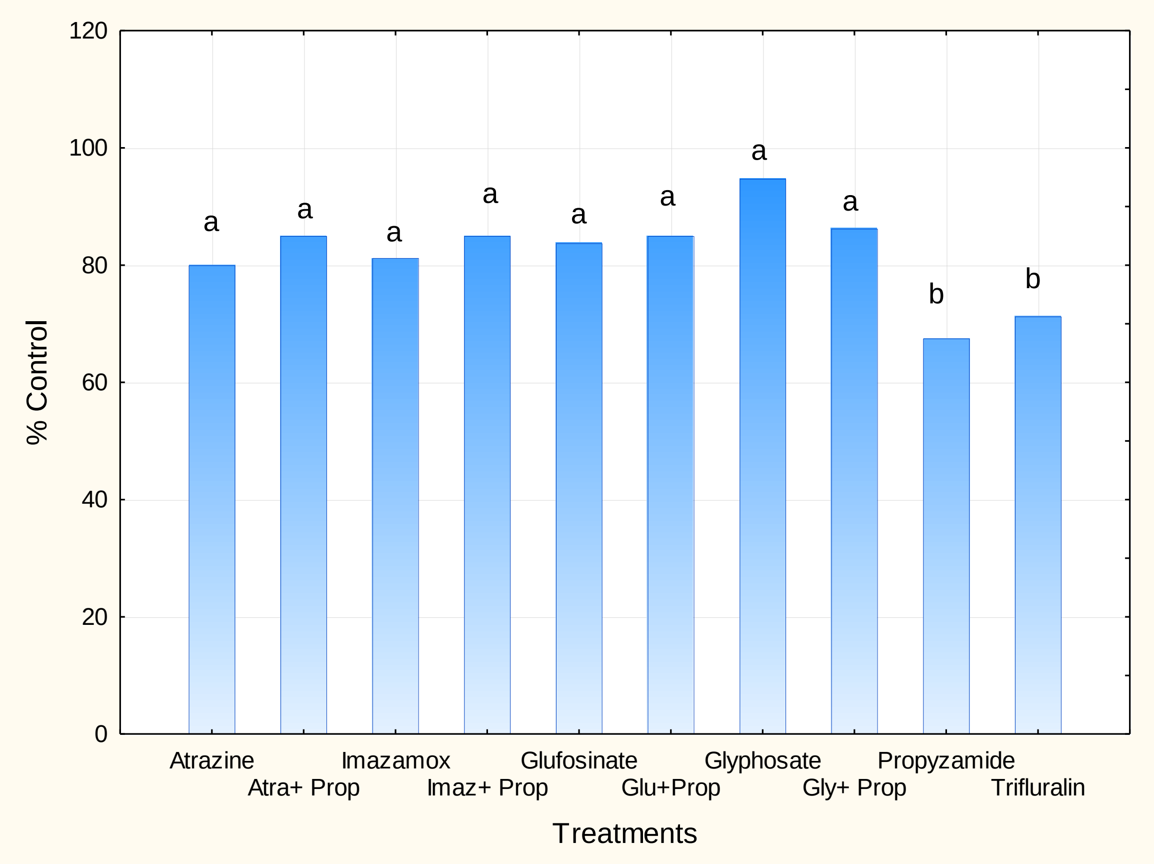

Figure 5.21: The effect of different herbicide treatments on the control of weeds at Roodebloem 6 weeks after treatment in 2013. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

There was no significant interaction of treatments and time (Table 5.25), but the main factor, Treatments, showed significant differences in 2014 (p =0.000). Figure 5.22 illustrates the effectiveness of adding propyzamide to atrazine efficacy. However, it appears that propyzamide reduced glyphosate efficacy as compared to when only glyphosate was applied.

Table 5.25: Analysis of variance on the control of weeds at Roodebloem 6 and 10 WAT (2014)

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	27723.0	9	3080.3	6.8308	0.000001
Time (weeks)	66.6	1	66.6	0.1477	0.702085
Treatment*Time (weeks)	3949.5	9	438.8	0.9731	0.471171
Error	27056.8	60	450.9		

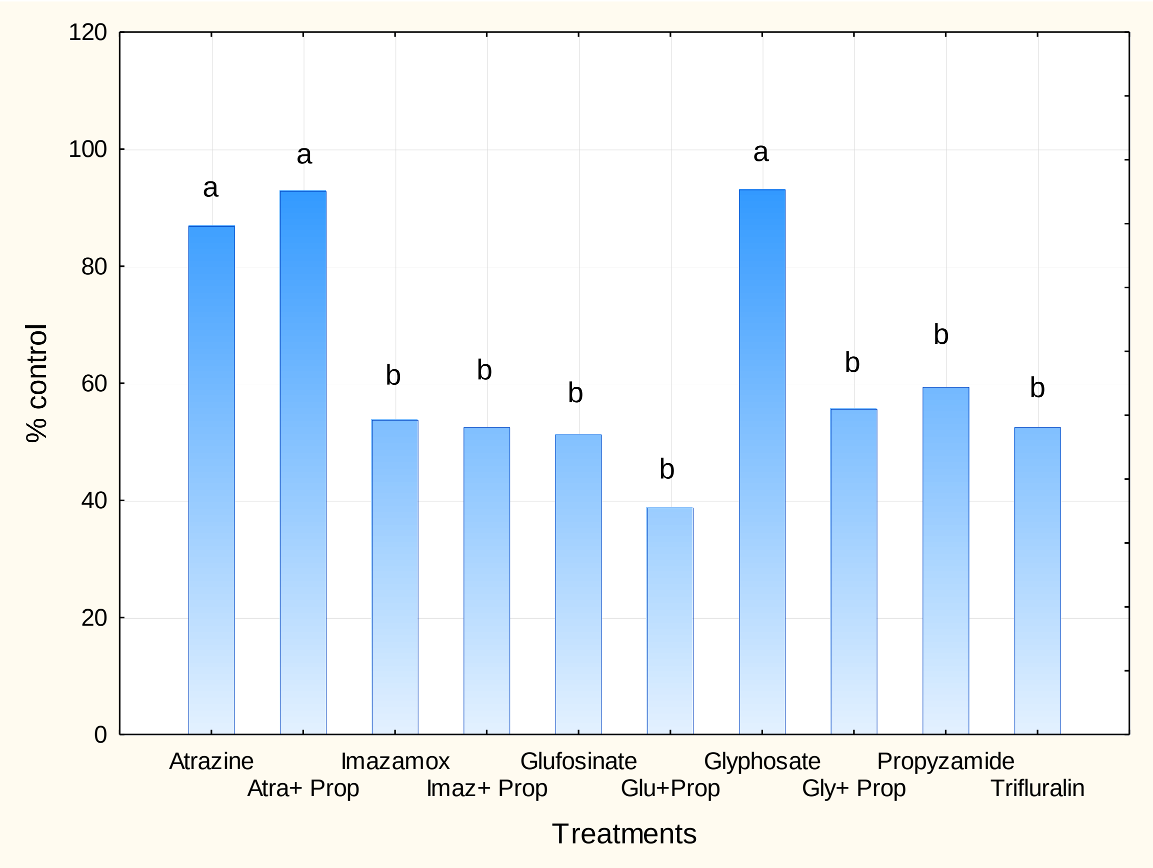

Figure 5.22: The effect of different herbicide treatments on the control of weeds at Roodebloem (average of 6 and 10 weeks after treatment) in 2014. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

The results of 2013 from Langgewens show no significant interaction of treatment and time on weed control in the field at 6 and 10 weeks. However, a significant effect was detected on the treatments applied (Table 5.26). Propyzamide did not significantly influence the efficacy of any of the four herbicides (Figure 5.23). Imazamox and its mixture with propyzamide appeared to give better control than the other herbicides tested.

Table 5.26: Analysis of variance on the control of weeds at Langgewens 6 and 10 weeks after treatment in 2013

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	27212.5	9	3023.6	11.9353	0.000000
Time (week)	5.0	1	5.0	0.0197	0.888745
Treatment*Time (week)	2557.5	9	284.2	1.1217	0.361927
Error	15200.0	60	253.3		

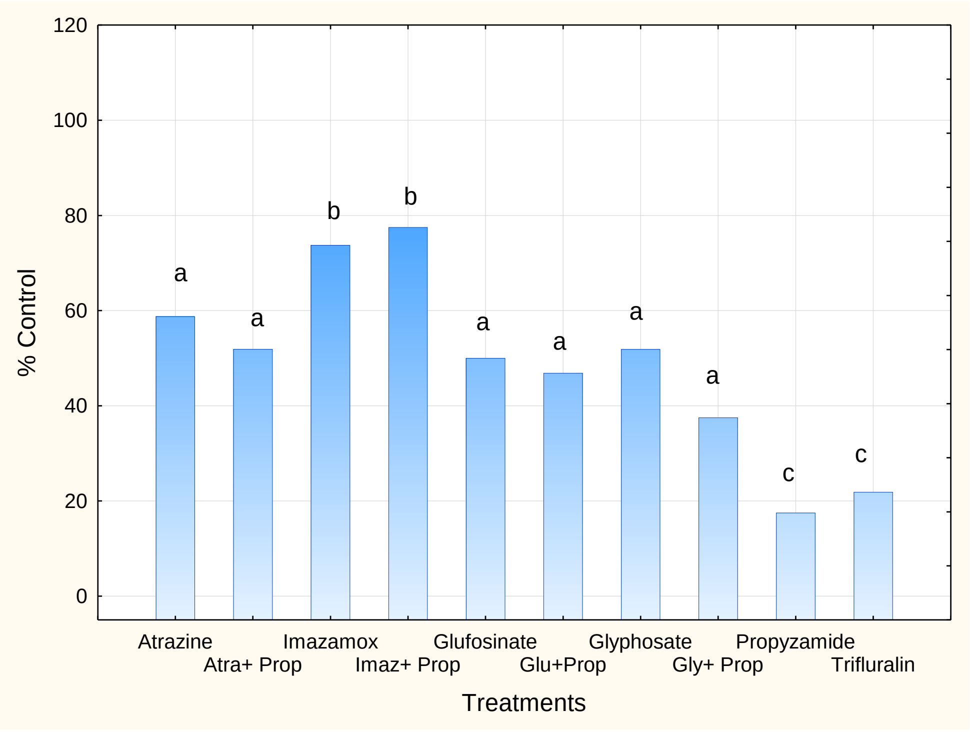

Figure 5.23: The effect of different herbicide treatments on the control of weeds at Langgewens 6 and 10 weeks after treatment in 2013. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

The results from the second trial in 2014 did not follow the same trend as the previous study conducted in 2013. Table 5.27 indicates the significant interaction (p= 0.000) of treatment and time. Imazamox was the only herbicide where addition of propyzamide significantly increased efficacy of the herbicide (Figure 5.24). Similarly, imazamox was the only herbicide where a significant increase in percentage control took place from 6 WAT to 10 WAT. In all other cases, there were no significant differences between herbicides with or without imazamox and also no significant differences in terms of percentage control at 6 and 10 WAT.

Table 5.27: Analysis of variance for the control of weeds at Langgewens 6 and 10 weeks after treatment in 2014

Effect	SS	Degr. of Freedom	MS	F	p
Treatment	30662.8	9	3407.0	10.5133	0.000000
Time (weeks)	4882.8	1	4882.8	15.0675	0.000261
Treatment*Time (weeks)	12557.8	9	1395.3	4.3057	0.000239
Error	19443.7	60	324.1		

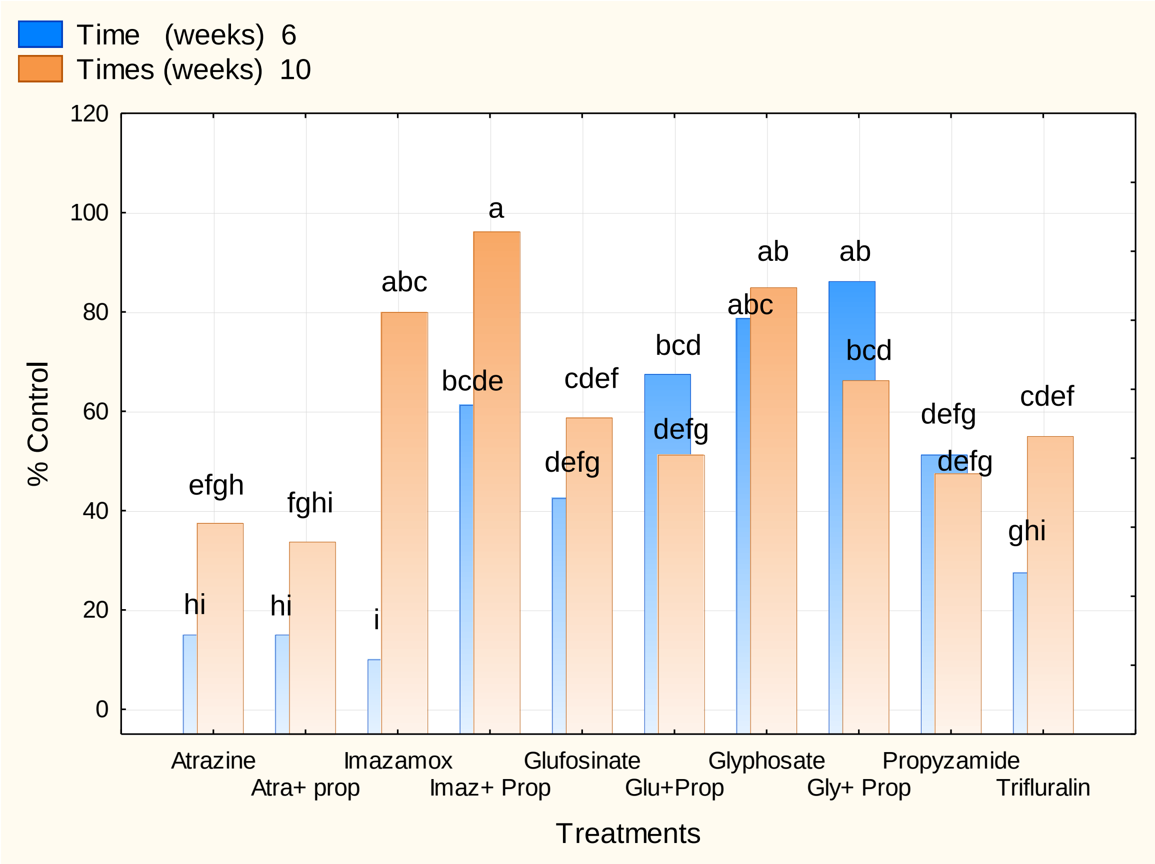

Figure 5.24: Interaction of treatments and time on the control of weeds at Langgewens 6 and 10 weeks after treatment in 2014. The same letters above treatment bars indicate no significant differences between treatments at p = 0.05. (Atra (Atrazine), Imaz (Imazamox), Glu (Glufosinate ammonium), Gly (Glyphosate), Prop (Propyzamide).

5.4 Discussion

5.4.1 Glasshouse study

Trial 1: Weedy ryegrass (Lolium spp)

Preliminary results from the study conducted in 2013 indicated an inconsistency on ryegrass control with propyzamide combined with atrazine, glufosinate ammonium, glyphosate and imazamox. Propyzamide works best in moist and wet soils. Atrazine gave more effective control of ryegrass when applied alone than in a mixture with propyzamide. It is clear that propyzamide reduced the atrazine efficacy. Although poor control of ryegrass was observed when atrazine were mixed with propyzamide, imazamox gave better control of ryegrass when mixed with propyzamide. According to Hydrick and Shaw (1994), glyphosate applied with chlorimuron controlled weeds better than glyphosate applied alone. Propyzamide increased glyphosate control although it was not significant. Imazamox efficacy on ryegrass was improved by propyzamide because the ryegrass population used in the study was discovered to be resistant to imazamox. It is therefore inconclusive whether propyzamide will improve the efficacy of imazamox on non-resistant weeds.

Trial 2: Commercial ryegrass (Lolium multiflorum cv. Agriton 2014)

Results from the different herbicides used in the second trial were analysed separately. Atrazine killed ryegrass plants more effectively when it was applied alone than in mixture with propyzamide. The interaction of propyzamide and atrazine was antagonistic. Shaw and Arnold (2002) reported antagonism of CGA 277476, cloransulam-methyl, imazaquin, and pyrithiobac on the control of hemp sesbania. Similar results of antagonism were observed by Koo et al. (2000) on barnyardgrass control with propanil and pyribenzoxim.

There were also significant negative effects of propyzamide on the control of ryegrass by glufosinate ammonium and the expected calculated control percentages indicated antagonism between propyzamide and glufosinate ammonium too. Glufosinate ammonium in mixture with clethodim reduced the control of goosegrass compared to the control obtained when clethodim was applied alone (Burke et al. 2005).

An interesting trend was observed with glyphosate and propyzamide. At low dosage rates (0.5x) there was strong antagonism of glyphosate and propyzamide and at the higher rate of 0.75x the antagonism was still evident but not as pronounced (Table 5.11). At 1x there was no antagonism observed. Koo et al. (2000) reported antagonistic interaction of pyribenzoxim and propanil when mixed at high dosage rate of 4 kg ha^{-1}. In this instance the antagonism was a result of high dosage rates of propanil.

Similarly, at low dosage rates (0.5x) there was antagonism observed between imazamox and propyzamide but at higher dosage rates this interaction appeared to turn into synergism (Table 5.14 and Figure 5.10).

Addition of propyzamide appeared to have a consistent negative effect on efficacy of atrazine and to a lesser extent glufosinate ammonium in the glasshouse trials.

5.4.2 Field study

Field trial 1: Effect of different treatments on weed control in unplanted fields

In order to prolong the longevity of herbicides once they are applied on a large scale, it is necessary to devise appropriate control programs to delay development of resistance. Rotation of herbicides with different mode of action is one such method. It is also effective to mix herbicides with different mode of actions provided the herbicides do not influence the efficacy of one another (Koo et al. 2000). Addition of propyzamide increased the efficacy of atrazine at Welgevallen (Figure 5.12). This is in complete contrast to what was found in the glasshouse trials. This could be as a result of weed density or the type of weeds found on the field at Welgevallen. It is also possible that the two herbicides could react differently when applied as post emergence leaf-applied herbicides as in the glasshouse study or when applied as pre-emergence soil applied herbicide. Additionally, the increased percentage control could be because of a better residual effect which keeps plots cleaner after six weeks than when propyzamide was not added.

Herbicides applied at high or lower rates can increase and reduce the efficacy when combined together. Baghestani et al. (2008) reported an increase in wheat yield when bromoxynil was applied with MCPA and clodinafop propargyl. At Roodebloem and Langgewens the application of proyzamide with the four herbicides tested did not result in significant positive or negative effects on the efficacy of the herbicides. Only glyphosate at Roodebloem was positively influenced by propyzamide in terms of dry mass reduction (Figure 5.15). Norris et al. (2001) have found that application of glyphosate in a tank mixture did not increase barnyard control when compared to glyphosate alone. Glyphosate applications made to large weeds or under adverse environmental conditions can result in unsatisfactory weed control. This might be the case with the poor control of weeds in Welgevallen and Langgewens. As mentioned previously, propyzamide is known to work well under cool and moist condition, this could explain why it did not work when it was mixed with other herbicides such as imazamox, glufosinate ammonium and glyphosate.

Addition of propyzamide increased the efficacy of glyphosate at Roodebloem in terms of dry mass reduction. Grichar and Prostko (2009) reported the effect of glyphosate combined with fungicide in controlling weeds in soybeans. Similar results were reported on the addition of imazethapyr to low doses of glyphosate that improved control of *Amaranthus rudis* and *Ipomoea hederacea*, but did not improve control of *Setaria faberi* and *Abutilon theophrasti* (Li et al. 2002). Herbicides atrazine, glufosinate ammonium and imazamox gave poor control of weeds when applied alone and in mixture with propyzamide. The observed poor control could be as a result of the level of weeds infestation on the field.

Field trial 2: Effect of different herbicide treatments on plots planted with herbicide resistant canola cultivars

Generally, weed control with triazine herbicides depends on soil moisture and rainfall around the time of spraying. Application of atrazine on plots that were planted with TT (triazine resistant) cultivars controlled weeds effectively at 6 WAT. According to Oilseeds WA (2006) triazine tolerant canola tolerates high levels of atrazine when applied at the seedling stage. However, the findings of the current study revealed a reduced control at 10 WAT. This could be a result of the weeds growing bigger than canola and eventually suppressing it. This explains the early effectiveness of atrazine at 6 WAT and the reduction at 10 WAT. Nonetheless, weed control by trifluralin and propyzamide increased by 20% after 10 weeks at Welgevallen in 2013 (Figure 5.18). This could be the result of residual action of trifluralin and propyzamide. Addition of propyzamide generally increased the efficacy of all the herbicides at 6 WAT in 2013 at Welgevallen (Figure 5.18) but it was significant only in the case of glufosinate ammonium. However, at 10 WAT addition of propyzamide caused a decrease in efficacy of glufosinate ammonium and glyphosate. According to Martin et al. (2001) canola should be kept weed free 17- 38 days after crop emergence (DAE). In 2014 propyzamide caused a significant increase in the efficacy of atrazine (Figure 5.19), similar to 2012. Most treatments were more effective at 6 weeks than at 10 weeks, probably because of weed growth or the seeds were dormant in the ground and as soon as the conditions became conducive they started growing.

At Roodebloem in 2013 very few weeds germinated. It could be a herbicide carry-over effect from the previous year. In 2014 time did not affect the efficacy of treatments. Addition of propyzamide caused a significant decrease in the efficacy of glyphosate (Figure 5.22).

At Langgewens in 2013, similar to the 2013 Roodebloem data, the addition of propyzamide to the four herbicides did not have any significant effect on the efficacy of the herbicides. In 2014 time did not affect the herbicide efficacy at 6 and 10 weeks. Imazamox efficacy was significantly increased at 6 WAT in combination with propyzamide. Imazamox efficacy was improved at 10 weeks.

Conclusions

The result suggests that propyzamide negatively affected atrazine efficacy on ryegrass in the glasshouse, but it did show a positive response when added to atrazine in the field experiments. Propyzamide positively affected efficacy of imazamox on ryegrass in the glasshouse, due to the fact that the ryegrass population used was resistant to imazamox. Overall, it does not appear to be economically feasible to apply propyzamide in mixtures with any of the herbicides*.*

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CHAPTER 6

GENERAL CONCLUSIONS

Glufosinate ammonium became available as post emergence herbicide in crops after the introduction of glufosinate ammonium resistant crops such as canola, cotton, corn, rice, soybean and sugarbeet (Duke 2005). As a relatively new herbicide in weed control, the introduction of glufosinate ammonium as post emergence application in herbicide resistant cultivars is still low compared to other well-known herbicides.

It is well established that environmental conditions and growth stage can have an important influence on the efficacy of herbicides. In order to obtain effective control of weeds, glufosinate ammonium should be applied to small growing weeds rather than large weeds. Small plants are generally easier to control than older plants. However, this was not true for the results observed in the first trials conducted on commercial and weedy ryegrass. Herbicide rates as low as 2.5 L ha^{-1} controlled the ten week old plants. Furthermore, weedy ryegrass followed the same trend as the commercial ryegrass. Therefore, if a 2.5 L ha^{-1} rate is able to control the ten week old plants then the recommended rate of 7.5 L ha^{-1} rate should also be effective.

Temperature in particular seems to have an effect on glufosinate ammonium efficacy. The findings of the current study indicated that glufosinate ammonium works effectively under cool temperatures for the control of ryegrass. This effect could be due to size of the ryegrass plants at spraying. Plants that were growing under cool temperatures were smaller than those growing under warm temperatures.

Adjuvants are used to enhance the herbicide efficacy in weed control. The most used fertiliser adjuvant is ammonium sulphate (AMS) which conditions hard water and makes it easier for the herbicide to penetrate into the plant for better weed control. Ammonium sulphate works effectively when combined with glyphosate herbicides. Addition of AMS increases the plant's ability to absorb herbicides. Young et al. (2003) reported that addition of AMS to glyphosate increased the absorption in velvetleaf. Addition of AMS fertiliser to bentazon enhanced the efficacy of bentazon on common cocklebur (*Xanthium pensylvanicum*) and black nightshade (*Solanum nigrum*) control (Abouzena et al. 2009). Previously, glufosinate ammonium plus AMS has proved to be more effective on horsenettle (*Solanum carolinense* L.) and common milkweed (*Asclepias syriaca*) control than when applied alone (Pline et al. 1999).The results of this study proved that AMS increase glufosinate ammonium efficacy in commercial ryegrass cultivars and the Eendekuil ryegrass population at specific dosage rates but not on the Moorreesburg and Hopefield populations. This could be because of the some levels of resistance the two populations have shown to glufosinate ammonium. In addition Maschhoff et al. (2000) reported increased glufosinate ammonium efficacy on *Echinochloa crus-galli*, *Setaria faberi* and *Abutilon theophrasti* when AMS was added to glufosinate ammonium. The increased control was species dependent.

The results of the current study on herbicide mixtures revealed that propyzamide did not influence the efficacy of any of the herbicides in the field. However, the glasshouse trial showed a different response of imazamox and glyphosate to the addition of propyzamide on the control of ryegrass. The ryegrass species used in the trial was resistant to imazamox, therefore addition of propyzamide influenced the efficacy of imazamox on ryegrass in the glasshouse. Interaction of atrazine and propyzamide resulted in antagonism, addition of propyzamide reduced atrazine efficacy compared to when only it was applied. The same trend was observed on glufosinate ammonium in a glasshouse for the control of ryegrass. Propyzamide addition did not influence the efficacy of atrazine, imazamox, glyphosate and glufosinate ammonium to a large extent. Therefore it is not economically viable to mix propyzamide with the herbicides.

Therefore it is imperative to investigate a full range of temperatures from 5 to 30 ^{0}C to determine the real optimal temperature for the best efficacy of glufosinate ammonium on ryegrass. More work should also be undertaken to determine the poor performance of AMS under field conditions. Environmental conditions should also be taken into consideration when applying propyzamide alone and also in combination with other herbicides.

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