**Glyphosate resistance in wild oats (*Avena fatua* L)**

By

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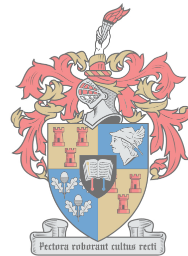


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# Declaration

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## ABSTRACT

Herbicide resistance is the ability of weed species to thrive and reproduce following applied recommended dosage of the herbicide that is toxic to the wild type. There is a world-wide occurrence in agriculture of weeds with high genetic diversity that have developed resistance to weed management, ryegrass (*Lolium spp.*) and wild oat (*Avena fatua*) included. Wild oats has developed resistance to commonly used herbicides which include clodinafop-propargyl, diclofop-methyl, fenoxaprop-P-ethyl, fluazifop-P-butyl, imazamox, iodosulfuron-methyl-sodium, sethoxydim, sulfosulfuron, and tralkoxydim in South Africa. There is an opportunity of using glyphosate to alleviate wild oats weed resistance problems when used in a pre-plant application. The herbicide has an uncommon mode of action but also has no wild oat resistance yet proven in South Africa.

There are precautions to be considered when applying glyphosate since it is a post emergence herbicide. Most precise recommended dosage rates of herbicides can be developed by determining the effect of environmental factors, different location and plant growth of weeds on efficacy of glyphosate. The principle objective of the study was to determine the effective dosage rate of glyphosate for the control of wild oats from different wild oat populations, after different germination processes and at different temperatures. Studies on the influence of three wild oat populations and two germination processes on glyphosate efficacy were carried out in Chapter 3. Screening of nine wild oat populations to observe the level of sensitivity to glyphosate was done in Chapter 4. Influence of temperature on glyphosate efficacy in six wild oat populations was investigated in Chapter 5. A study to establish if changes in temperature during the duration of the experiment influence the efficacy of glyphosate on wild oat plants was carried out in Chapter 6. A summary of and recommendations about the entire study are given in Chapter 7.

In chapter 3 wild oat populations from Malmesbury, Prieska and Eendekuil were germinated after treatment with ammonia gas, gibberellic acid and water and the germination rate and –percentage were calculated. Glyphosate (360 g a.e. $L^{-1}$ formulation) at dosage rates of 0, 270, 540 and 1080 g a.e $ha^{-1}$ were applied on the resulting wild oat populations treated with gibberellic acid and pure water that was growing in a glasshouse set at 20/25 °C night/day temperature. The germination test proved that ammonia and gibberellic acid treatments improved germination of the Malmesbury and Prieska populations compared to the water treatment. Gibberellic acid treatments had no influence on the survival of wild oat seedlings treated with glyphosate. Almost 100% control of the three wild oat populations was

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accomplished with 270 g a.e. $ha^{-1}$ of glyphosate indicating that there were no glyphosate resistant plants present in the samples tested. The results also proved that gibberellic acid can be used as a treatment to enhance germination of dormant wild oat seeds without influencing the efficacy of glyphosate.

In the first experiment of Chapter 4 wild oat populations from Lindley, Bethlehem A, Bethlehem B, Clarens, Prieska, Eendekuil A and B were germinated with gibberellic acid (1Mm $GA_3$) and the resulting seedlings treated with 0, 270, 540 and 1080 g a.e $ha^{-1}$ glyphosate dosage rates. Glasshouses were set at 20/25 °C night/day temperatures. Results of this study indicated that populations which were tested, showed no signs of resistance to glyphosate. The Prieska wild oat population showed some tolerance to glyphosate only at the 270 g a.e $ha^{-1}$ dosage rate. All populations showed high sensitivity to glyphosate at the recommended 540 g a.e $ha^{-1}$ dosage rate.

In the second experiment in Chapter 4 two wild oat populations from Malmesbury and Eendekuil C that previously showed signs of possible resistance were germinated with gibberellic acid (1Mm $GA_3$) and treated with 0, 270, 540 and 1080 g a.e $ha^{-1}$ glyphosate dosage rates. Glasshouses were set at 20/25 °C night/day temperatures. Results of this study indicated that the Malmesbury and Eendekuil C populations showed no resistance to glyphosate.

In Chapter 5 wild oat populations from Prieska, Bethlehem A, Malmesbury, Eendekuil A, B and C were germinated with gibberellic acid (1Mm $GA_3$) and exposed to 0, 180, 360, 540 and 720 g a.e $ha^{-1}$ glyphosate dosage rates. Plants were grown at four different temperature levels: 10/15 ºC, 15/20 ºC, 20/25 ºC and 25/30 ºC. Significant three-way interactions ($p<0.05$) between population, glyphosate dosage rates and temperature was noted in terms of the survival percentage, fresh plant mass $pot^{-1}$ and dry plant mass $pot^{-1}$. The Bethlehem A and Prieska populations had the highest survival rate percentage when glyphosate at 180 g a.e $ha^{-1}$ was applied, this was revealed under glasshouse temperatures of 20/25 ºC (Bethlehem A), 15/20 ºC (Prieska) and 25/30 ºC (Prieska). The lowest survival rate percentage appeared when all the population were sprayed with 360, 540 and 720 g a.e $ha^{-1}$ under all four temperatures in each glasshouse. These wild oat populations did not show resistance characteristics based on the results found.

In Chapter 6 wild oat populations from Bethlehem A and Malmesbury were germinated with gibberellic acid (1Mm $GA_3$) and exposed to 0, 180, 360, 540 g a.e. $ha^{-1}$ glyphosate dosage rates. The plants were grown at two different temperature regimes 15/20 ºC and 25/30 ºC. Then

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half of the plants grown in each glasshouse were switched from 15/20 ºC to 25/30 ºC and *vice versa* on the day that glyphosate was applied. Significant three-way interactions between populations, dosage rate and temperature regime occurred when the temperatures were changed. The Bethlehem A population showed the highest survival percentage of 50% when switched from 15/20 ºC to 25/30 ºC temperature at 180 g a.e. $ha^{-1}$ dosage rate. Malmesbury in contrast showed the highest survival percentage of 46% when switched from 25/30 ºC to 15/20 ºC at 180 g a.e. $ha^{-1}$ dosage rate. Low survival percentages occurred at dosage rates of 360 g a.e. $ha^{-1}$ and no plants survived at 540 g a.e. $ha^{-1}$ dosage rates. The wild oat populations therefore did not show any sign of glyphosate resistance based on the results found.

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## UITTREKSEL

Onkruiddoderweerstand is die vermoë van 'n onkruid om te oorleef en voort te plant na die toediening van die geregistreerde dosis van 'n onkruiddoder wat dodelik is teen die oorspronklike individue van die onkruid. Daar is 'n wye verspreiding van onkruide met hoë genetiese variasie in landbou, raaigras (*Lolium* spp.) en wildehawer (*Avena fatua*) ingesluit, wat weerstand teen chemiese middels ontwikkel het. Wildehawer het weerstand teen algemeen toegediende onkruiddoders soos clodinafop-propargyl, diclofop-methyl, fenoxaprop-P-ethyl, fluazifop-P-butyl, imazamox, iodosulfuron-methyl-sodium, sethoxydim, sulfosulfuron en tralkoxydim in Suid Afrika ontwikkel. Glifosaat kan gebruik word om probleme met onkruiddoderweerstand in wildehawer teen te werk in voorsaai toedienings. Die onkruiddoder het 'n unieke meganisme van aksie en daar is tans nog nie weerstand daarteen in wildehawer in Suid Afrika bewys nie.

Glifosaat moet met sorg aangewend word omdat dit 'n na-opkoms onkruiddoder is. Die mees akkurate dosisse vir onkruiddoders kan bepaal word deur die invloed van omgewingsfaktore, verskillende lokaliteite en plantgroeistadia van onkruide op die effektiwiteit daarvan vas te stel. Die hoofdoelwit van hierdie studie was om die effektiewe dosis van glifosaat vir wildehawerplante van verskillende wildehawerpopulasies, na verskillende ontkiemingsbehandelings en by verskillende temperature vas te stel. Ondersoeke na die invloed van drie wildehawerpopulasies en twee ontkiemingsbehandelings op die effektiwiteit van glifosaat is in Hoofstuk 3 uitgevoer. Nege wildehawerpopulasies is in Hoofstuk 4 ondersoek om die vlak van sensitiwiteit vir glifosaat vas te stel. Die invloed van temperatuur op die sensitiwiteit van ses wildehawerpopulasies is in Hoofstuk 5 ondersoek. 'n Ondersoek om vas te stel of verandering in groeitemperatuur na toediening van glifosaat die effektiwiteit van die glifosaat beïnvloed, is in Hoofstuk 6 gedoen. 'n Opsomming van die studie asook voorstelle vir verdere ondersoeke word in Hoofstuk 7 gegee.

In Hoofstuk 3 is saad van wildehawerpopulasies van Malmesbury, Prieska en Eendekuil ontkiem na behandeling met ammoniakgas, gibberelliensuur en suiwer water en die ontkiemingspersentasie en –tempo is bepaal. Glifosaat (360 g a.b. $L^{-1}$ formulasie) teen dosisse van 0, 270, 540 and 1080 g a.b $ha^{-1}$ is toegedien op die wildehawersaailinge wat gevestig het na die ontkiemingsbehandelings met suiwer water en gibberelliensuur en gegroei het in 'n glashuis met 20/25 °C nag/dag temperature. Die ontkiemingstoetse het bewys dat behandeling met ammoniakgas en gibberelliensuur die ontkieming van die Malmesbury en Prieska populasies verhoog het in vergelyking met die suiwer water behandeling. Gibberelliensuur behandelings

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het geen invloed op die oorlewing van wildehawer gehad wat met glifosaat behandel is nie. Amper 100% beheer van die drie wildehawerpopulasies is verkry met toediening van 270 g a.b. $ha^{-1}$ van glifosaat wat aandui dat daar geen glifosaatbestande plante aanwesig was in die monsters wat getoets is nie. Die resultate het ook bewys dat gibberelliensuur gebruik kan word om dormante wildehawersaad se ontkieming te bevorder sonder dat dit die effektiwiteit van glifosaat beïnvloed.

In die eerste eksperiment van Hoofstuk 4 is sade van wildehawerpopulasies van Lindley, Bethlehem A, Bethlehem B, Clarens, Prieska en Eendekuil A en B ontkiem met gibberelliensuur (1Mm $GA_3$) en die gevestigde saailinge is behandel met 0, 270, 540 and 1080 g a.b $ha^{-1}$ glifosaatdosisse. Glashuise is gereguleer by temperature van 20/25 °C nag/dag temperature. Die resultate van die studie het aangedui dat die populasies wat getoets is geen tekens van weerstand teen glifosaat getoon het nie. Die Prieska wildehawerpopulasie het tekens van toleransie teen glifosaat getoon by die 270 g a.b $ha^{-1}$ dosis. Al die populasies het volkome sensitiwiteit teenoor glifosaat getoon by die aanbevole dosis van 540 g a.b $ha^{-1}$.

In die tweede eksperiment van Hoofstuk 4 is twee vermoedelik weerstandbiedende wildehawerpopulasies van Malmesbury en Eendekuil C ontkiem met gibberelliensuur (1Mm $GA_3$) en behandel met 0, 270, 540, 1080 g a.b $ha^{-1}$ glifosaat dosisse. Glashuistemperature is beheer by 20/25 °C nag/dag. Die resultate het getoon dat die Malmesbury en Eendekuil C populasies geen weerstand teen glifosaat getoon het nie.

In Hoofstuk 5 is wildehawerpopulasies van Prieska, Bethlehem A, Malmesbury, Eendekuil A, B en C ontkiem met gibberelliensuur (1Mm $GA_3$) en blootgestel aan 0, 180, 360, 540 and 720 g a.b $ha^{-1}$ glifosaat dosisse. Plante is laat groei in glashuise by vier temperatuurvlakke nl. 10/15 ºC, 15/20 ºC, 20/25 ºC en 25/30 ºC nag/dag. Betekenisvolle drie-rigting interaksies ($p<0.05$) tussen populasie, glifosaat dosis en temperatuur is waargeneem in terme van die persentasie oorlewing, vars plantmassa $pot^{-1}$ en droë plantmassa $pot^{-1}$. Die Bethlehem A en Prieska populasies het die hoogste oorlewingspersentasie getoon wanneer glifosaat teen 180 g a.b $ha^{-1}$ toegedien is. Dit is waargeneem by temperature van 20/25 ºC (Bethlehem A), 15/20 ºC (Prieska) en 25/30 ºC (Prieska). Die laagste oorlewingstempo is waargeneem toe al die populasies gespuit is met 360, 540 and 720 g a.b $ha^{-1}$ by al vier temperatuurvlakke. Hierdie wildehawerpopulasies het dus ook geen teken van weerstand getoon nie.

In Hoofstuk 6 is wildehawerpopulasies van Bethlehem A en Malmesbury ontkiem met gibberelliensuur (1Mm $GA_3$) en blootgestel aan 0, 180, 360, 540 g a.b. $ha^{-1}$ glifosaat dosisse.

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Die plante is laat groei by twee verskillende temperatuurvlakke nl 15/20 $^{o}$C and 25/30 $^{o}$C nag/dag temperature in glashuise. Die helfte van die plante in elke glashuis is toe geskuif van 15/20 $^{o}$C na 25/30 $^{o}$C en *vice versa* op die dag dat glifosaat toegedien is. Betekenisvolle drie-rigting interaksies ($p<0.05$) tussen populasie, dosis en temperatuurvlak regime is waargeneem waar die temperature gewissel is. Die Bethlehem A populasie het die hoogste oorlewingspersentasie van 50% getoon toe dit geskuif is van 15/20 $^{o}$C na 25/30 $^{o}$C temperature teen die 180 g a.b. ha$^{-1}$ dosis. Die Malmesbury populasie, in teenstelling, het die hoogste oorlewingspersentasie van 46% getoon toe dit geskuif is van 25/30 $^{o}$C na 15/20 $^{o}$C teen die 180 g a.b. ha$^{-1}$ dosis. Lae oorlewingspersentasies het voorgekom by dosisse van 360 g a.b. ha$^{-1}$ en geen plante het oorleef by dosisse van 540 g a.b. ha $^{-1}$ nie. Gebaseer op hierdie resultate het die wildehawerpopulasies geen weerstand teen glifosaat getoon nie.

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# Preface

This dissertation consists of seven chapters. Chapter 1 gives brief background information of the study, supports the reason for conducting the study and outlines the objectives of the study. A review of literature that is relevant to the study is given in Chapter 2. Chapters 3 to 6 consists of experiments presented in complete paper format with an introduction, specific objectives, materials, method, results, discussion and conclusion. Chapter 7 summarises the findings from the experiments as well as provide recommendations. All the references cited in the study are found in the reference list at the end of each chapter. An appendices section containing outputs of statistical analyses of data and weather data presented in the paper is placed at the end of this dissertation.

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# CHAPTER 1

## Introduction

### 1.1. Background

Chemical weed control as a method of weed control has caused tremendous relief from the difficult task of mechanical weed control. Chemical weed control initiated with the use of acids, heavy metals and salts (Cobb and Kirkwood 2000). These chemical weed control products was initially non-selective products, but selective auxins herbicides in the form of 2,4-D and MCPA revolutionised weed science (Pieterse 2010). These products were highly effective as they controlled large amounts of weed species in crops such as wheat (Cobb and Kirkwood 2000).

The production of these two products encouraged the continuous development of many new products and there are currently more than 140 different discovered chemical formulations registered as herbicide products in South Africa (Anonymous 2004). The increased production and availability of herbicides resulted in farmers becoming too dependent on herbicides, and traditional weed control methods such as crop rotation, tillage, hand pulling etc. were neglected.

This resulted in large-scale use of herbicides (Gressel 1991). The dependence on herbicides rapidly increased without bearing in mind the effects that might be implemented by the continuous use of the herbicides, i.e. the capacity of weed species to adapt to such harsh environmental conditions (Gressel 1991). The appearance and evolution of herbicide resistant weed species was one of the crucial consequences of the large-scale use of herbicides (LeBaron and McFarland 1990; Lorraine-Colwill et al. 2001).

Therefore, herbicide resistance is a rapidly continuous developing world-wide problem that cause significant crop yield losses, which threatens the capability to successfully control weed populations and production costs are increased (Owen 2010; Powles and Yu 2010; Tranel et al. 2011). Herbicide resistant weed species are also most common in both annual and perennial crops in South Africa, but particularly in the south-western Cape Province (Pieterse 2010).

However, resistance to selective herbicides appear to be superior in annual crops, whereas resistance to non-selective herbicides, especially glyphosate, is a significant aspect obstructing effective weed management in perennial crops such as vineyards (Eksteen 2007). According to Heap (2014), 220 weed species world-wide has currently evolved to become resistant to at least

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one herbicide. Currently a lot of research is focused on the ability of herbicides to effectively control weed populations and plants that show a certain level of resistance to herbicides.

A large number of weed species globally evolved to become resistant to at least one herbicide. This evolution of resistance limits options available in the management of weed infestations in agricultural fields and both smallholder and large-scale farmers are affected by high weed population densities that are causing huge losses in their crops (Heap 2014). Weeds in crops cause yield reductions due to competition.

Weeds are anticipated to cost the Australian grain industry Australian $3.3 billion per year in lost yield. Weeds are costing Australian grain growers an average of $146 ha$^{-1}$ in expenditure and yield losses. Average expenses on weed control, including both non-herbicide and herbicide practices, is $113 ha$^{-1}$. Weeds are causing up to 2.76 million tonnes of grain in yield losses. Wild oats (*Avena* spp.) is one of the most expensive weeds in terms of total yield losses (GRDC 2017). In South Africa, the decrease in crop production due to all weed infestations is 16.6% (Oerke et al 1994).

Wild oats also infect grain samples and costs of cleaning are estimated at $1Million per year (Medd 1996). Wild oat is one of the most economically harmful weeds especially in production of small grains in North America where they produce an annual loss of over $500 million due to application of herbicides to manage resistance (Beckie et al. 2012).

Wild oats is a greatly competitive and difficult to control weed species, which has a huge potential to become a herbicide resistant weed. In South Africa, it has been found to be resistant to two mechanism of action herbicide groups viz. acetolactate synthase (ALS) inhibitor and acetyl-CoAcarboxylase (ACC-ase) inhibitors (Cairns and Hugo 1986). Resistance to the well-known systemic herbicide, glyphosate has not been reported (www.weedscience.org). Wild oats is considered good forage for animal feed, whereas it is a harmful and highly competitive weed species in crops (Cairns and Hugo 1986).

According to Heap (2014) the first case of herbicide resistance in South Africa was discovered by Cairns and Hugo (1986) who found a wild oat (*A. fatua L*) population that was resistant to diclofop-methyl. World-wide, the reduction of highly effective herbicides used in the management of weed population due to herbicide resistance has had economic consequences in many crop production systems (Sinde et al. 2004).

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This resulted into limited options of managing and controlling weed populations consequently causing the exponential growth of weeds, particularly the most aggressive wild oats. The manifestations of herbicide resistance have become a common incidence in South Africa where wild oat weed species are categorised among the most troublesome weeds (Cairns and Hugo 1986).

The ARC report mentioned that wild oats weed species are common weeds in the southern parts of South Africa. It is also a winter weed which has become a major problem in the irrigation areas of the Northern Cape (ARC Report 2014). Although no confirmed cases of wild oats resistant to glyphosate has been reported, circumstantial evidence point to the possibility of glyphosate resistant populations in the south-western and Northern Cape. This study therefore aims to investigate the possible occurrence of glyphosate resistant wild oats in South Africa.

## 1.2. Study objectives

a. As wild oats are often dormant

- i) To find the most effective pre-germination treatment to germinate wild oat seeds for herbicide resistance testing. (Chapter 3)
- ii) To investigate whether the selected pre-germination treatment have any effect on the plants that can influence glyphosate efficacy. (Chapter 3)

b. Screening wild oat populations for glyphosate resistance. (Chapter 4)

c. To establish if temperature influences the efficacy of glyphosate on wild oat plants. (Chapter 5)

d. To establish if change of temperature influence the efficacy of glyphosate on wild oat plants. (Chapter 6)

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## References

Anonymous. 2004. A guide to the use of herbicides. 18th edition. Directorate: Food Safety and Quality Assurance, Department of Agriculture, Republic of South Africa.

ARC (Agricultural Research Council) report. 2014. http://www.arc.agric.za/arc-sgi/Pages/Crop%20Protection/Weed-Control-in-Small-Grain-Production.aspx [accessed 20 June 2017]

Cairns ALP, Hugo KJ. 1986. Differential tolerance of Western Cape wild oat biotypes to diclofop-methyl and mixtures containing diclofop-methyl. Final report, Department of Agronomy and Pastures, University of Stellenbosch, South Africa.

Cobb AH, Kirkwood RC. 2000 (eds). Herbicides and their Mechanisms of Action. Sheffield Academic Press, United Kingdom; CRC Press, United State, Africa and Canada. pp 1–24.

Beckie HJ, Francis A, Hall LM. 2012. The Biology of Canadian Weeds. 27. *Avena fatua* L. (Updated). *Canada Journal Plant Science.* 92: 1329–1357.

Eksteen FH. 2007. Resistance of ryegrass (*Lolium* spp) to paraquat and glyphosate in the Western Cape. PhD dissertation, Stellenbosch University, South Africa.

GRDC (Grains Research and Development Corporation). 2017. Comprehensive study reveals cost of weeds to northern growers. https://grdc.com.au/news-and-media/news-and-media-releases/north/2016/04/comprehensive-study-reveals-cost-of-weeds-to-northern-growers [accessed 17 May 2017].

Gressel J. 1991. Why get resistance if it can be prevented or delayed. In: Caseley JC, Cussans GW, Atkin RK (eds). *Herbicide resistance in weeds and crops.* Butterworth Heinemann, Oxford. pp 1-26.

Heap I. 2014. Global perspective of herbicide-resistant weeds. *Pest Management Science* 70: 1306–1315.

Lebaron HM, Mcfarland J. 1990. Overview and diagnosis of herbicide resistance in weeds and crops. In: Green M, LeBaron HM, Moberg WM (eds). *Managing resistance to agrochemicals, from fundamental research to practical strategies.* ASC Symposium Series, Washington D.C.

Lorraine-Colwill DF, Powles SB, Hawkes TB, Preston C. 2001. Inheritance of glyphosate resistance in *Lolium rigidum* (Gaud). *Theoretical and Applied Genetics* 102: 545-550.

Stellenbosch University https://scholar.sun.ac.za

Medd RW, 1996. Ecology of Wild Oats. *Plant Protection Quarterly* 11: 185-187.

Oerke EC, Dehne HW, Schonbeck F, Weber A. 1994. *Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops*. Elsevier, Amsterdam.

Owen MDK. 2010. Herbicide resistance. In: Kempken F, Jung C (eds). *Genetic modification of plants*. Agriculture, Horticulture and Forestry 64. Springer-Verlag, Berlin. pp 159–177.

Pieterse PJ. 2010. Herbicide resistance in weeds: a threat to effective chemical weed control in South Africa. *South African Journal of Plant and Soil* 27: 66-73.

Powles S, Yu Q. 2010. Evolution in Action: Plants Resistant to Herbicides. *Annual Review of Plant Biology* 61: 317-347.

Sinde J, Jones R, Hester S, Odom D, Kalisch C, James R, Cacho O. 2004. The Economic Impacts of Weeds in Australia. CRC for Australia Weed Management. *Technical Series*. p 8.

Tranel PJ, Riggins CW, Bell MS, Hager A.G. 2011. Herbicide resistances in *Amaranthus tuberculatus*: A call for new options. *Journal of Agricultural and Food Chemistry* 59: 5808–5812.

www.weedscience.org. [accessed 06 August 2018].

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# CHAPTER 2

## 2. Literature Review

### 2.1. *Avena* species (wild oats) history and origin

The first person to describe oats was Tounefort, who in 1700 established the genus *Avena* (Malzew 1930). Later, Linnaeus (1753) distinguished four oat species: *Avena sterilis, A. fatua, A. sativa* and *A. nuda*. The very first indication of wild oats was discovered from the 12$^{th}$ century Egyptian family (1788-2000 B.C) (Cairns and De Villiers 1984). Tackolm and Drar (1941) recognized these wild oats as *Avena sterilis* and Avena *fatua.* It is assumed that these wild oats were weeds based on Coffman (1961a and b) who pointed out that the earliest Egyptians had no activity of oat production. While the exact native species of wild oats is still an on-going issue, researchers reached a mutual agreement that it originated in South-west Asia. *Avena fatua or A. sterilis*, all hexaploid (oats that have six sets of chromosomes) oats, contain one native centre which is in South-west Asia. According to Malzew (1930), oats were discovered as weeds in the earliest cereal samples. Compared to barley and wheat, oats cultivation is apparently a more recent practice. In this manner oats spread happened together with barley to the western-most parts of Europe by 1500 B.C (Cairns and De Villiers 1984).

The articulations of the florets and its inheritance have been an important aspect in the evolution of oats (King 1966). Mutation that took place in the earliest population of weeds which preferred florets with no articulation (non-articulate florets), resulted in a selective advantage and the grains ended up harvested with the main crop and was afterwards sown in the following years (Sampson 1954). These repetitive cycles of selection resulted in the cultivation and approval of various *Avena* spp. as crops. Thurston (1954) cited that in *A. fatua* species the process of germination usually occurs in both autumn and spring. It is widely distributed in the cooler climatic conditions of the northerly altitudes in Asia and Europe. *Avena ludoviciana* on the other hand is a serious weed which is restricted only to southern England and the warmer parts of Europe. In addition Baum et al (1972) discovered *A. sterilis* to be the most global species adapted to a wide variety of conditions. These species have been reported to be a strong competitor with the main crops. *Avena fatua* have been discovered to have entered the northern most parts of the Mediterranean location. Baum et al. (1972) viewed *A. fatua* as of a western and central Asiatic rather than of Mediterranean origin

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## 2.2 Wild oat species distribution in the Western Cape

*Avena fatua*, *A. sterilis* and *A. barbata* were identified in Cape wheat fields (Louw 1930). He commented that although *A. barbata* was identified to be fairly widespread, it did not grow and infested crop fields in large numbers. Four wild oat species in the Western Cape was found by Cairns (1974), with *A. fatua, A. ludoviciana, A. sterilis and A. barbata* contributing approximately 70%, 20%, 5% and 5% respectively to the samples collected in cereal fields. *Avena fatua* is commonly found in the southern Cape but appeared less frequently in other provinces of the country (Henderson and Anderson 1966). Thurston and Phillipson (1976) confirmed the identification of the wild oat species present in the Western Cape and commented that the samples from this site revealed similarities with Australian wild oat species. Differences amongst the four wild oat species present were in the colour of the seed (Cairns 1974).This was notably so in the case of *A. fatua* and *A. sterilis* whereas *A. ludoviciana* and *A. barbata* were more uniform in colour. *Avena sterilis*, were heavily populated in the higher rainfall areas around Stellenbosch, though *A. fatua* mostly occurred throughout the region including the Swartland, which is commonly a drier area, where it was dominant. This suggests that the local *A. fatua species* is more drought resistant compared to other species (Cairns1974).

## 2.3*. Avena fatua* (Wild oats)

Wild oats (*Avena fatua*) is a noxious weed in many areas. The wild oat plant has been used for bread making and flavouring alcoholic drinks and was considered a good animal feed (Fogelfors 1984). However wild oats plants are greatly competitive and uncontrolled wild oats can decrease yields up to 80%. An increase in yield losses occur when wild oats start to emerge at the same time as the crop. Wilds oats also lowers the quality of most field crops (Department of Agriculture and Food 2015).

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**Figure 2.1:** Mature wild oat plants in a wheat field.
(Grains Research and Development Corporation 2015)

**Taxonomy and Nomenclature** (ITIS Standard Report 2017)

Kingdom: Plantae
Taxonomic Rank: Species

Synonym(s):
*Avena fatua* var. glabrata Peterm.
*Avena fatua* var, vilis (Wallr) Hausskn
*Anelytrum avenaceum Hack.*
*Avena intermedia* Lindgr.
*Avena intermedia* T. Iestib.
*Avena lanuginose* Gilib.
*Avena meridionalis (*Malzev) Roshev
*Avena paten* St-Lag.
*Avena pilosa Scop.*
*Avena septentrionalis Malzev*
*Avena vilis* Wallr
*Avena fatua* ssp. Meridonalis Malzev.
*Avena fatua* var. intemedia Hartman.
*Avena fatua var. intemedia* Husn.
*Avena fatua var. intemedia* Vasc.
*Avena fatua* var. vilis (Wallr) Malzev.

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*Avena sativa* var. *fatua* (L.) Flori
*Avena sativa var. sericea Hook.f.*

Common Name(s): Wild oat
Wild oats
Flax grass
Oat grass
Wheat oats

**Taxonomic Hierarchy**

| | |
|---|---|
| Kingdom | Plantae – plantes, Planta, Vegetal, plants |
| Subkingdom | Viridiplantae |
| Infrakingdom | *Streptophyta* – land plants |
| Superdivision | *Embryophyta* |
| Division | *Tracheophyta* – vascular plants, tracheophytes |
| Subdivision | *Spermatophytina* – spermatophytes, seed plants, phanérogames |
| Class | *Magnoliopsida* |
| Superorder | *Lilianae* – monocots, monocotyledons, monocotyledons |
| Order | Poales |
| Family | Poaceae – grasses, graminées |
| Genus | *Avena* L. – oat, oats |
| Species | *Avena fatua* L. – wild oat, wild oats, flax grass, oat grass, wheat oats |
| Subspecies | *Avena fatua* ssp. *fatua* L. |

Wild oat grows upright and is a cool season annual grass with open-branched, nodding flower clusters. Wild oat inhabits agricultural lands, grassland, crop fields, orchards, vineyards, gardens, roadsides, and other disturbed fields. It makes good forage for livestock feed. Wild oats can become troublesome in agriculture when it invades and lowers the quality of a field crop, or competes for resources with the crop plants. It takes very few wild oat plants to cause a significant reduction in the yield of wheat or cultivated oat field, even though the seeds are a type of oat (Moore 1968; Welsh 1974).

Wild oats has a self- pollinated reproductive behaviour. Self-pollination is the transfer of pollen from the anther to the stigma of the same flower or another flower on the same plant (Auld and Medd 1987). Self-pollination restricts the variation of progeny. Self-pollination can be

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advantageous, permitting plants to spread beyond the range of appropriate pollinators or produce offspring in spaces where pollinator populations have been seriously decreased. According to Orson (2015) wild oat are naturally self-pollinated, however some out-crossing can occur. This result in seed from different habitats and from within the same habitat having different biology. Different field stocks of similar wild oats can germinate at different times, creating variation of emergence patterns between and within fields.

Negative aspects such as competitiveness, producing large number of seeds, easily developing resistance to herbicides, avoiding early herbicide applications through later germination, representing large cost to cropping, easily spread as contaminants of grain, hay and machinery and acts as a host for a number of important cereal diseases and pest contribute to wild oats being a major weed (GRDC 2010). Wild oats is often more competitive than wheat (Martin and Field1988). Shoot biomass and competitiveness are enhanced by ploughing and high levels of fertilization with nitrogen (Bozic 1986) and phosphorus (Konesky et al 1989). Wild oats has a high uptake of phosphorus and nitrogen due to its large root system (Haynes et al 1991).

Wild oats is a host of cereal cyst nematode (*Heteroderma avenae*) and cereal stem nematode (*Ditylenchus dipsaci*). They can be infected with barley yellow dwarf virus and are susceptible to attack by several insects and fungi that affect cereals (Orson 2015). Wild oats is a host of Root lesion Nematode (*Pratylenchus thorneii*) allowing some build-up of numbers. It is also a carrier of other nematodes and the bacteria related with annual rye grass toxicity (Medd 1996).

### 2.4. Glyphosate

Glyphosate is a well-known non-selective, systemic herbicide. The main purpose of this herbicide is to kill weeds and grasses that compete with crops. Glyphosate was brought into the market in 1974. Therefore, glyphosate has been in commercial use for 43 years now. Crops that are transgenic glyphosate resistant (maize, canola, cotton etc.) were introduced in 1995 to agriculture (Duke and Powles 2009). These crops have been genetically modified and allowed for glyphosate to be used in weed management as a selective herbicide on glyphosate-resistant crops (Yu et al. 2007). Glyphosate is worldwide known and applied as a highly effective, systemic, broad-spectrum type of herbicide which controls annual and perennial weeds (Yu et al. 2007; Duke and Powles 2009).

A glyphosate [N-(phosphonomethyl)-glycine] molecule (Figure 2.2) is a derivative of the amino acid glycine which is the smallest amino acid molecule found in proteins (Nandula 2010).

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According to Székács and Darvas (2012), to encourage an optimal translocation in the plant, the molecule is most often formulated in the form of ammonium, isopropylammonium, potassium, sodium or trimesium salts in order to further increase its solubility in water.

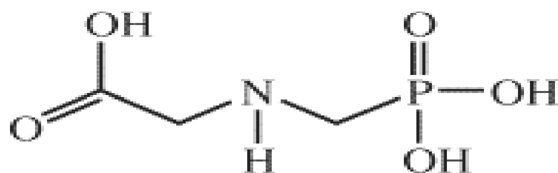


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**Figure 2.2:** The chemical structure of glyphosate (Székács and Darvas 2012).

In the glyphosate molecule (Figure 2.2) a phosphonomethyl group will substitute one of the amino hydrogen atoms of glycine. When the glyphosate is absorbed by plants it restricts and inhibits the activity of the enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) which is an enzyme at work within the chloroplasts of plants and this enzyme appears at the start of shikimic acid pathway that converts simple carbohydrates. This enzyme creates an essential part of the shikimate biosynthesis pathway which leads to the production of shikimic acid. The shikimic acid paves the way of the formation of the aromatic amino acids tryptophan, tyrosine and phenylalanine (Yu et al. 2007; Preston et al. 2009; Maeda and Dudareva 2012).

### 2.4.1. Glyphosate mechanism of action

Glyphosate herbicide kills plants that are susceptible by interfering with the syntheses of the combination of the aromatic amino acids phenylalanine, tyrosine and tryptophan and it does this by binding and blocking the action of 5- enolpyruvylshikimate-3-phosphate synthase (*EPSPS)* enzyme of the shikimate pathway (Figure 2.3). Constraining the enzyme causes shikimate to gather in plant tissues and transfers the energy and resources away from other processes. The growth of plants can cease within hours of application while it takes several days for the leaves to start to turn yellow (Hock and Elstner 2004)

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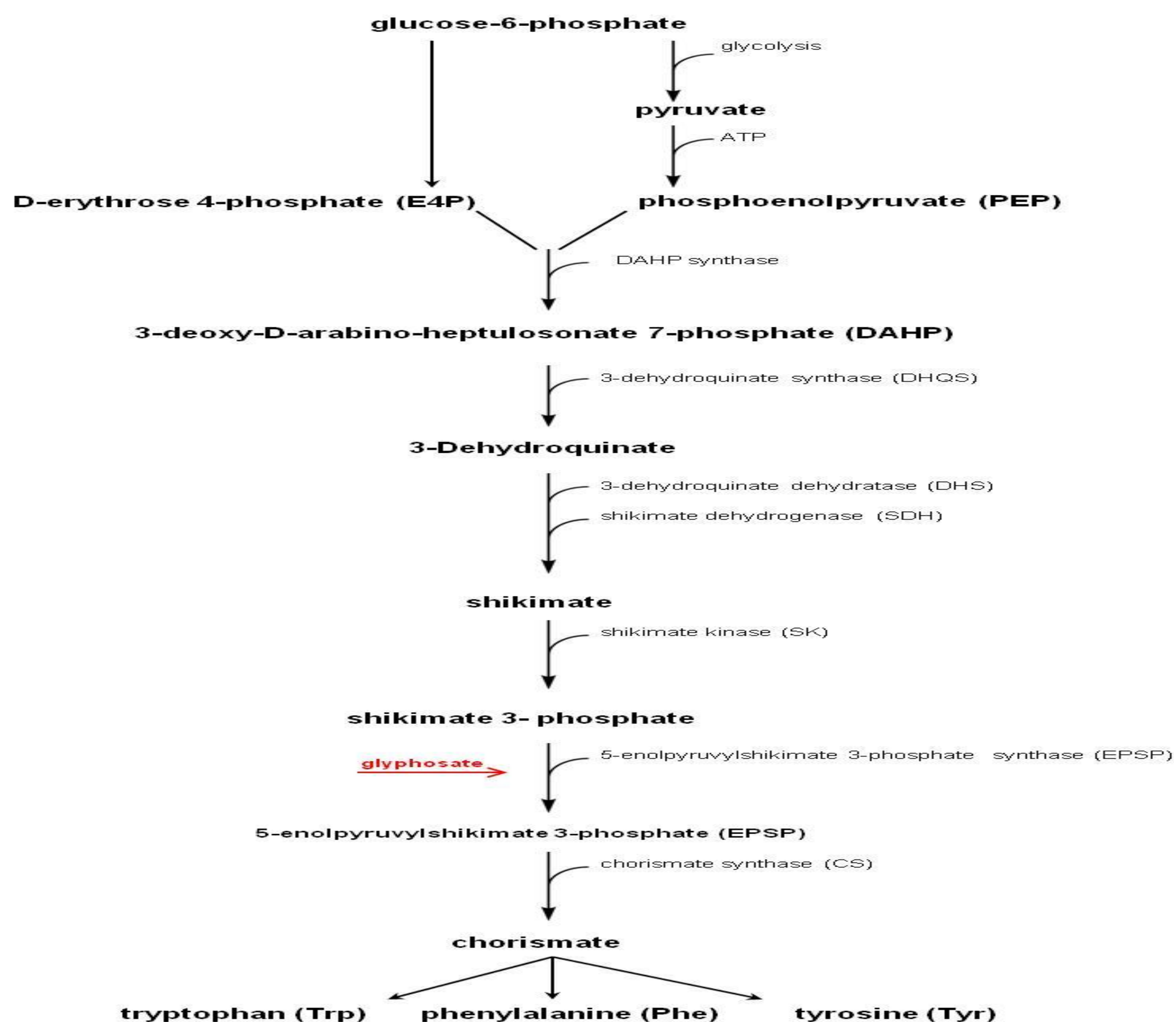


**Figure 2.3:** The shikimate pathway (from Azania et al. 2013).

The EPSPS enzyme catalyzes the reaction of shikimate-3-phosphate (S3P) and phosphoenolpyruvate to form 5-enolpyruvyl-shikimate-3-phosphate (EPSP) (Steinrücken and Amrhein1980). The inhibition of EPSPS by glyphosate is accomplished by interfering with the shikimate pathway (Pavlović et al. 2011; Maeda and Dudareva 2012). The molecule that binds EPSPS is the PEP which successfully completes the shikimate pathway (Székács and Darvas 2012). The accumulation of shikimic acid in the chloroplast is due to the inhibition of EPSPS. These result in a little amount of EPSPS and metabolic products being produced. The decrease leads to a high decline in the important aromatic amino acids which are usually produced by plants (Powles and Preston 2006; Yu et al. 2007).

The critical building blocks for protein in both animal and plant living cells are amino acids which consist of tryptophan, phenylalanine and tyrosine. The production of plant products like

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alkaloids and auxins are limited to function for a short period without the presence of these amino acids. The productions of this product are crucially important for plant development, production and defense mechanism etc. (Maeda and Dudareva 2012). The effects of glyphosate can be visually identified by the continuous wilting, stunted growth, discoloration, morphological deformation and wrinkling of leaves of the affected plant which usually develop a browning of the above soil surface growth and deterioration of below soil surface plant material (Schuette 1998).

### 2.4.2 Uptake and translocation of glyphosate in plants

Glyphosate is mainly applied through foliar application as it is absorbed through foliage and it is a common post-emergence herbicide. The glyphosate is sprayed directly onto the leaves; the molecules of glyphosate are absorbed into plant cells from the leaf surface. Thereafter the molecules are translocated to the meristematic tissues via the phloem where the molecule works in the meristem (Schuette 1998). The effectiveness of glyphosate as a post-emergence herbicide depends on its ability to reach living plant material and on the dosage rate. Climatic conditions and vigour of plants also greatly influence phloem transport and translocation of glyphosate to the meristem tissue (Nandula 2010).

## 2.5 Herbicide resistance

Herbicide resistance is defined by Moss (2002) as the inherited ability of plants to survive and reproduce following exposure to a herbicide dose normally lethal to the wild type.The term herbicide resistance usually indicates the occurrence of resistance in weed species (Anderson 1996).

### 2.5.1. Different types of herbicide resistance

There are six different types of resistance as follows:

#### 2.5.1.1. Cross-resistance

Cross-resistance is well documented or known (Burnet et al. 1991; Burnet et al. 1993; Burnet et al. 1994a). Cross-resistance is a term that may be used to indicate resistance to at least two or more herbicides from a variety of chemical groups resulting from the presence of a single resistance mechanism (Gressel 1988). Burnet et al. (1994b) stated that, cross-resistance is a phenomenon whereby weed species become resistant to distinct classes of herbicides as an outcome of selection by a chemically unrelated herbicide.

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This phenomenon has been observed in several different instances, with the perfect example being that of cross-resistance to chlorsulfuron in a biotype of rigid ryegrass (*Lolium rigidum)* subjected to diclofop and trifluralin (Heap and Knight 1986; Heap and Knight 1990). Cross-resistance mostly occurs to herbicide groups of which the population has never been subjected to yet. Currently populations of rigid ryegrass display resistance across 16 varieties of herbicide chemical classes with 11 different modes of action (Preston et al. 1996). Cross-resistance takes place where at least one mechanism of resistance accommodates numerous herbicide compounds as a result of a singular sector. The terminology cross-resistance is commonly used to indicate an evolutionary occurrence caused by a single selection (Powles and Preston 2004).

#### 2.5.1.2. Target site cross-resistance

In most cases, target-site resistance develops under a regime of high herbicide dosage rates, confers a high degree of resistance and it is commonly controlled by a single or relatively few genes and has up to date received by far the greater amount of attention by the herbicide industry (Gressel 1991). Single-gene resistance was the first to appear and is also the easiest to understand. According to Devine (1997), the enzyme acetylcoenzyme A carboxylase (ACCase) is the target site of two major groups of synthetic herbicides, the aryloxyphenoxypropionates and cyclohexanediones. As cited by Devine et al. (1993) the target site is usually the site to which the herbicide binds, or with which it interferes in some manner, resulting in death of the plant.

A yellow starthistle plant population in Washington State evolved resistance to picloram, a picolini acid herbicide when that population was subsequently exposed to clopyralid, another picolini acid herbicide to which the population showed resistance to (Prather et al 2000). Numerous cases of herbicide resistance in weeds is influenced by a single mutation in the targeted enzyme or protein or a place in the plant where herbicides bind and disrupt normal functions of a plant; this resulted in herbicide resistance or cross-resistance. Infrequently does a single plant show resistance to several herbicides that affect different target fields (Prather et al 2000).

Although genetic modification takes place in the target fields of the key enzymes, the herbicide might still be able to be effective. The mutant enzyme usually acts with decreased efficacy, resulting in decreased fitness of the mutant when it competes with the wild biotype. (Gressel 1993).

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Target site cross-resistance is a result of a modification of the herbicide-binding site, which block the different herbicides from binding to the target. This form of cross-resistance will take place in herbicides with similar target site of action that bind to the similar domain on the target. Target site cross resistance does not necessarily result in resistance to all herbicide classes with a similar mode of action (De Wet and Cairns 2005).

In most target site resistance cases weeds have resistance to at least one of a group of herbicides. Those herbicides can be related or have related chemical families. Weeds may possess one mechanism that provides the ability to withstand herbicide from a variety of chemical families. For instance, a mutation in the enzyme acetolactate synthase (ALS) may influence resistance to the sulfonylurea and imidazolinone herbicide families. There is quite a variety of mutations and amino acids substitutions that provides a plant with resistance to at least one family of ALS inhibitor herbicides (USDA 2017).

*Rapistrum rugosum* (turnip weed) is a weed species, which is most usually controlled by tribenuron-methyl (TM), a sulfonylurea (SU) which belongs to the acetolactate synthase (ALS) inhibiting herbicides group. Numerous cases of unexplained control failure of *R. rugosum* by TM have been noticed (Hatami et al 2016). An experiment was conducted by Hatami et al (2016) where the concentration of TM for 50% inhibition of ALS enzyme activity *in vitro* showed a high level of resistance to the herbicide (Resistant Factors were from 28 to 38) and cross-resistance to sulfonyl-aminocarbonyl-triazolinone (SCT), pyrimidinyl-thiobenzoate (PTB) and triazolopyrimidine (TP) occurred. Different TM resistance levels between the *R. rugosum* populations were found. ALS mutation (Pro197Ser) is likely to be the cause of the cross resistance found in those populations to four of the five families of the ALS inhibitors group.

#### 2.5.1.3. Non-target site resistance and non-target site cross-resistance

Non-target site resistance is resistance due to several mechanisms other than a target-site modification. Non-target site resistance can be caused by mechanisms such as enhanced metabolism, decreased rates of herbicide translocation, sequestration and other mechanisms (Powles and Yu 2010; Preston 2004; Délye 2013). These mechanisms decrease the amount of herbicide reaching the target site. Non-target site cross-resistance occurs when a single mechanism provides resistance across herbicides with a variety of modes of action. Such mechanisms are usually not related to the herbicide target site. Examples are cytochrome P450-based non-target site cross resistance and glutathione transferees-based resistances,

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which degrade a spectrum of herbicides that have different fields of action (Powles and Shaner 2001).

In plants that display non-target-site resistance (NTSR) to Acetohydroxyacid/ALS inhibitor synthase (AHAS) herbicides, the exact amount of herbicide from a dosage that reach the AHAS is decreased to under the toxic level which allow plants to withstand the herbicide dosage. For non-target-site resistance to AHAS inhibitor herbicides, variable herbicide foliar uptake has not been observed as a resistance mechanism, with only a few exceptions where a slight effect of this mechanism was pointed out (White 2002). Differential herbicide translocation has also not been indicated to influence resistance to AHAS inhibitor herbicides (Riar et al 2013).

#### 2.5.1.4. Intergroup cross-resistance

Intergroup cross-resistance takes place when only one or a single evolutionary pressure selects resistance to herbicides which is different in both familiar and unfamiliar target fields. This may occur by co-ordinately changing a single enzyme or a group of enzyme that degrade a variety of herbicides, as well as by unknown mechanisms (De Wet and Cairns 2005).

#### 2.5.1.5.. Multiple-resistance

Multiple-resistance (MR) implicates that numerous resistance mechanisms evolved separately in time, due to separate selections by independent herbicides, occurring in the same individuals. Multiple-resistance may be used to define the result of multiple evolutionary occurrences due to constant sequence selections. Multiple-resistance can be described as the expression in individuals and populations of more than one resistance mechanism. Multiple-resistance weed species may contain from two to many different resistance mechanisms and may also show resistance to less or more herbicides. The easiest situation is where a single weed species exhibit two or more different resistance mechanisms, which confer resistance to one herbicide, or a group of herbicides. Several complicated cases where two or more different resistance mechanisms have been selected in a constant sequence by different herbicides and conferred resistance to a group of herbicides have been already shown (Powles and Preston 2004).

Plants of rigid ryegrass in Australia have multiple resistance to a number of herbicides in the cyclohexanedione, sulfonylurea, dinitroanline, triazine, substituted urea and triazole classes to which the weed has not been exposed. These classes include all of the herbicides currently registered in areas where this weed is a problem. The mechanism of MR in rye grass include

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changes to the herbicide fields of action and the detoxification of herbicide by plant enzymes called cytochrome $P_{450}$ mixed function. This family of enzyme is similar to those found in many insects resistant to insecticides (Prather et al 2000).

Weeds with multiple resistances generally inherit more than one mechanism that endow weeds the ability to withstand herbicide from different chemical families. In this case, herbicide alternatives become very limited. A *Kochia scoparia* population was discovered that was target-site resistant to the PS II inhibitor herbicide, triazine, and to ALS inhibitor herbicides. The plant carried two mutations, one for resistance to each class of herbicide (USDA 2017). Weed populations also may have multiple herbicide-resistance mechanisms. For instance, a rigid ryegrass population in Australia possess target-site resistance to ACCase and to ALS inhibiting herbicides. Rigid ryegrass has metabolism-based resistance to a number of other herbicides from different chemical classes. (USDA 2017).

#### 2.5.1.6. Creeping multi-factorial resistance

This certain type of resistance is characterized by a gradual, increasing or creeping increase in the $LD_{50}$ of the entire population as a result of several applied treatments. Different researchers have ascribed such creeping resistance due to gradual accumulation of genes, alleles that each provides small increases in resistance. This has been well reported in Australia for diclofop-methyl resistance in field populations of rigid ryegrass weeds. However, low rates are usually used and no target site resistance was initially discovered. Creeping resistance have been discovered sooner even though the target site resistances have masked their incidences, up until the uncontrolled creeping resistance covered most of the wheat fields in Australia (Powles and Preston 2004).

### 2.6. Glyphosate resistance

Preston et al. (2009) reported that Italian ryegrass (*L. multiflorum*) is specifically resistant to glyphosate. Rigid ryegrass was distributed to numerous countries on more than five continents across the world. Agriculture comprises of intensive production systems which apply survival pressure on any available weed species. An intensive application of agriculture weed management techniques by means of glyphosate mostly facilitates the evolution of glyphosate resistance specifically when these practices are misused. In Italian and rigid ryegrass, according to Preston et al. (2009), at least two mechanisms of resistance have been identified. In 1996, the first reported case of glyphosate resistance in ryegrass was a population of glyphosate

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resistant rigid ryegrass biotype in Australia (Pratley et al 1996). Glyphosate resistance has been pointed out worldwide including the USA (Perez-Jones et al 2005), South Africa, Israel, Italy, Spain, France (Heap 2011) and the most recent cases appeared in Australia (Owen and Powles 2010). Biotypes of Italian ryegrass resistant to glyphosate were also discovered in Chile (Perez and Kogan 2003), USA (Perez-Jones et al. 2005), Spain, France, Brazil and Argentina (Heap 2011).

### 2.6.1. Cases of glyphosate resistance in rigid ryegrass.

In 1996, rigid ryegrass resistance was initially reported in Australia and rigid ryegrass resistance was again reported in 2001 in South Africa. These occurrences have since been widely observed in numerous other countries including Israel, USA, France and Italy (Heap 2009). Numerous greenhouse trails were conducted with the main purpose to study dose response and inheritance characteristics of the progeny of a cross between resistant and susceptible lines (Simarmata et al. 2005).

Rigid ryegrass reproduces by cross fertilization. Repetitive selections through four and eight generations respectively had to be carried out by Simarmata et al. (2005) before finally a homozygous susceptible and a homozygous resistant parent was bred. In the end, Simarmata et al. (2005) discovered that this rigid ryegrass from California had nuclear, semi-dominant inheritance characteristics. They discovered that resistance inheritance comprises of clearly more than one mutant allele and that it is transferred through pollen.

Wakelin and Preston (2006) studied numerous populations of rigid ryegrass in Australia. Their study also led to the conclusion that the nuclear genome was a factor responsible for encoding glyphosate resistance in each of the eight populations of rigid ryegrass in their studies. However, amongst the populations studied, the level of dominance which was observed had a range from partial to total. In contrast to Simarmata et al. (2005), Wakelin and Preston (2006) concluded that a single dominant allele is responsible for the control of glyphosate resistance.

This study of Simarmata et al. (2005) was followed by Simarmata and Penner (2008) who studied the role of glyphosate metabolism in the plants bred for resistance. They again assessed the effect of glyphosate on the actions of EPSPS and the EPSPS gene in resistant and susceptible rigid ryegrass biotypes. There were no significant differences found in the metabolism of a glyphosate molecule between the two biotypes. However, actions of EPSPS reflected significantly more inhibition in the susceptible than in a resistant biotype (Simarmata and Penner 2008).

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Furtehrmore, Pavlović et al. (2011), discovered that rigid ryegrass accumulated more than the normal amount of shikimic acid in susceptible populations than in resistant ones. When shikimate accumulates, it is a clear sign that glyphosate is reaching and inhibiting the target enzyme (Powles and Preston 2006). The assumption was therefore made that the EPSPS insensitivity of the resistant biotype was the most probable resistance mechanism of this population of ryegrass found in California (Simarmata and Penner, 2008).

#### 2.6.2. Cases of glyphosate resistance in Italian ryegrass

In 2001, glyphosate resistance in Italian ryegrass was firstly reported in Chile (Perez and Kogan 2003). Resistance in Italian ryegrass was subsequently also observed in numerous different countries such as Brazil, Spain, Japan, Switzerland, USA, Italy and New Zealand (Heap 2009). In central Chile a history of weed control in fruit orchard systems of about 10 years of intense glyphosate dependence selected for glyphosate resistance in annual ryegrass populations (Vila-Aiub et al. 2008). No differences in leaf absorption and translocation of glyphosate in susceptible and resistant varieties were found (Vila-Aiub et al. 2008). The foundation of the resistance was ascribed to target-site EPSPS gene mutation.

#### 2.6.3. Case of glyphosate resistance in perennial ryegrass (*Lolium perenne*)

Glyphosate resistance in perennial ryegrass was first reported in 2008 in Argentina with subsequent reports in New Zealand in 2012 and Portugal in 2013 (Ghanizadeh et al. 2013). Yanniccari et al. (2012) conducted a trial of glyphosate application on a population of perennial ryegrass from Argentina. They assessed germination, chlorophyll content, shikimic acid concentration and survival of these resistant populations as compared to a susceptible population as control. Results led to the conclusion that chlorophyll content in resistant and susceptible populations varied greatly. The resistant population only exhibited a slight decrease in chlorophyll content whereas the susceptible populations’ chlorophyll content decreased rapidly upon glyphosate application (Yanniccari et al. 2012). Congruent to results found in the other studies, Yanniccari et al. (2012) also found that accumulation of shikimate post-application are higher in susceptible biotypes than in resistant ones.

### 2.7. Numerous herbicide resistances in wild oat

Herbicide resistance is a global problem which is increasing over the last couple of years that results in substantial yield losses, hinders effective weed control and cause a large increase in production cost (Owen 2010; Powles and Yu 2010; Tranel et al. 2011). Although the majority of the 388 herbicide-resistant weed biotypes documented worldwide to date are resistant to only

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one mode of action, approximately 30% are resistant to two or more modes of action, with 44% of these appearing since 2005 (Heap 2016).

Multiple resistance leads to difficult and challenging weed control since changing to a different mode of action may not be suitable in managing multiple resistant biotypes. Wild oat populations were characterized to be resistant to members of three different mode-of-action families – repetition. An overall. Vila-Aiub et al. (2008) stated that the large cost are increasing when (i) the physiological mechanisms of herbicide resistance transfer resources away from the process that supports reproduction and growth, (ii) resistance-conferring mutations in herbicide target enzymes interfere with normal plant function or metabolism, (iii) altered ecological interactions takes place such as plants that are resistant to herbicides becoming less attractive to pollinators or more susceptible to disease or herbivores.

### 2.7.1. Resistance in *Avena fatua*

**Table 1.1:** The occurrence of herbicide resistant *Avena fatua* in the world (adapted from www.weedscience.org)

| | **Location** | **Herbicide type** | **Group** | **Infested agricultural fields** | Year |
|---|---|---|---|---|---|
| 1 | Argentina | clodinafop-propargyl, diclofop-methyl, and fenoxaprop-P-ethyl | ACCase inhibitors (A/1) | Spring Barley, and Wheat | 2010 |
| 2 | Australia | clodinafop-propargyl, diclofop-methyl, flamprop-methyl, and mesosulfuron-methyl | Multiple Resistance: 3 Fields of Action<br>ACCase inhibitors (A/1)<br>ALS inhibitors (B/2)<br>Antimicrotubule mitotic disrupter (Z/25) | Chickpea, Spring Barley, and Wheat | 2006 |
| 3 | Belgium | clodinafop-propargyl, and fenoxaprop-P-ethyl | ACCase inhibitors (A/1) | Winter wheat | 1996 |

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| | | | | | |
|---|---|---|---|---|---|
| 4 | Brazil | clodinafop-propargyl | ACCase inhibitors (A/1) | | 2010 |
| 5 | Canada | fenoxaprop-P-ethyl, imazamethabenz-methyl, imazapyr, pyroxasulfone, quizalofop-P-ethyl, sulfentrazone, and triallate | Multiple Resistance: 5 Fields of Action<br>ACCase inhibitors (A/1)<br>ALS inhibitors (B/2)<br>PPO inhibitors (E/14)<br>Long chain fatty acid inhibitors (K3/15)<br>Lipid Inhibitors (N/8) | Spring wheat | 2015 |
| 6 | Chile | clodinafop-propargyl, diclofop-methyl, haloxyfop-methyl, and tralkoxydim | ACCase inhibitors (A/1) | Wheat | 1998 |
| 7 | France | iodosulfuron-methyl-sodium, mesosulfuron-methyl, metsulfuron-methyl, and pyroxsulam | ALS inhibitors (B/2) | Wheat | 2006 |
| 8 | Germany | cycloxydim, fenoxaprop-P-ethyl, flupyrsulfuron-methyl-sodium, mesosulfuron-methyl, and pinoxaden | Multiple Resistance: 2 Fields of Action<br>ACCase inhibitors (A/1)<br>ALS inhibitors (B/2) | Sugar beets | 2012 |
| 9 | Iran | clodinafop-propargyl, and diclofop-methyl | ACCase inhibitors (A/1) | Wheat | 2007 |
| 10 | Mexico | clodinafop-propargyl, cycloxydim, diclofop-methyl, fenoxaprop-P-ethyl, fluazifop-P-butyl, sethoxydim, and tralkoxydim | ACCase inhibitors (A/1) | Wheat | 1998 |

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| | | | | | |
|---|---|---|---|---|---|
| 11 | Poland | fenoxaprop-P-ethyl, iodosulfuron-methyl-sodium, metsulfuron-methyl, pinoxaden, propoxycarbazone-sodium, and sulfometuron-methyl | Multiple Resistance: 2 Fields of Action<br>ACCase inhibitors (A/1)<br>ALS inhibitors (B/2) | Spring Barley, and Spring wheat | 2011 |
| 12 | South Africa | clodinafop-propargyl, diclofop-methyl, fenoxaprop-P-ethyl, fluazifop-P-butyl, imazamox, iodosulfuron-<br>methyl-sodium, sethoxydim, sulfosulfuron, and tralkoxydim | Multiple Resistance: 2 Fields of Action<br>ACCase inhibitors (A/1)<br>ALS inhibitors (B/2) | Wheat | 1986 |
| 13 | Turkey | diclofop-methyl, fenoxaprop-P-ethyl, and tralkoxydim | ACCase inhibitors (A/1) | Wheat | 2011 |
| 14 | United Kingdom | fenoxaprop-P-ethyl, flamprop-M-isopropyl, fluazifop-P-butyl, imazamethabenz-methyl, mesosulfuron-methyl, pinoxaden, pyroxsulam, and tralkoxydim | Multiple Resistance: 3 Fields of Action<br>ACCase inhibitors (A/1)<br>ALS inhibitors (B/2)<br>Antimicrotubule mitotic disrupter (Z/25) | Canola, Cereals, and Wheat | 1994 |
| 15 | United States | difenzoquat, and triallate | Multiple Resistance: 2 Fields of Action<br>Lipid Inhibitors (N/8)<br>Cell elongation inhibitors (Z/8) | Cereals | 1993 |

Resistance in *A. fatua* occurs to seven different herbicide groups within 15 locations throughout the world (Table 1). *Avena fatua* is responsible for the infestation of agricultural

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systems such as spring barley, winter wheat, wheat, cereals, canola, sugar beets, chickpea etc. Resistance was first reported in 1986, but currently herbicide resistance in *A. fatua* has become a major problem, especially in wheat and other cereal fields (Table 1).

### 2.7.2. Worldwide occurrence of herbicide resistance

When the very first evolution of resistance in weeds was discovered, it was largely ignored as they lacked importance. This was due to the occurrence on one or two farms that experienced herbicide resistance. The widespread increase in herbicide resistance was not by the distribution or spreading of weed seeds or pollen, it was due to evolution (Gressel 1993).

The locations which were affected by herbicide resistance were inadequately documented due to a shortage of confirmed and well investigated data. The fact that resistant seed is spreading unrestricted during each season, contributes to making it more challenging to keep a history record of the problem. There have been records of few detailed appearances of herbicide resistance existing in the late 1960s. It was initially examined during 1968 when Ryan (1970) reported that *Scenecio vulgaris* developed resistance to two herbicides, atrazine and simazine. Although, ever since the identification of triazine resistance in *S. vulgaris* populations, there has been a constant increase in the amount of resistant weed species throughout the world (Moss and Rubin 1993).

It is estimated that triazine resistant weeds infested about one million hectares in the USA and at least two million hectares in other countries in the world (LeBaron 1991). An observation of rigid ryegrass infestations amongst crops in fields or in high concentrated cropped locations of about 250 $km^2$ in South Australia, in 1994 showed that 40% of all fields examined contained diclofop-methyl resistant populations (Nietschke et al. 1996). Jutsum and Graham (1995) reported that resistance was discovered in more than 100 grass and broad-leaved species in over 40 countries.

LeBaron (1991) stated that about 113 herbicide resistant weed species had evolved in numerous locations globally by the year 1990. Resistant weed species occurs in approximately 10 of the 50 states of the USA (LeBaron 1991). In Australia, wild oats and ryegrass which were resistant to aryloxyphenoxypropionates and cyclohexanediones infested millions of hectares (Morrison and Bourgeois 1995). According to Christopher et al. (1991) resistance to diclofop-methyl was discovered in rigid ryegrass in 1982, by Heap and Knight (1982). Today herbicide resistance occurs in 251 species (146 dicots and 105 monocots). There are recently 479 different cases of herbicide resistant weeds worldwide. Weeds species have evolved resistance

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to 23 of the 26 known herbicide fields of action and to 162 different herbicides. Herbicide resistant weeds have been discovered and confirmed in 91 crops in a total of 69 countries. New well observed and investigated cases of herbicide resistant weeds have been contributed by approximately 531 weed scientists (Heap 2017). There was 461 or more cases of resistant weed species covering all available herbicides modes of action (Heap 2015). Today there is an increment of 4 weed species which are resistant to herbicide and an increment of 18 different cases of herbicide resistant as compared to the year 2015.

### 2.7.3. Herbicide resistance in South Africa

The initial confirmed case of herbicide resistance to diclofop-methyl in the Western Cape Province was in *A. fatua* (Cairns and Hugo 1986). A total area of about 6000 hectares was estimated as being herbicide resistant to ACCase inhibitors (Smith 2001). Three grass weed species in the Western Cape Province are of high economic value regarding resistance, namely ryegrass, wild oats and ripgut brome (*Bromus diandrus)* and have been proven to be resistant to grass weed herbicides (Smith 2001). According to Lemerle et al. (1995), ryegrass is a highly competitive weed species in wheat production and yield decreases due to competition with ryegrass, can be higher than 75%, depending mainly on type of cultivar, sowing date and seeding rate. Afentouli and Eleftherohorinos (1996) discovered a decrease of up to 60% of wheat yield if competition occurs. Carlson and Hill (1985) stated that wheat yield decreased continuously as populations of wild oats increases. Recently, herbicide resistance in wheat production has only been investigated in the winter rainfall area of South Africa. The case of resistance is more related with herbicide usage and crop production fields. By investigating herbicide sales during 1996, certain conclusions can be made regarding a report of resistance for the future (Smit 2001).

Cairns and Laubscher (1986) initially reported cases of herbicide resistance in South Africa and established evidence on herbicide resistance to diclofop-methyl in wild oats in the Western Cape. Since then reports of resistance to herbicides in weeds increased. During 1998 and 1999 resistance of ryegrass to ACCase and ALS inhibitors was confirmed (Smit and De Villiers 1998; Smit et al 1999).

In addition, Kellermann (2002) also confirmed resistance to ACCase and ALS inhibitors in ryegrass. Resistance of little canary grass (*Phalaris minor*) to ACCase inhibitors was reported by Smit and Cairns (2001) and this was confirmed by Pieterse who again reported resistance to ALS inhibitors in the very same species (Heap 2009). A short time after ALS inhibitors were

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registered for ripgut brome control in wheat fields in South Africa, reports of unsatisfactory control started to appear. Resistance to these herbicides was later confirmed (Fourie 2004).

In 2001, Cairns and Eksteen reported ryegrass resistance to glyphosate (Heap 2009). One year later resistance in ryegrass to paraquat was also reported by Cairns and Eksteen (Heap 2009). Paraquat resistance in ryegrass was also confirmed by Yu et al. (2004). In 2003, multiple resistance to both glyphosate and paraquat herbicides as well as ACCase inhibitors in a ryegrass biotype was reported by Cairns (Heap 2009). This was also confirmed by Eksteen (2007) and Eksteen et al. (2005).

The inherited traits of these mechanisms were described by Yu et al. (2007). In 1993, herbicide resistance reported in broadleaved weeds in South Africa were first reported by Botes and Van Biljon in smooth pigweed (*Amaranthus hybridus*) to the triazines (Heap 2009). Smooth pigweed resistance to triazine was also confirmed by Sereda et al. (1996). According to Smit and Cairns (2001) resistance of wild radish (*Raphanus raphanistrum*) to chlorsulfuron developed and weed species resistant to several other ALS inhibitors were also reported by McDermott and Pieterse (Heap 2009).

## References

Afentouli GC, Eleftherohorinos IG. 1996. Little seed canary grass *(Phalaris minor)* and short-spiked canary grass *(Phalaris brachystachys)* interference in wheat and barley. *Weed Scienc*, vol. 44. 560-565.

Anderson WP. 1996. Weed Science: Principles and Applications (3rd edn). Waveland Press Publisher. pp 125-138

Auld BA, Medd RW. 1987. *Weeds: An illustrated botanical guide to the weeds of Australia*. Elseriver.

Azania ACM, Pinto LR, Adriano CR, Perecin D, Azania AP. 2013. The use of glyphosate in sugarcane: A Brazilian experience. *Herbicides Current research and case studies in use. Croatia: InTech*.https://www.intechopen.com/books/herbicides-current-research-and-case-studies-in-use/the-use-of-glyphosate-in-sugarcane-a-brazilian-experience [accessed 08 May 2017].

Stellenbosch University https://scholar.sun.ac.za

Baum BR, Fleischman G, Martens JW, Rajhathy T, Thomas H. 1972. Notes on the habitat and distribution of *Avena* species in the Mediterranean and Middle East. *Canadian Journal of Botany* 50*:* 1385-1397.

Bozic D. 1986. The influence of agricultural technology on the aboveground biomass of weeds in winter wheat. *Agrochemicals Journal.*No.5-6: 309-320.

Burnet MWM, Barr AR, Powles SB. 1994a. Chloroacetamide resistance in rigid ryegrass (*Lolium rigidum*). *Weed Science*, vol. 42. 153-157.

Burnet MWM, Hart Q, Holtum JAM, Powles SB.1994b. Resistance to nine herbicide classes in a population of *Rigid Ryegrass (Lolium rigidum).Weed Science,* Vol.42. 369-377*.*

Burnet MWM, Hildebrand OB, Holtum JAM, Powles SB. 1991*.* Amitrole, triazine substituted urea, and metribuzin resistance in a biotype of rigid ryegrass *(Lolium rigidum). Weed Science,* vol. 39. 317-323*.*

Burnet MWM, Loveys BR, Holtum JAM, Powles SB. 1993. A mechanism of chlorotoluron resistance in *Lolium rigidum. An international Jouranl of Plant Biology* 190: 1821-89*.*

Cairns ALP. 1974. Biology and control of *Avena fatua* L. in the Western Cape. MSc thesis, University of Stellenbosch, South Africa.

Cairns ALP, De Villiers OT. 1984. The physiology of seed dormancy and germination in wild oats (*Avena fatua* L.).PhD thesis, Stellenbosch University, South Africa.

Cairns AL, Hugo KJ. 1986. Differential tolerance of Western Cape wild oat biotypes to diclofop-methyl and mixtures containing diclofop-methyl. In: Proceedings of the 1986 South African Weed Science Society Congress. Pretoria.

Cairns AL, Laubscher EW. 1986. Differential tolerance of Western Cape wild oat to diclofop-methyl and mixtures containing diclofop-methyl. Final report. Stellenbosch: Department of Agronomy and Pastures, Stellenbosch University.

Carlson HL, Hill JE.1985. Wild oat *(Avena fatua)* competition with spring wheat: Plant density effects. *Weed Science* 33: 176-181.

Christopher JT, Powles SB, Liljegran DR, Holtum JAM. 1991. Cross-resistance to herbicides in annual Ryegrass *(Lolium rigidum). Plant Physiology* 95: 1036-1043.

Stellenbosch University https://scholar.sun.ac.za

Coffman FA. 1961a. Origin and History. In: Coffman FA (ed), *Oats and Oat Improvement*, Agronomy Monograph. ASA, Madison, WI. doi:10.2134/agronmonogr8.c2, 8: 15-40

Coffman FA. 1961b. World importance and distribution. In: Coffman FA (ed) *Oats and Oat Improvement,* Agronomy Monograph. ASA, Madison WI. doi:10.2134/agronmonogr8.c1 8: 1-14

Department of Agriculture and Food. 2015. Wild oats.https://www.agric.wa.gov.au/grains-research- development/wild-oats. [accessed 2016 23 February 23].

De Wet H, Cairns ALP.2005. Paraquat and glyphosate resistance in *Conyza bonariensis* in the Western Cape in the Republic of South Africa. MScAgric thesis, Stellenbosch University, South Africa.

Délye C. 2013. Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. *Pest Management Science* 69:176–187.

Devine MD, Duke SO, Fedtke C. 1993. *Physiology of herbicide action*. PTR Prentice-Hall. *Inc., New Jersey*. pp: 441.

Devine MD. 1997. Target-site based resistance to ACCase inhibitors. In *Weed and Crop Resistance to Herbicides*. In: De Prado R, Jorrin J, Garcia-Toress L (eds).Springer Netherlands. pp: 61-69.

Duke SB, Powles SO. 2009. Glyphosate-resistant crops and weeds. *Journal of Agro Biotechnology Management and Economics* 12:346-457.

Eksteen FH, Cairns ALP, Pieterse, PJ. 2005. Resistance of ryegrass (*Lolium* spp.) against glyphosate and paraquat/diquat in orchards and vineyards. *Technical Yearbook 2005/6*.

Eksteen FH. 2007. Resistance of ryegrass (*Lolium* spp.) to paraquat and glyphosate in the Western Cape. PhD Agriculture thesis, Stellenbosch University, South Africa.

Fogelfors H. 1984. Useful weeds? Part 14. *Lantmannen* p 105.

Fourie JH. 2004. An investigation into the emergence of herbicide resistance in *Bromus diandrus* Roth. (In Afrikaans with English abstract) MSc thesis, Stellenbosch University, South Africa.

Stellenbosch University https://scholar.sun.ac.za

Ghanizadeh H, Harrington KC, James TK, Woolley DJ. 2013. Confirmation of glyphosate resistance in two species of ryegrass from New Zealand vineyards. *New Zealand Plant Protection*, vol. 66. 89-93.

Grains Research and Development Corporation. 2010. Australia Government. Wild oats.http://www.grdc.com.au/Resources/IWMhub/Secti on-8-Profiles-of-common-weeds-of-cropping/Wild-oats. [accessed 13 March 2016].

Grain Research and Development Cooperation. 2015. Mature wild oat plant (Photo A. Storrie).https://grdc.com.au/Resources/IWMhub/Section-8-Profiles-of-common-weeds-of-cropping/Wild-oats [accessed 10 August 2016].

Gressel J. 1988. Multiple resistances to wheat selective herbicides*:* new challenges for molecular biology. In*:* Oxford Surveys of Plant Molecular and Cell Biology, S. pp 195-203*.*

Gressel J. 1991. Why get resistance? It can be prevented or delayed. In: Caselay JC, Cussans GW, Atkin RK (eds). *Herbicide resistance in weeds and crops*. Butterworth Heinemann, Oxford. pp: 1-26

Gressel J. 1993. Management of herbicide resistant weeds in crop production. *International Crop Science*: 121-127.Gressel J, Segel LA. 1990. Modelling the effectiveness of herbicide rotations and mixtures as strategies to delay or preclude resistance. *Weed Technology.* Vol 61. 86-198.

Hall LM, Holtum JAM, Powles SB. 1994. Mechanisms responsible for cross resistance and multiple resistance. In: Powles S.B, Holtum JAM. (eds). *Herbicide resistance in plants: biology and biochemistry.*: Lewis Publishers, Boca Raton FL. pp 243-261.

Henderson M, Anderson JG. 1966. Common weeds of South Africa. Department of Agriculture Botanical Survey Memoir 37: 6-7.

Hatami ZM, Gherekhloo J, Rojano-Delgado AM, Osuna MD, Alcántara R, Fernandez P, Sadeghipour HR, De Prado R. 2016. Multiple mechanisms increase levels of resistance in *Rapistrum rugosum* to ALS herbicides. *Frontiers in Plant Science*, vol. 7. p 7.

Haynes B, Koide RT, Elliott G. 1991. Phosphorus uptake and utilization in wild and cultivated oats (*Avena* spp). *Journal of Plant Nutrition* 14: 1105-1118.

Stellenbosch University https://scholar.sun.ac.za

Heap IM, Knight R, 1982. A population of ryegrass tolerant to the herbicide diclofop-methyl. *Australian Journal* of *Agricultural Research* 48: 156-157.

Heap IM, Knight R. 1986. The occurrence of herbicide cross-resistance in a population of annual ryegrass *(Lolium rigidum)* resistant to diclofop-methyl. *Australian Journal* of *Agricultural Research* 37:149-156.

Heap IM, Knight R. 1990. Variation in herbicide cross-resistance among populations of annual ryegrass *(Lolium rigidum)* resistant to diclofop-methyl. *Australian Journal* of *Agricultural Research* 48: 156-157.

Heap I. 2009. International survey of herbicide resistant weeds. http://www.weedscience.com. [accessed 26 April 2017].

Heap I. 2011. The international survey of the herbicide resistant weeds.http://www.weedscience.com. [accessed on 28 March 2017]

Heap I. 2015. International Survey of Herbicide Resistant Weeds. Annual Report Internet. Available at: http://www.weedscience.com [accessed 08 May 2017].

Heap I. 2016. The international survey of herbicide resistant weeds. Available at http://www.weedscience.org [accessed 08 May 2017].

Heap I. 2017. The International Survey of Herbicide Resistant Weeds. Available www.weedscience.org [accessed 08 May 2017].

Hock B, Elstner EF (eds). 2004. *Plant Toxicology*, Fourth Edition. CRC Press. ISBN 978-0-203-02388-4.

Integrated Taxonomic Information System. 2017. Wild oat (Avena *fatua*). Standard Report, Taxonomic serial NO: 41458. https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=41458#null [accessed 14 April 2017]

Jutsum AR, Graham JG. 1995. Managing weed resistance: Activities of the herbicide resistance action committee*. Pest Outlook* 6: 30-33.

Kellermann JL. 2002. An investigation of herbicide resistance of *Lolium* spp. in the South Western Cape. MSc Agriculture thesis, University of Stellenbosch, Stellenbosch.

Stellenbosch University https://scholar.sun.ac.za

King LJ. 1966. *Weeds of the world*. International Scientific Press Incorporated, New York.

Konesky DW, Siddiqi MY, Glass ADM, Hsiao AI. 1989. Genetic differences among barley cultivars and wild oat lines in endogenous seed nutrient levels, initial nitrate uptake rates, and growth in relation to nitrate supply. *Journal of Plant Nutrition* 12:9-35.

Lebaron HM. 1991. Distribution and seriousness of herbicide resistant weed infestations worldwide. In: Gaseley JG, Gussans GW, Atkin RK (eds). *Herbicide resistance in weeds and crops*. Butterworth-Heinemann Ltd, Oxford. pp 27-43.

Lemerle D, Verbeek B, Coombes N. 1995. Losses in grain yield of winter crops from *Lolium rigidum* competition depend on crop species, cultivar and season. *Weed Research* 35: 503-509.

Linnaeus C. 1753. Species plantarum, 2 vols. *Laurentii Salvii, Holmiae*. https://books.google.co.za/books?id=h7coAAAAYAAJ&pg=PA3&dq=Linnaeus+1753+avena+fatua&hl=en&sa=X&ved=0ahUKEwjagOrF5tXOAhXJD8AKHUZCB84Q6AEIHjAB#v=onepage&q=Linnaeus%201753%20avena%20fatua&f=false [accessed 16 August 2016]

Louw JG. 1930. A dissertation about wild oats mainly with regard to classification and germination. MSc thesis, Stellenbosch University, South Africa.

Maeda H, Dudareva N. 2012. The shikimate pathway and aromatic amino acid biosynthesis in plants. *Annual Review of Plant Biology* 63: 73–105.

Malzew AI. 1930. Wild and cultivated oats. *Bulletin of Applied Botany, Genetical Plant Breeding Supplement* 38 Leningrad. pp 473-503.

Martin MPLD, Field RJ. 1988. Influence of time of emergence of wild oat on competition with wheat. *Weed Research*, United Kingdom 28:111-116.

Medd RW. 1996. Ecology of wild oats. *Plant Protection Quarterly* 11: 185-187.

Moore DM. 1968. The vascular flora of the Falkland Islands. *British Antarctic Survey Scientific Reports* 60: 1–202.

Morrison IN, Bourgeois L. 1995. Approaches to managing ACCase inhibitor resistance in wild oat on the Canadian prairies, vol. 2.

Stellenbosch University https://scholar.sun.ac.za

Moss SR. 2002. Herbicide - resistant weeds. In: R.E.L. Naylor (ed.):. *Weed management handbook.* 9th edn, Blackwell Science, Oxford.

Moss SR, Rubin B. 1993. Herbicide-resistant weeds: a worldwide perspective. *Journal* of *Agricultural Science,* Cambridge 120: 141-148.

Nandula VK (ed). 2010. *Glyphosate resistance in crops and weeds: history, development, and management*. John Wiley and Sons.

Nietschke B, Llewellyn R, Mathews JM, Powles SB, Reeves TG. 1996. A survey of herbicide resistant *Lolium* and *Avena* species in South Australia. In: Asghar M (ed). University of Southern Queensland. In: Proceedings of the 8th Australian Agronomy Conference. pp: 691-692.

Orson J. 2015. Wild oats. http://www.fwi.co.uk/academy/lesson/wild-oats. [accessed 08 May 2017].

Owen MDK. 2010. Herbicide resistance. Editors: Kempken F, Jung C Genetic modification of plants. *Biotechnology. Agriculture. For*. 64. Springer-Verlag, Berlin 159–177.

Owen MJ, Powles SB. 2010. Glyphosate-resistant rigid ryegrass (*Lolium rigidum*) populations in the Western Australian grain belt. *Weed Technology,* vol. 24. 44–49

Pavlović D, Reinhardt C, Elezović I, Vrbničanin S. 2011. Identification of glyphosate resistance in *Lolium rigidum* Gaudy. *Pesticides and Phytomedicine* 26: 393–399.

Perez A, Kogan M. 2003 Glyphosate-resistant *Lolium multiflorum* in Chilean orchards. *Weed Research,* vol. 43. 12–19

Perez-Jones A, Park KW, Colquhoun J, Mallory-Smith CA, Shaner D. 2005. Identification of glyphosate-resistant ryegrass (*Lolium multiflorum*) in Oregon. *Weed Science,* vol. 53. 775–779.

Powles S, Yu Q. 2010. Evolution in action: plants resistant to herbicides. *Annual Review of Plant Biology*, vol. 61. pp 317-347.

Powles SB, Preston C. 2006. Evolved glyphosate resistance in plants: Biochemical and genetic basis of resistance. *Weed Technology* 20: 282–289.

Stellenbosch University https://scholar.sun.ac.za

Powles SB, Preston, C. 2004. Herbicide cross resistance and multiple resistance in plants. www.plantprotection.org. [Accessed 08 May 2017]

Powles SB, Shaner DL. 2001. Herbicide resistance and world grains. CRC Press, Florida.

Prather TS, Ditomaso JM, Holt JS. 2000. Herbicide resistance definition management strategies. University of California. Division of agriculture and natural resources. https://anrcatalog.ucanr.edu/pdf/8012.pdf [accessed 28 April 2017]

Pratley J, Baines P, Eberbach P, Incerti M, Broster J. 1996.Glyphosate resistance in annual ryegrass. In: Virgona J, Michalk D (Eds.), Proceedings of the 11th Annual Conference of the Grasslands Society of New South Wales, The Grasslands Society of NSW, Australia. p 126.

Preston C, 2004. Herbicide resistance in weeds endowed by enhanced detoxification: complication for management.. *Weed Science* 52: 448–453.

Preston C, Tardif FJ, Powles SB. 1996. Multiple mechanisms and multiple herbicide resistance in *Lolium rigidum.* American Chemical Society Publisher. pp 117-129.

Preston C, Wakelin AM, Dolman FC, Bostamam Y, Boutsalis P. 2009. A decade of glyphosate-resistant *Lolium* around the world: Mechanisms, genes, fitness, and agronomic management. *Weed Science* 57: 435–441.

Riar DS, Norsworthy JK, Srivastava V, Nadula V, Bod JA, Scott RC. 2013. Physiological and molecular basis of acetolactate synthase inhibiting herbicides resistance in barnyard grass (*Echinochloa crus-galli). Journal Agriculture Food Chemistry* 61:278–289.

Ryan CF. 1970. Resistance of common groundsel to simazine and atrazine. *Weed Science* 18: 614-616.

Sampson DR. 1954. On the origin of wild oats. Botanical Museum Leaflet, Harvard University 16: 265-303.

Schuette J. 1998. Environmental fate of glyphosate. Environmental Monitoring and Pest Management. Department of Pesticide Regulation. Sacramento, CA.

Sereda B, Erasmus DJ, Coetzer RLJ. 1996. Resistance of *Amaranthus hybridus* to atrazine. *Weed Research* 36: 21-30.

Stellenbosch University https://scholar.sun.ac.za

Simarmata M, Penner D. 2008. The basis for glyphosate resistance in rigid ryegrass (*Lolium rigidum*) from California. *Weed Science* 56: 181–188.

Simarmata M, Bughrara S, Penner D. 2005. Inheritance of glyphosate resistance in rigid ryegrass (*Lolium rigidum*) from California. *Weed Science* 53: 615–619.

Smit JJ, Smit HA, De Villiers BL.1999. Differential efficacy of tralkoxydim and diclofop-methyl on a suspected resistant ryegrass (*Lolium rigidum* Gaud.) biotype. *South African Journal Plant Soil* 16: 169-172.

Smith JJ, Cairns ALP. 2001. Resistance of *Raphanus raphanistrum* to chlorsulfuron in the Republic of South Africa. *Weed Research* 41: 41-47.

Smit JJ, De Villiers BJ. 1998. *Lolium* spp. resistance to ACC-ase inhibitors in wheat (*Triticum aestivum* L.) within the Republic of South Africa. *South Africa Journal Plant and Soil* 27: 25th Anniversary Edition, vol. 15. 1983-2008

Smith JJ. 2001. Distribution and Severity of Herbicide Resistance in the Republic of South Africa. MSc thesis, University of Natal Pietermaritzburg, South Africa.

Steinrücken HC, Amrhein N.1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase". *Biochemical and Biophysical Research Communications* 94: 1207–1212.

Székács A, Darvas B. 2012. Forty Years with Glyphosate. In: Hasaneen MN (ed).*Herbicides-properties, synthesis and control of weeds*, Intechopen publishers. pp 247–284.

Tackholm V, Drar M. 1941. Flora of Egypt 1 Cairo Fouda I. University Bulletin Faculty of Science 17: 574.

Thurston JM.1954. A survey of wild oats *(Avena fatua* and *Avena ludoviciana)* in England and Wales in 1951. *Annals of Applied Biology* 41: 619-636.

Thurston JM, Phillipson A. 1976. Wild oats distribution. In: Jones DP (ed), Wild Oats in World Agriculture. Agricultural Research Council (London). pp 19-64.

Tranel PJ, Riggins CW, Bell MS, Hager AG. 2011. Herbicide resistances in *Amaranthus tuberculatus*: A call for new options *Journal of Agricultural and Food Chemistry* 59: 5808–5812.

Stellenbosch University https://scholar.sun.ac.za

USDA (United State Department Of Agriculture). 2017. Herbicide Resistance: Mechanisms, Inheritance, and Molecular Genetics. http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=998688941&topicorder=6&maxto=7&minto=1 [accessed 03 March 2016]

Vila-Aiub MM, Vidal RA, Balbi MC, Gundel PE, Trucco F, Ghersa CM. 2008. Glyphosate-resistant weeds of South American cropping systems: An overview. *Pest Management Science* 64: 366–371.

Wakelin AM, Preston C. 2006. Inheritance of glyphosate resistance in several populations of rigid ryegrass (*Lolium rigidum*) from Australia. *Weed Science* 54: 212–219.

Welsh SL. 1974. Anderson's flora of Alaska and adjacent parts of Canada. Provo: Brigham Young University Press xvi. Illustrations, portrait, map.p 724.

White AD, Owen MDK, Hartzler MG, Cardina J. 2002. Common sunflower resistance to acetolactate synthase-inhibiting herbicides. *Weed Sci*ence 50:432–437.

Yanniccari M, Istilart C, Giménez DO, Castro AM. 2012. Glyphosate resistance in perennial ryegrass (*Lolium perenne* L.) from Argentina. *Crop Protection* 32:12–16.

Yu Q, Cairns ALP, Powles S. 2007. Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. *An international Journal of Plant Biology* 225: 499–513.

Yu Q, Cairns ALP, Powles SB. 2004. Paraquat resistance in a population of *Lolium rigidum*. *Functional Plant Biology* 31: 247-254.

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# CHAPTER 3
## GERMINATION PRE-TREATMENT OF WILD OATS AND THE EFFECT ON GLYPHOSATE SUSCEPTIBILITY

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**Abstract**

**Glyphosate plays an important role in controlling weeds. Occurrence of glyphosate resistance in weeds necessitates the testing of weed species, including wild oats (*Avena* spp), for glyphosate resistance. Seed pre-treatments to break seed dormancy and improve seedling establishment for testing are sometimes needed. It is important to know if seed pre-treatments can influence the sensitivity of the resulting seedlings to herbicides. An experiment was conducted to evaluate germination rate and germination percentage of three wild oat populations treated with ammonia gas, gibberellic acid and distilled water. The second experiment consisted of seedlings from the respective wild oat populations derived from the most time-effective of the germination pre-treatments, being treated with three different glyphosate dosage rates (0, 270, 540 and 1080 g a.e $ha^{-1}$). The percentage survival of the seedlings was assessed six weeks after glyphosate application. The ammonia treatment, and to a lesser extent, gibberellic acid treatment, improved the germination of two of the four wild oat populations but the gibberellic acid treatment proved to be more time-effective. Gibberellic acid pre-treatment of the seeds did not influence the efficacy of glyphosate on the seedlings and no sign of resistance to glyphosate in any of the three populations tested was observed.**

**Keywords**: germination percentage, glyphosate, seed treatment, survival rate, wild oats.

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## 3.1. Introduction

Dormant seeds are described as seeds that cannot germinate in a certain period of time under environmental conditions that are normally favourable for the germination process to take place in the seed (Baskin and Baskin 2004). Most seeds are dormant at maturity and, furthermore, there are different natural mechanisms which may cause delay in the germination process (Nikolaeva 2001; Baskin and Baskin 1998). Seed dormancy can be classified as morphological dormancy where delay of germination is caused by the expected period of embryo growth and radicle emergence after the mature seed has been dispersed. Seed dormancy that occurs in a freshly matured dormant seed is described as primary dormancy, which develops during seed maturation on the parent plant (Hilhorst et al.1998). Wild oat (*Avena fatua* L) seeds are usually dormant at the time of being detached from the parent plant and even though fully viable, these dormant seeds will not germinate when exposed to conditions that stimulate germination of the non-dormant seeds (Simpson 1978). According to Simpson (1990), only seeds of wild oat that have the genetic characteristics of primary dormancy can be induced into secondary dormancy. Wild oat seeds also have secondary dormancy that keeps the seed from germinating under unfavourable conditions such as high summer temperatures (Warrick and Baughman 2003).

Wild oat seeds are described as notorious seeds because they have high dormancy levels. Wild oat seed may remain dormant for up to ±6 years if left near the soil surface or when they are worked deeply into the soil. Most of the seeds that survive for long periods in the soil in persistent seed banks are usually water-impermeable seeds (Roberts 1986). Several seed species in the seed bank have physical dormancy characteristics which helps them adapt and it allows seed germination to take place over time and space (Baskin and Baskin 2014). Thurston (1957) observed the dormancy behaviour of different populations of wild oat seeds and came to conclusion that dormancy percentages range from 95% to 0%.

Studies of wild oat seed dormancy and germination have been studied for several years. Weed scientists pursue simultaneous germination of all weed seeds in the soil to enable farmers to control the weed population with a single application and avoid endless applications of herbicides (Cairns 1984). Basic studies on wild oats have been complicated because harvested seeds from the fields differ mainly in their degree of dormancy (Hay 1962). Comparison of dormancy of wild oats from different locations within a climactic area have been done by Miller et al. (1982) working in Minnesota, Patterson (1976) in West Australia, and Imam and Allard (1965) working in California. These three locations had different conditions in terms

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of rainfall, soil type, temperature and agricultural practices but photoperiod and optimum growing season did not differ (Cairns 1984). Ecospecies in areas which receives rainfall in winter or during cold conditions (West Australia and California), usually germinate in autumn and in those areas ecospecies which germinate in spring would be eliminated by long dry summers (Cairns 1984). Different environmental conditions could trigger germination in seeds, as some seeds react to seasonal temperatures differently (Van Assche et al. 2003).

A wide range of optimum germination temperatures has been reported for wild oat seed collected in different parts of the world. Friesen and Shebeski (1961) discovered that the optimum germination temperature of wild oat samples collected in Canada was 21°C, while Quail and Carter (1968) reported an optimum germination temperature of 15°C for wild oat samples collected in Australia. Whalley and Burfitt (1972) however, recorded an optimum germination temperature of 20°C for wild oat harvested in Australia. Kochi (1968), working in Germany, revealed that only 10% of the wild oat seeds germinating at 15°C germinated at 20°C. Temperatures above 15°C resulted in a gradual decline in germination up to 35°C where germination ceased.

Sawhney and Naylor (1979) revealed that temperature serves as one of the most essential environmental factors which interacts strongly with genotype regarding the level of dormancy. Meanwhile Peters (1978) examined the dormancy of different lines of wild oats grown at 15°C and 20°C in a dark growth chamber. The total mean germination percentage after two months was lower in seeds grown at 15°C and higher in seeds grown at 20°C. Seeds were thereafter stored for three months at either 15°C or 20°C. The mean germination percentage for the wild oat seed matured at 15°C had increased with 11,7% and for seeds that matured at 20°C increases with 47,7%.

Temperature is therefore one of the important factors for germination in combination with water, as water is an important requirement for the normal germination process of seeds. However different volumes of water in which seeds imbibes, has significant effects on breaking seed dormancy (Simpson 1978). An experiment which was conducted by Hsiao and Simpson (1971), showed that the response of wild oat seed to different wavelengths of light could be affected by the quantity of water the seeds were imbibed in. Small and large volumes of water restrict germination when compared to the medium range of volumes. The interaction of light with low volumes of water inhibit germination in the light compared to darkness, however large volumes of water together with white light, promoted germination compared to darkness.

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Cumming and Hay (1958) found that immersing non-dormant seed in water promoted secondary dormancy in the seeds. Naylor and Simpson (1961) found that leaching excised dormant embryos with large volumes of water, led to increased gibberellin which promoted germination process of the seeds. They attributed this effect to the leaching out of a gibberellin inhibitor.

Breaking seed dormancy is commonly released by manipulating the environmental conditions to influence germination. Growth hormones regulators are also used to release seed dormancy. The major influence of gibberellic acid ($GA_3$) on the breaking of dormancy in wild oats is well documented (Cairns 1974). It was initially reported by Green and Helgerson (1957) that $GA_3$ could break the dormancy of freshly harvested wild oat seed. They discovered the optimum concentration of $GA_3$ to be 50 ppm. Black and Naylor (1959) grew exised panicles in different range of $GA_3$ concentrations and discovered the seed to be non-dormant when compared to the water controls. A fascinating finding was made by Peters (1978) who discovered that the a-amylase content of wild oat seeds which had been exposed to drought stress conditions during their maturation was significantly higher than in seeds from well-watered plants.

Wild oats have been used regularly to investigate and observe the mechanism of dormancy due to the high level and persistence of dormancy expressed and its important status as a weed. The mechanism of $GA_3$, water and $NH_3$ gas of breaking dormancy and stimulating germination has been clearly explained by Cairns (1974). However, these pre-treatments, in particular $GA_3$, may have some physiological effect on plant growth after the germination process, as wild oat seedlings germinated with $GA_3$, typically has a tall, lanky appearance compared to those germinated in pure water. This poses a question whether the perceived accelerated growth and probably physiological and metabolical processes can influence the response of such plants to herbicides. There are numerous publications that have been dedicated to wild oat dormancy, yet the effect of the dormancy breaking pre-treatments on the sensitivity to herbicides of the resulting seedlings has not been investigated. The overall objective of this study was to determine a quick and effective pre-treatment to facilitate good germination of wild oat seed and to assess if the selected pre-treatment has any influence on the efficacy of glyphosate on the wild oat seedlings.

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## 3.2. Material and Methods

### 3.2.1. The experimental site

The first part of the experiment was conducted in a laboratory and the second part was conducted in a glasshouse at the Stellenbosch University Welgevallen experimental farm. The site is located at 33° 56´33" S and 18° 51´56" E at an altitude of 136 m above sea level.

### 3.2.2. The treatments and experimental design

In the first part of the experiment, seeds from wild oat (*Avena fatua L.*) populations were selected based on the suspected resistance and susceptibility of the populations to glyphosate. A randomized complete block design arranged as a 3×3 factorial with 6 replications was used for the experiment. The experimental factors were three wild oat populations from 1) Malmesbury (33.4655° S, 18.7185° E), 2) Prieska (29.7069° S, 22.7390° E) and 3) Eendekuil (32°41′S, 18°53′E) which were pre- treated in three ways (distilled water (control), 1Mm $GA_3$ and ammonia gas). In the second part of the experiment the germinated seeds from the first part of the experiment were transplanted into a glasshouse. Only the distilled water and $GA_3$ treatments were used for this part of the experiment. A randomized complete block design arranged as a 3×2×4 factorial with 6 replications was used for the experiment. The experimental factors were seed from three wild oat populations (Malmesbury, Prieska and Eendekuil as described above) which were germinated in two different ways (distilled water and 1Mm $GA_3$) and glyphosate dosage rates at four levels (0, 270, 540 and 1080 g a.e $ha^{-1}$). The glyphosate treatments were applied at 2-3 weeks after transplanting.

### 3.2.3. Trail establishment and management

**Seedling preparation and germination process**

Seeds of three wild oat populations (Malmesbury, Prieska and Eendekuil) were each treated in three different ways:

**i. Distilled water pre-germination treatment**

The selected seeds were germinated in 6 ml of distilled water in 90 mm diameter plastic petri dishes (20 per dish) lined with 2 filter papers (Munktell Grade 391 Filter Paper, Vos Instruments) (Munktell 2018), at a constant temperature of 20 ºC in the dark in a germination cabinet. The petri dishes were enclosed in a poly propylene bag to prevent evaporation of the germination solution.

**ii. 1 Milli molar of $GA_3$ hormone pre-germination treatment**

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The selected seeds were germinated in 6 ml of a prepared 1 Milli-molar of $GA_3$ solution (0.087 g of $GA_3$ mixed with 250 ml of distilled water), in petri dishes as described above.

**iii. Ammonia gas pre-germination treatment**

For this treatment, 1 g of NaOH (sodium hydroxide) and 1 g of $NH_4Cl$ (ammonium chloride) were mixed together in a petri-dish which was placed at the bottom of a dessicator. To this mixture was added a drop of water to produce ammonia gas ($NaoH + NH_4Cl = NaCl + H_2O + NH_3$). Seeds were quickly placed in the dessicator and the dessicator lid was tightly closed. Seeds were exposed to the ammonia gas for 30 minutes before the lid was opened. Seeds were removed and exposed to fresh air for 48 hours before the seed husks were removed carefully by hand. Seeds were then leached under running water using a sieve for 24 hours. After the leaching treatment seeds were pricked on the distal end to allow oxygen to infiltrate. Treated seeds were placed in petri dishes (20 per dish) lined with 2 filter papers; 6 ml of distilled water was added and the procedure as described above was followed.

**iv Planting**

In the second part of the experiment, after the germination process that lasted for about 1-2 weeks, seedlings were transferred to the glasshouse inside the petri dishes where they germinated but the lids were removed. Established seedlings developed a green pigment colour after 5-7 days. Seedlings were then transplanted into 8 cm x 8 cm pots (three per pot) containing a coarse sand/gravel mix in a glasshouse that was set at a night/day temperature of 20/25 ºC.

**v Irrigation**

An automated irrigation system was used to water the plants. The plants were irrigated at 9:00 am, 12:00 pm and 4:00 pm for one minute. The plants were irrigated with a balanced nutrient solution (Table 3.1).

**Table 3.1**: Composition of the nutrient solution used to feed the plants growing in pots

| **EC = 2.0** | | | |
|---|---|---|---|
| Element (Macro) | Concentration mg $L^{-1}$ | Fertilizer | 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 |

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| $N0_3^-$ | 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 |

**Pest and disease control**

No pests and diseases were experienced in the glasshouse.

**Herbicide application**

Glyphosate was applied at the 4$^{th}$ leaf growth stage (about 2-3 weeks after transplant) using a pneumatic pot sprayer at a pressure of 2 bars in 100 L $ha^{-1}$ of water.

## 3.3. Data collection

**Germination**

Germination was recorded when the seeds started to germinate in the growth chamber. A seed was considered germinated when the protruding radicle was 1 mm long. Germinated seeds were counted daily until no more seeds germinated for 12 consecutive days. Percentage germination and germination rate was calculated from the data. The calculation was done using the formulae:

**a. Germination percentage =** $\frac{N_{T\times100}}{N}$

Where $N_{T\,=}$ Number of germinated seeds

N= the total number of all the seeds used in the petri dishes (Anjum and Bajwa 2005)

**b. Germination rate =** $\sum_{i-1}^{k}\frac{ni}{Di.ni}\times100$

Where K= Final day

Di= Day of recording

ni= Number of seeds germinated on day Di

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i= Day one to day k (Pieterse and Cairns1986)

**Application of glyphosate**

On the day of spraying extra control plants were harvested (This was done to compare difference of seedling sizes before glyphosate was applied) and the following variables were recorded:

**i. Number of leaves per plant**

The number of leaves per plant were counted and recorded and the mean number of leaves of the plants per pot was calculated.

**ii. Leaf area ($cm^2$)**

Leaf area per plant was measured using a leaf area meter (Campbell Scientific Africa (Pty) Ltd, LI-Cor3100C Area Meter) (Li-Cor 2016) and the mean leaf area of the plants per pot was calculated.

**iii. Plant height (cm)**

A ruler was used to measure plant height of each plant. Plant height of stems and leaves was measured from above soil surface to the tip of the longest leaf. The mean plant height of plants per pot was calculated.

**iv. Fresh plant mass (g)**

From each pot, surviving plants were harvested above soil surface by means of scissors and put into small brown paper bags and the fresh plant mass was measured using an electrnic balance. The mean fresh mass per pot was calculated.

**v. Dry plant mass (g)**

After determining fresh plant mass, the small brown paper bags with plants were placed into an oven and dried at 80$^{0}$C for 48 hours. The dry plants were weighed on an electronic balace and mean dry mass per pot was calculated.

**Evaluation**

Six weeks after glyphosate application and ensuring that no regrowth of sprayed plants took place, the following variables were recorded:

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**i. Survival percentage**

Survival percentage was recorded six weeks after spraying. The calculation was done using the following formula:

$$\text{Percentage Survival \%} = \frac{\text{number of surviving plants per pot}}{3(\text{plants per pot})} \times 100\%$$

Plants that displayed any actively growing green leaves, no matter how small the leaves, six weeks after glyphosate application, were considered as surviving plants.

## 3.4. Data analysis

Data was subjected to analysis of variance using the STATISTICA 12 program (Stastica 2012). Means of significant main effects and interactions in the experiments were separated using Tukey $HSD_{0.05.}$

## 3.5. Results

In the first part of the experiment, a significant ($p<0.05$) interaction was recoded between seed treatment and different populations for the germination percentage and germination rate of wild oat seeds. In both the Prieska and Malmesbury populations the highest germination rate and -percentage were obtained when treated with ammonia gas (Figure 3.1 and Figure 3.2). In the Malmesbury and Prieska populations $GA_3$ gave significantly higher germination percentage than water, while the Eendekuil population showed no significantly different responses to any of the treatments (Figure 3.1). There was no significant difference between the Malmesbury, Prieska and Eendekuil populations when treated with $GA_3$ or distilled water with regard to germination rate (Figure 3.2).

In the second part of the experiment, there was significant ($p<0.05$) interaction between wild oat populations, seed treatment and glyphosate dosages in terms of survival percentage (Figure 3.3). Where no glyphosate was applied the Malmesbury population pre-treated with water gave the highest survival rate (96%). Where glyphosate was applied, there were no differences between dosage rates, seed treatments or populations (Figure 3.3).

Figure 3.4 showed the same trends in terms of fresh weight as was observed for survival rate in Figure 3.3. Again, there was no significant differences between populations, dosage rates and seed treatments where glyphosate was applied.

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The P≤ 0001 indicates that there is a significant three-way interaction between wild oat populations, seed treatment and glyphosate dosages in terms of dry mass (Figure 3.5). The interaction was again caused by the variable results where no glyphosate was applied (control treatment).

Table 3.2 show the vegetative parameters measured on the day of spraying. There were no significant differences in size of seedlings between the treatment combinations except for the Eendekuil seeds germinated in water, where the seedlings were significantly smaller than the rest.

**Table 3.2:** Vegetative growth parameters for three different wild oat populations which were germinated with water and gibberellic acid at the time of spraying

| Population | Seed germination process | Plant Height (cm) | Leaf Area ($cm^2$) | Dry plant mass (g) |
|---|---|---|---|---|
| Malmesbury | Water | 33.00 [a] | 41.23 [a] | 0.25 [a] |
| Malmesbury | Gibberellic acid | 33.58[a] | 40.90[a] | 0.18 [a] |
| Prieska | Water | 29.58 [a] | 37.32 [a] | 0.22 [a] |
| Prieska | Gibberellic acid | 31.16[a] | 38.84 [a] | 0.14 [a] |
| Eendekuil | Water | 14.30 [b] | 22.00 [b] | 0.01 [b] |
| Eendekuil | Gibberellic acid | 29.23 [a] | 37.6 [a] | 0.18 [a] |

Values in a column followed by the same letters indicate no significant differences between treatments at p = 0.05.

## 3.6. Discussion

**Germination percentage and -rate**

Malmesbury and Prieska wild oat populations gave the highest germination percentage and rate when treated with ammonia gas (Figure 3.1 and Figure 3.2). Sawhney and Naylor (1979) reported that differences in dormancy levels between different populations have a genetic basis;

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genotype expression is significantly influenced by the environment. They showed temperature to be the most important environmental factor which interacts strongly with genotype in the expression of dormancy. Remarkable differences in germination behaviour amongst different populations were also discovered by Naylor and Jana (1976).

Ammonia gas stimulated the highest germination percentage and -rate as compared to distilled water and $GA_3$. This could have been partly influenced by the seeds being treated with ammonia gas and having their seed coats removed. Thereafter seeds were pricked at the distal end which created an opening to allow oxygen to enter the seeds which reached the embryo without any constraints. This might have stimulated both the germination percentage and -rate. The oxygen may diffuse easily into the embryo with no constraints of the seeds hulls and pass through the punctured tiny hole. Although Cairns (1984) reported that structures such as the seed coat can restrict gas exchange, they are not the only determinants of dormancy.

The coat-imposed seed dormancy is due to either the permeability of the coat to water and gases, the mechanical prevention of radicle extension, or the seed coat preventing inhibitory substances from leaving the embryo or it may be supplying inhibitors to the embryo (Baskin and Baskin 2014).

Chen et al. (1981) concluded that even though wild oat hulls contain a variety of phenolic inhibitors, these substances has no influence in the regulation of wild oat seed germination. Cairns (1984) also reported that, it can be speculated that $NH_3$ has an effect on permeability. Ammonia gas treatments resulted in high germination percentage rates. Cairns (1974) discovered that a mixture of $PH_3$, $NH_3$ and $CO_2$ stimulated the germination of dormant wild oat seeds. Cairns (1984) reported that it was noticeable that germination was significantly stimulated only in those treatments containing $NH_3$. These gases were injected separately into test tubes containing seeds in order to observe their potential of breaking dormancy separately. Ammonia was thus noted as the gas which elevate seed dormancy. They also discovered that $NH_3$ stimulates the synthesis of a-amylase in germinating seeds of wild oats earlier than when germination is stimulated by $GA_3$ or when naturally non-dormant seeds germinate. Cairns (1984) discovered that in endosperm halves that had been leached under running tap water for 48 hours, pre-treatment by $NH_3$ led to a significant increase in a-amylase synthesis.

In the Malmesbury population gibberellic acid resulted in significantly higher germination percentage than distilled water (Figure 3.1). Gibberellic acid is well known for enhancing growth and production. Gibberellic acid is utilized for the stimulation of germination of difficult dormant

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seed. The outcome of $GA_3$ on the release of dormancy in wild oat seeds has been well documented (Cairns 1974). According to Green and Helgerson (1957) $GA_3$ has the ability to release dormancy of newly harvested wild oat seeds. Black and Naylor (1959) cultivated and grew loose branching clusters of wild oats in a range of $GA_3$ concentrations and discovered that the seed that was produced were not dormant when compared to the water controls. Hsiao (1979) discovered that maximum germination of dormant wild oat seeds could be obtained by the application of $GA_3$ which helps to overcome resistance of the seed coat by increasing the growth potential of the embryo (Baskin and Baskin 2004; Baskin and Baskin 2014). Scarification may however increase sensitivity to $GA_3$ (Baskin et al. 2006).

Germinating seeds in 6 ml of distilled water did not release dormancy and had the lowest germination percentage and -rate. However, the amount of water in which seeds are imbibed, has a crucial influence on seed dormancy (Simpson 1978). Dormancy in wild oats can be released by soaking the seeds in boiled water (Hay and Cumming 1959). Hay (1962) reported that seeds were soaked for 96 hours in water and then placed on moist filter paper and less than 10% of seeds germinated. Opik and Simon (1963) discovered that seed which were treated with water had a higher percentage of non-germinated seeds than germinated seeds.

There was no significant difference in the germination rate between the Malmesbury, Prieska and Eendekuil wild oat populations when treated with $GA_3$ and water (Figure 3.2). This could have been influenced by the intact seed coat in these two treatments. The seed coat serves as constraint for oxygen to diffuse into the embryo and also prevents water to be imbibed easily by the seeds. According to Simpson (1978) seed coats of the seeds can restrict gas exchange. The effect of oxygen, carbon dioxide and water are essential for germination and shortages of these elements also decrease the rate of germination by affecting the metabolism of the embryo. Hay and Cumming (1959) reported that there were seeds which did not escape dormancy after soaking them in water. Seeds were viable at the end of the experiment since removal of seed coat and puncturing the seeds coats resulted in almost a complete germination. Hsaio (1979) found the effect of puncturing a hole in the seed led to greater penetration of $GA_3$.

Seed coat restriction and impermeability to water are the most essential causes of the low germination in most seed species (Torres et al. 2008; Verma and Kasera 2006; El Balla et al. 2011; Baskin and Baskin 2014). The seed coat serves as a layer to protect the embryo physical, temperature-related or water damages and it also ensure that the plant seed remain in a state that the plant seed remain in a state of dormancy until conditions are right for the plant embryo

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to germinate (Kigel 1995; Fenner and Thompson 2005; Baskin and Baskin 2014; Martínez-Fernández et al. 2014).

For the purpose of this experiment, $GA_3$ treatment was considered the most suitable treatment. Although ammonia gas treatments caused better and faster germination in two of the populations, the time and labour required to aerate, leach and dehull the wild oat seed negated the advantage in seed germination percentage and seed germination rate. The $GA_3$ treatment was therefore considered the most effective method to break dormancy of large batches of wild oat seed to provide seedlings for testing for herbicide resistance.

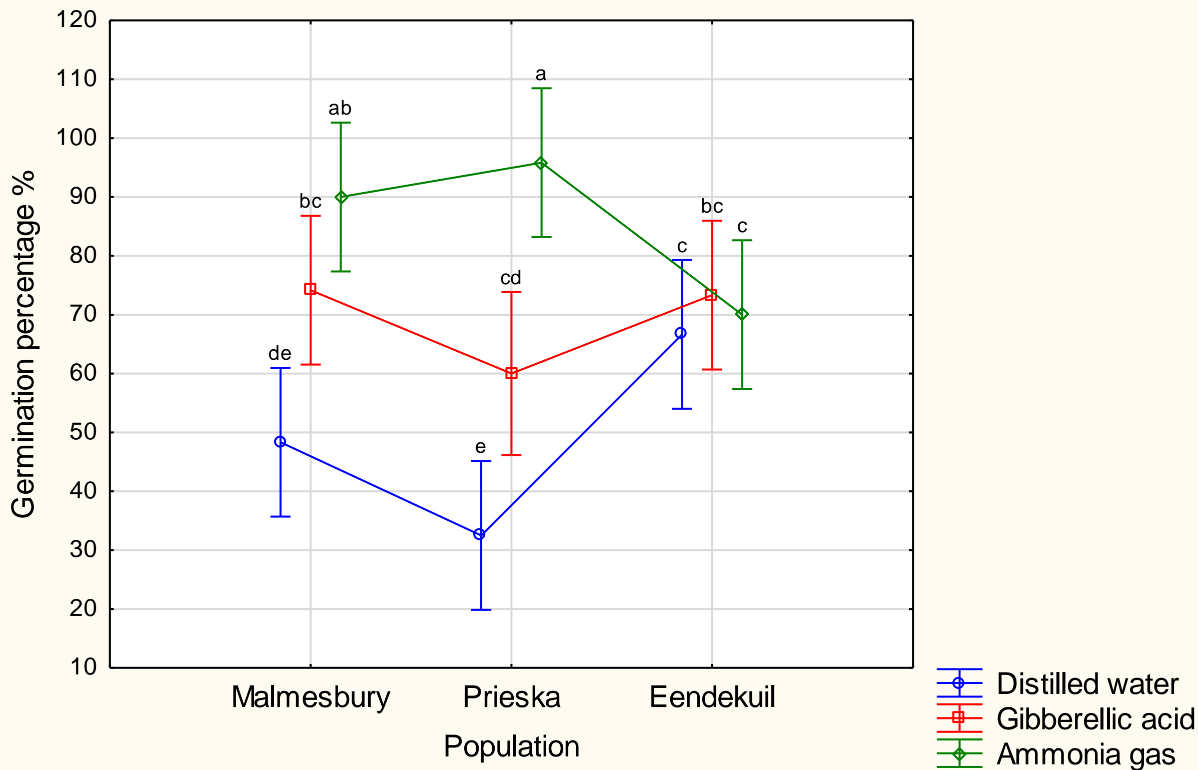


**Figure 3.1:** Interaction effects between seed treatment and three wild oat populations on germination percentage (%). (Different letters indicate significance between means at p=0.05)

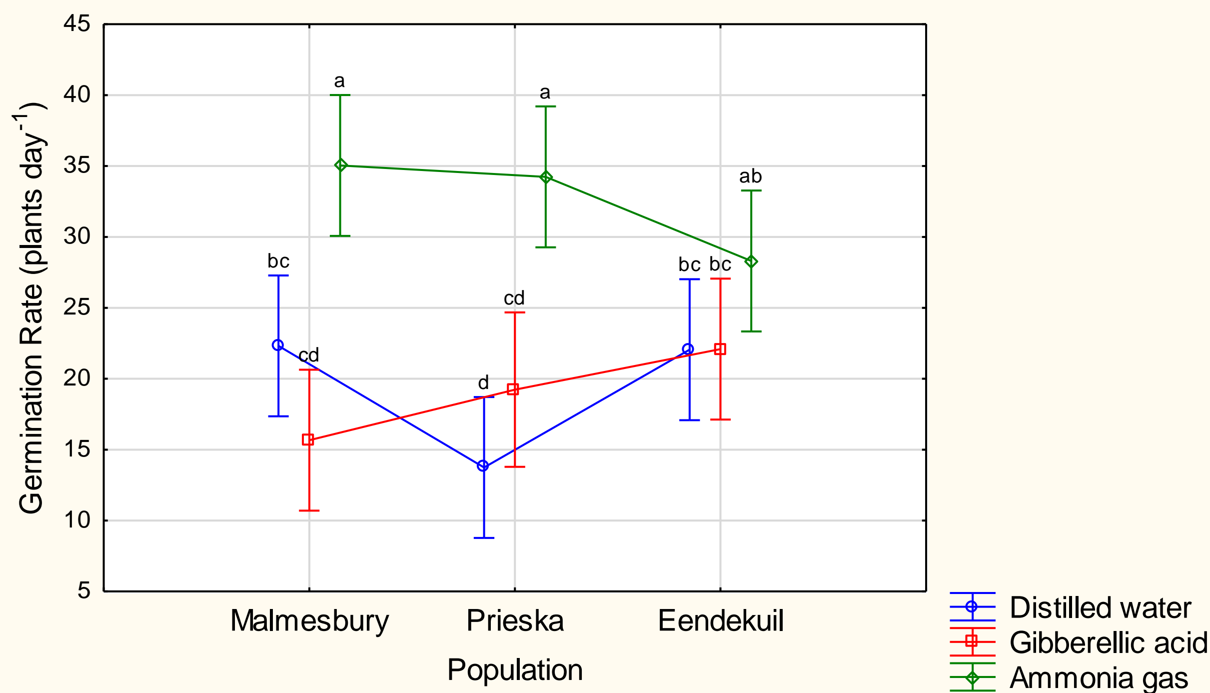


**Figure 3.2:** Interaction effects between seed treatment and three wild oat populations on germination rate. (Different letters indicate significance between means at p=0.05)

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**Survival percentage**

A significant ($p<0.05$) interaction between the three wild oat populations, two seed treatments and four glyphosate dosages was recorded (Figure 3.3). In general, control of wild oats increased as dosage rate of glyphosate increased from 0 g a.e $ha^{-1}$ to 1080 g ai $ha^{-1}$ dosage rates. This was observed for the Malmesbury, Prieska and Eendekuil populations. High survival rates occurred when no glyphosate was applied in all three wild oat population; however the lowest survival rate was noticed in all dosage rates of 270, 540 and 1080 g a.e $ha^{-1}$. According to Smeda and Putnam (2010) an increase of dosage rate from the control dosage to near the recommended dosage rate is expected to control weed population effectively.

All three populations treated with water and gibberellic acid showed no significant decrease in survival rate percentage with an increased dosage rate compared to the lowest glyphosate rate of 270 g a.e. $ha^{-1}$. Seedlings derived from treatment with gibberellic acid, appeared quite tall, very thin and lanky with long leaves. Table 3.2 however shows that in both Malmesbury and Prieska populations no significant difference in plant height, leaf area and dry mass occurred although there is a trend of slightly higher plant height when treated with $GA_3$. In contrast, dry mass showed a lower trend (although not significantly) in $GA_3$ treated plants. The Eendekuil population germinated in water cannot be compared with the rest as it revealed unexplainable results. It is maybe possible that the vigour of the Eendekuil seeds was lower compared to the other two populations resulting in poor seedling establishment. Treatment with $GA_3$ might have triggered better germination of seed. The trend of weak seedlings from the water treated Eendekuil seeds is also evident when the results of the fresh and dry mass of the non-sprayed control plants in Figures 3.4 and 3.5 is considered.

These differences in plant size at the time of spraying had no influence on survival of wild oat seedlings when treated with glyphosate. Glyphosate is sprayed directly onto leaves and the molecules of glyphosate are absorbed into plant cells from the leaf surface (Schuette 1998). As $GA_3$ treated seedlings appeared to have long and tall leaves this might have increased absorption of glyphosate on the leaf surface. The effectiveness of glyphosate depends amongst others, on size and spread of leaf surface area and humidity (Nandula 2010). Thereafter the glyphosate molecules are translocated to the meristematic tissues via the phloem and the molecule are then active in the meristem (Schuette 1998). However, Table 3.2 shows that there were no significant differences in leaf area of water and $GA_3$ treated plants. Gupta and Chakrabarty (2013) stated that a gradual increase of meristem tissue development, stem elongation and seed germination highly rely on $GA_3$ mechanism.

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Seedlings derived from seed treated with distilled water, showed a normal length and healthy leaves and did not influence survival of wild oat seedlings when treated with glyphosate. Vigour of plants has a great influence in phloem transport and distribution of glyphosate to the meristem tissue (Nandula 2010). Nonetheless, other studies showed that some crop species, including soya bean and maize, have the ability to slowly degrade glyphosate into carbon dioxide and aminomethylphosphonic acid (AMPA), following the exact pattern of glyphosate metabolism suggested for soil bacteria (Coupland 1985; Franez et al. 1997).

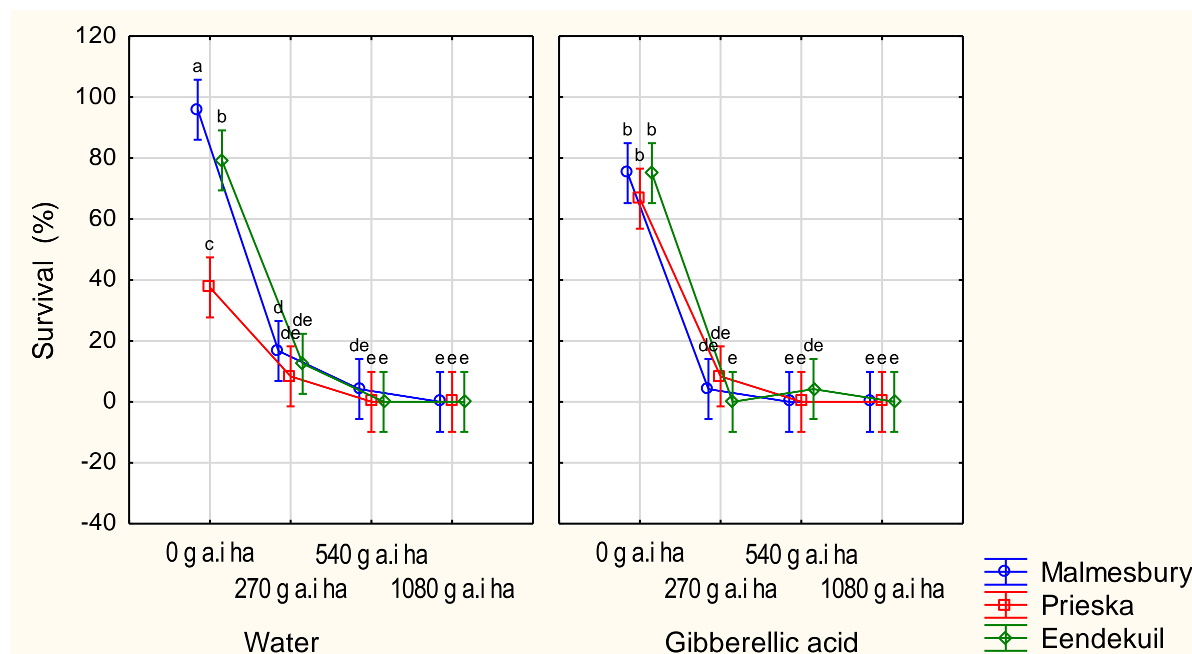


**Figure 3.3:** Three way interaction between three wild oat populations, two seed treatments and four glyphosate dosage rates in terms of survival percentage. (Different letters indicate significance between means at p=0.05)

**Fresh plant mass (g)**

Fresh mass was determined by weighing surviving plants after application with glyphosate. Figure 3.4 showed that the highest fresh weight of surviving plants occurred when no glyphosate was applied and the lowest fresh mass occurred when any dosage rate of glyphosate was applied on the plants. The increase of glyphosate dosage rates influence the glyphosate content in plant tissue which is most likely to improve efficacy of glyphosate treated seedlings (Feng et al. 2004). The effectiveness of glyphosate is mainly due to the dosage rate

(Nandula 2010). Based on the experimental results even the lowest concentration of 270 g a.e. $ha^{-1}$ of glyphosate showed a high level of control. Thus Feng et al. (2004) showed that plant tissue is influenced when glyphosate concentrations reached sufficient levels to cause inhibition of activity of EPSPS. The increased efficacy of glyphosate amongst the three populations is probably due to the fact that the seedlings were growing vigorously.

The pre-treatment of seeds most probably influence vigorous growth. Gibberellic acid is a plant hormone which affects gene expression and transcriptions, cell division and plant growth (Srivastava 2002). The efficacy of glyphosate as an herbicide depends on sufficient amounts reaching living plant material, which strongly influence plant health (Nandula 2010). Lester et al. (1972) discovered that $GA_3$ stimulated stem elongation, allowing more vertically orientated leaf blades. However, it was not evident that it influenced the efficacy of glyphosate in this trial.

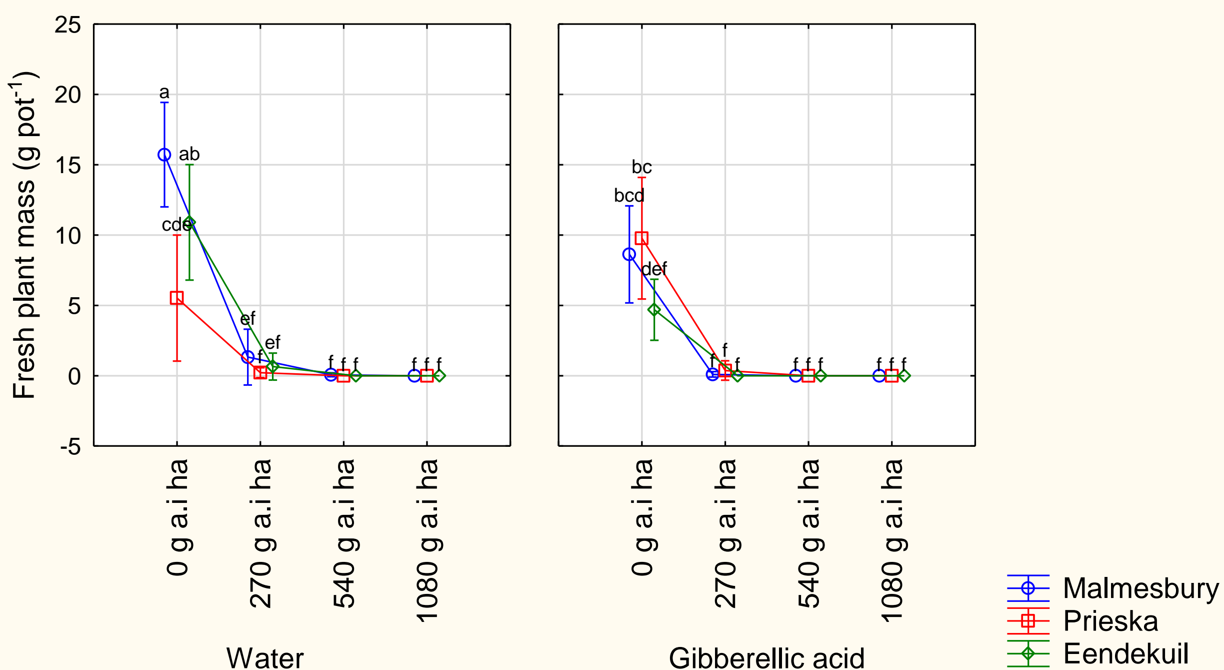


**Figure 3.4:** Three way interaction between three wild oat populations, two seed treatments and four glyphosate dosages in terms of fresh mass $pot^{-1}$. (Different letters indicate significance between means at p=0.05)

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**Dry plant mass (g)**

There was no rate-response decrease in the efficacy of glyphosate recorded for dry mass of plants. In all populations pre-germination treatments did not show a clear trend of decrease in dry mass with increase in glyphosate dosages (excluding the control treatment). These results show that all the wild oat populations were completely controlled and no resistance behaviour wasobserved. High concentrations of glyphosate in the tissue content are due to the increase in the dose rate of glyphosate (Feng et al. 2004). Any surviving tissue provides the opportunity to regrow under favourable conditions (Feng et al. 2003). It clearly shows that all the applied dosages killed the plants and no plant tissue survived.

The efficacy of glyphosate in all populations treated with different pre-germinations is probably due to the fact that the seedlings were growing actively which could have influenced translocation of glyphosate. Translocation of glyphosate is mainly via the phloem, following along the sugar gradient from source cells with high concentration of sugar such as leaf cells to continuously growing sink cells with low concentration of sugar such as root cells (Gouger and Geiger 1984; Feng et al. 2004). Plant leaf area influence efficient absorption of glyphosate. Franez et al. (1997) expressed that when plant cells absorb glyphosate through the plasma membrane in the symplast both active and passive transport diffusion mechanisms take place. Franez et al. (1997) showed that in most plants glyphosate is rapidly translocated; it mostly invades into the symplast and is translocated extensively through the entire parts of the plant through the phloem, following the same distribution pattern as photo assimilates.

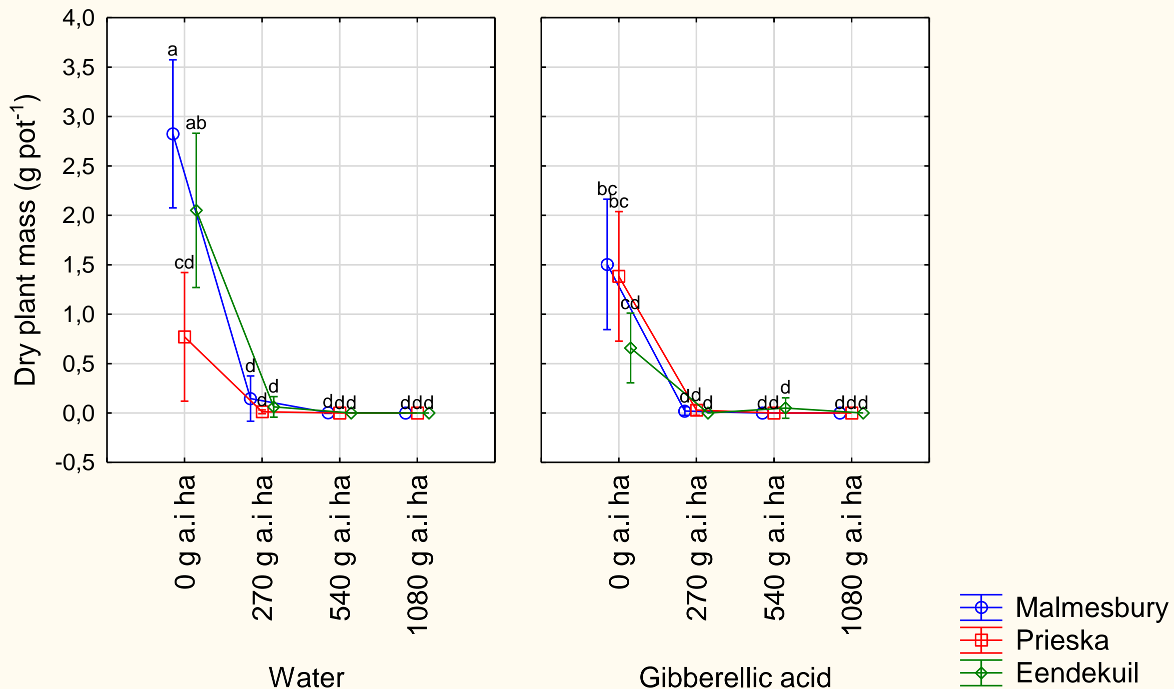


**Figure 3.5:** Three way interaction between three wild oat populations, two seed treatment and four glyphosate dosages in terms of dry mass pot$^{-1}$. (Different letters indicate significance between means at p=0.05)

## 3.7. Conclusion

Ammonia treatment, and to a lesser extent, $GA_3$ treatment, improved the germination of the two wild oat populations. It did not appear as if $GA_3$ treatment, which results in tall, lanky seedlings, influenced the survival of wild oat seedlings treated with glyphosate. Therefore gibberellic acid can safely be used as a pre-germination treatment to improve wild oat seed germination to provide sufficient wild oat seedlings to be tested for resistance to glyphosate. The fact that almost 100% control was achieved in all three wild oat populations with 270 g a.e. ha$^{-1}$ of glyphosate (half the recommended dose), means that there is no indication of significant resistance to glyphosate in any of the three populations tested.

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## References

Anjum T, Bajwa R. 2005. Importance of germination indices in interpretation of allelochemical effects. *International Journal of Agriculture and Biology* 7: 1560-8530.

Baskin CC, Baskin JM. 1998. *Seeds: Ecology, biogeography, and evolution of dormancy and germination.* San Diego, Academic Press.

Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. *Seed Science Research*. 14: 1–16.

Baskin CC, Baskin JM. 2014. *Seeds: ecology, biogeography, and, evolution of dormancy and germination.* Elsevier.

Baskin CC, Thompson K, Baskin JM. 2006. Mistakes in germination ecology and how to avoid them. *Seed Science Research* 16: 165–168.

Black M, Naylor JM. 1959. Prevention of the onset of seed dormancy by gibberellic acid. *Nature* (London) 184: 468-469.

Cairns ALP. 1974. Biology and control of *Avena fatua* L. in the Western Cape. MSc Thesis, University of Stellenbosch, Stellenbosch.

Cairns ALP. 1984. The physiology of seed dormancy and germination in wild oats (*Avena fatua* L.). PhD thesis, Stellenbosch University, South Africa.

Chen FS, MacTaggar JM, Elofson RM. 1981. Chemical constituents in wild oat *(Avena fatua)* hulls and their effect on seed germination. *Canadian Journal of Plant Science* 62: 155-161.

Coupland TM. 1985. Metabolism of glyphosate in plants. In: Grossboard E, Atkinson D (eds). *The herbicide Glyphosate*. London: Butterworths.pp 25-34.

Cumming BG, Hay JR. 1958. Light and dormancy in wild oats. *Nature* (London) 182: 609-610.

El Balla MMA, Saidahmed AI, Makkawi M. 2011. Effect of moisture content and maturity on hardseededness and germination in okra (*Abelmoschus esculentus* L. *Moench*.). *International Journal of Plant Physiology and Biochemistry* 3: 102–107.

Stellenbosch University https://scholar.sun.ac.za

Feng PCC, Chiu T, Sammons RD. 2003. Glyphosate efficacy is contributed by its tissue concentration and sensitivity in velvetleaf (*Abutilon theophrasti*). *Pesticide Biochemistry and Physiology* 7: 83-91.

Feng PCC, Tran M, CHIU T, Sammons RD, Heck GR, Cajacob CA. 2004. Investigations into glyphosate-resistant horseweed (*Conyza canadensis*): retention, uptake translocation, and metabolism. *Weed Science*. 52: 498–505.

Fenner M, Thompson K. 2005. *The ecology of seeds*. Cambridge, UK: Cambridge University Press.

Franez JE, Mao MK, Sikorski JA. 1997. *Glyphosate a unique global herbicide.* ACS Monograph 189. Washington DC. American Chemical Society.

Friesen G, Shebeski LH. 1961. The influence of temperature on the germination of wild oat seed. *Weeds* 9: 634-638.

Gouger JA, Geiger DR. 1984. Carbon partitioning and herbicide transport in glyphosate-treated sugarbeet (*Beta vulgaris*). *Weed Science* 32: 546–551.

Green JG, Helgerson, EA. 1957. The effect of gibberellic acid on dormant seed of wild oat. In: Proceedings of the 14$^{th}$ Northern Central Weed Control Conference. p 39.

Gupta R, Chakrabarty SK. 2013. Gibberellic acid in plant: still a mystery unresolved. *Plant Signalling and Behaviour 8:* 9.

Hay JR, 1962. Experiments on mechanism of induced dormancy in wild oats, *Avena fatua* L. *Canadian Journal of Botany* 40: 191-202.

Hay JR, Cumming BG.1959. A method for inducing dormancy in wild oats *(Avena fatua* L.). *Weeds* 7: 34-40.

Hilhorst HWM, Groot SPC, Bino RJ.1998. The tomato seed as a model system to study seed development and germination. *Acta Botanica Neerlandica Journal* 47: 169–183.

Hsiao AI. 1979. The effect of sodium hypochlorite and gibberellic acid on seed dormancy and germination of wild oats *(Avena fatua). Canadian Journal of Botany* 57: 1729-1734.

Stellenbosch University https://scholar.sun.ac.za

Hsiao AI, Simpson GM. 1971. Dormancy studies in seed of *Avena fatua* 7. The effects of light and variation in water regime on germination. *Canadian Journal of Botany* 49: 1347-1357.

Imam AG, Allard DR. 1965. Population studies in predominantly self-pollinating species VI. Genetic variability between and within natural populations of wild oats in different habitats in California. *Genetics* 1: 49.

Kigel J. 1995. Seed germination in arid and semiarid regions. In: Kigel J, Galili G (eds). *Seed development and germination*. New York, USA: Marcel Dekker. pp 645–699

Kochi W. 1968. Environmental factors affecting the germination of some annual grasses. Proceedings of the 9th British Weed Control Conference, 18-20 November, Brighton. pp 14-19.

Lester DC, Carter OG, Kelleher FM, Laing DR. 1972. The effect of gibberellic acid on apparent photosynthesis and dark respiration of simulated swards of *Pennisetum clandestinum* Hochst. *Australian Journal of Agricultural Research* 23: 205-213.

Li-Cor. 2016. LI-3100C Area Meter. Campbell Scientific Africa (Pty) Ltd https://licor.co.za/?portfolio=li-3100c

Martínez-Fernández V, Martínez-García F, Pérez- García F. 2014. Census, reproductive biology, and germination of *Astragalus gines-lopezii* (Fabaceae), a narrow and endangered endemic species of South West Spain. *Turkish Journal of Botany* 38: 686–695.

Miller SD, Nalewaja JD, Mulder CEG.1982. Morphological and physiological variation in wild oat. *Agronomy Journal* 75: 771-775.

Munktell. 2018. Munktell Grade 391 Filter Paper Vos Instruments. https://www.vosinstrumenten.nl/onderwijs/disposables-filters/filters/rondfilters/munktell-grade-391.html

Nandula VK. 2010. *Glyphosate resistance in crops and weeds: history, development, and management.* John Wiley and Sons.

Naylor JM, Jana S. 1976. Genetic adaption for seed dormancy in *Avena fatua. Canadian Journal of Botany* 54: 306-312.

Stellenbosch University https://scholar.sun.ac.za

Naylor JM, Simpson GM. 1961. Dormancy studies in *Avena fatua* 2. A gibberellin-sensitive inhibitory mechanism in the embryo. *Canadian Journal of Botany* 39: 281-295.

Nikolaeva MG. 2001. Ecological and physiological aspects of seed dormancy and germination (review of investigations for the last century). *Botanicheskii Zhurnal Journal* 86: 1–14.

Opik H, Simon EW. 1963. Water content and respiration rate of bean cotyledons. *Journal of Experimental Botany* 14: 299-310.

Patterson JG, 1976. The distribution of *Avena* species naturalized in Western Australia. *Journal of Applied Ecology*. 13: 257-263.

Peters NCB. 1978. Factors influencing the emergence and competition of *Avena fatua* in spring barley. PhD thesis, University of Reading, England.

Pieterse PJ, Cairns ALP. 1986. An effective technique for breaking the seed dormancy of *Acacia longifolia*. *South African Journal Plant Soil* 3: 85-87.

Quail PH, Carter OG. 1968. Survival and seasonal germination of seeds of *Avena fatua* and *A. ludoviciana*. *Autralian Journal of Agricultural Research* 19: 721-729.

Roberts HA. 1986. Seed persistence in soil and seasonal emergence in plant species from different habitats. *Journal of Applied Ecology 23: 639–656.*

Sawhney R, Naylor JM. 1979. Dormancy studies in *Avena fatua* 9. Demonstration of genetic variability affecting the response to temperature during seed development. *Canadian Journal of Botany* 57: 59-63.

Schuette J. 1998. Environmental fate of glyphosate. Available: http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/glyphos.pdf [accessed 22 March 2014].

Simpson GM, 1978. Metabolic regulation of dormancy in seeds - a case history of the wild oat *(Avena fatua).* Dormancy and Developmental Arrest: Experimental Analysis in Plants and Animals. In: Clutter EM (ed). pp 167-220.

Simpson GM.1990. *Seed dormancy in grasses*. Cambridge, Cambridge University Press.

Smeda RJ, Putnam AR. 2010. Influence of temperature, rainfall, grass species, and growth stage on efficacy of fluazifop. *Weed Technology* 4: 349–355.

Stellenbosch University https://scholar.sun.ac.za

Srivastava LM. 2002. *Plant growth and development: hormones and environment*. Academic Press. p 140.

Statistica. 2012. Data analysis software system Version 12, Statsoft Inc. www.statsoft.com

Thurston JM.1957. Morphological and physiological variation in wild oats (*Avena fatua* L. and *A. Ludoviciana Dur*) and hybrids between wild and cultivated oats. *Cambridge*: *The Journal of Agricultural Science* 49: 259-274.

Torres YA, Long MA, Zalba MS. 2008. Reproduction of *Pavonia cymbalaria* (Malvaceae), a native species with ornamental potential. *Phyton, International Journal of Experimental Botany 77*: 151–160.

Van Assche, JA, Debucquoy KLA, Rommens, WAF. 2003. Seasonal cycles in the germination capacity of buried seeds of some Leguminosae (Fabaceae). *New Phytologist Journal* 158: 315–323.

Verma V, Kasera PK. 2006. Effect of different seed and fertilizer treatments on growth and biomass of *Sida cordifolia* in semi-arid desert region of country. *Journal of Medical and Aromatic Plant Sciences* 28: 23–26.

Warrick BE, Baughman T. 2003. Wild Oat Control in Texas. Wild Oat Control Publication, extension Agronomists. Texas A&M Research and Extension Centre at San Angelo http://sanangelo.tamu.edu/extension/agronomy/agronomy-publications/wild-oat-control-publication/ [accessed 06 February 2016].

Whalley RDB, Burfitt JM. 1972. Ecotypic variation in *A. fatua*, *A. sterilis* (*A. ludoviciana*) and *A. barbata* in New South Wales and Southern Queensland. *Australian Journal of Agricultural Research* 23: 799-810.

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# CHAPTER 4
## SCREENING OF WILD OAT POPULATIONS FOR GLYPHOSATE RESISTANCE

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### Abstract
**Glyphosate has been proved to be ineffective in certain weedy ryegrass (*Lolium* spp.), buckhorn plantain (*Plantago lanceolata*) and hairy fleabane (*Conyza bonariensis*) populations in South Africa. Although glyphosate resistance in wild oats (*Avena fatua*) has not yet been proven, circumstantial evidence point to the possibility of glyphosate resistant populations in the south-western and northern Cape. Seven wild oat populations with no indication of being resistant to glyphosate from Lindley, Eendekuil A and B, Bethlehem A and B, Clarens and Prieska were selected for the first experiment. For the second experiment alleged resistant populations from Malmesbury and Eendekuil C were also selected. Seeds from all these populations were germinated in 1mM of gibberellic acid solution. At the four leaf growth stage, seedlings were treated with four glyphosate dosage rates (0, 270, 540, 1080 g a.e ha$^{-1}$). Survival percentage was calculated six weeks after glyphosate application. The Prieska population had the highest survival percentage (42%), while the Eendekuil B population had the lowest survival percentage (0%) at 270 g a.e ha$^{-1}$. The Eendekuil C and Malmesbury populations showed high susceptibility to glyphosate at different dosage rates including the lowest rate applied (270g a.e ha$^{-1}$). No plants survived at 540 g a.e ha$^{-1}$ and 1080 g a.e ha$^{-1}$ dosage rates. None of the populations that were tested was resistant to glyphosate at the registered recommended rate of 540 g a.e ha$^{-1}$.**

**Keywords**: Glyphosate, resistance, screening, survival rate, wild oats.

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## 4.1. Introduction

Herbicides serve as the most essential aspects in controlling weeds. Herbicides have imposed efficient, easy and simple labour techniques of weed control as compared to the previous techniques such as hand pulling and hoe weeding. In well-established agricultural production structures weeds are a crucial constraint (Oerke 2006) due to competition for valuable resources are important for plant growth (Bastiaans 2008). In the south-western part of South Africa, herbicides are frequently applied on crop fields. Herbicide resistance in South Africa has been proven in mainly in the Western Cape vineyards, orchard and wheat fields (Pieterse and Cairns 2008). Frequent, repetitive application of herbicides to control weeds, introduced new weed problems such as increased evolution of weeds which are resistant to certain herbicides (Pieterse and Cairns 2008).

For the evolution of an inherited trait like herbicide resistance in plant populations, there are certain requirements. Important requirements are heritable variation for the trait and natural selection. Both mutation and selection have a direct impact on evolution. This includes the combination of existing genetic variation, the rate of evolution and the intensity of selection (Jasieniuk et al. 1996). Herbicide resistance can be anticipated due to the results of natural selection (Heap 2014).

In addition to the above-mentioned factors which influence inherent resistance in plant populations, the type of herbicides and their method of application are also important. Three aspects of herbicide use which influence selection pressure for herbicide resistance is: frequency of using the herbicide, efficiency of the herbicide and the period or length of the effect of the herbicide (Gressel 2002). The rate at which herbicide resistance develop in a population of weeds is influenced by the frequency of naturally resistant individuals in the population as well as the characteristics of the herbicide applied (Pieterse 2010).

Gressel (1991) have defined herbicide resistance as a plant's inherited ability to thrive and continue to reproduce after being exposed to herbicide dosage application normally lethal to the wild type. The occurrence of herbicide resistance in weeds is an evolutionary ability which was first discovered in 1968 by an owner of an ornamental nursery (Gressel et al. 1982; Jasieniuk et al.1996). The first case of confirmed resistance was recorded to trazine application in groundsel (*Senecio vulgaris*). Seeds collected from the resistant biotype were proven as resistant to

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simazine and atrazine. In the year 1974, this created a problem in corn production (Gressel et al.1982). According to Heap (2009) herbicide resistance most commonly evolved to acetolactate synthase (ALS) inhibitors, triazines and Acetyl Coenzyme A Carboxylase (ACCase) inhibitors.

Cases of glyphosate resistance have been discovered globally which includes mainly the USA (Perez-Jones et al. 2005), South Africa, Israel, Italy, Spain and France (Heap 2011) and Australia (Owen and Powles 2010). Pratley et al. (1996) discovered the very first incidence of glyphosate resistance in rigid ryegrass (*Lolium rigidum*) biotypes in Australia. Preston et al. (2009) found that Italian ryegrass (*Lolium multiflorum*) was resistant to glyphosate. Glyphosate resistance in a *Lolium multiflorum* biotype was reported in Chile (Perez and Kogan 2003), United States of America (Perez-Jones et al. 2005), Spain, France, Brazil and Argentina (Heap 2011) and in 2001 rigid ryegrass resistance was also reported in South Africa. There were a number of greenhouse trails which were conducted to study dose response and inheritance characteristics of the progeny of a cross between resistant and susceptible lines (Simarmata et al. 2005). Final results of Simarmata et al. (2005) showed that resistance inheritance comprises of more than one mutant allele and that it is transported through pollen.

Wakelin and Preston (2006) also conducted studies on a number of rigid ryegrass populations in Australia. Their final results of the study led to the conclusion that the nuclear genome was a factor influencing the encoding of glyphosate resistance in each of the tested populations of rigid ryegrass. In contrast with what Simarmata et al. (2005) discovered, Wakelin and Preston (2006) concluded that a single dominant allele is the main factor which controls glyphosate resistance.

Simarmata and Penner (2008) conducted a follow-up study on the inheritance of resistance from the study of Simarmata et al. (2005). Simarmata and Penner (2008) studied again the effect of glyphosate on the actions of EPSPS and the EPSPS gene in resistant and susceptible rigid ryegrass biotypes. There were no significant differences found in the metabolism of a glyphosate molecule between the two biotypes. However, actions of EPSPS reflected more inhibition in the susceptible biotype as compared to the tested resistant biotype (Simarmata and Penner 2008).

Wild oats (*Avena* spp.) has not been confirmed as resistant to glyphosate yet (Heap 2017). However, the very first reported case of *A. fatua* resistance to diclofop-methyl was reported in the Western Cape Province (Cairns and Laubscher 1986). Aryloxyphenoxypropionates and

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cyclohexanediones resistance in wild oats (*A. fatua*) and ryegrass (*Lolium* spp.) was discovered in Australia (Morrison and Bourgeois 1995). Heap et al. (1993) reported that a total of four wild oat (*Avena fatua*) populations have an ability to thrive after aryloxyphenoxypropionate and cyclohexanedione herbicides was applied in the western part of Canada. *Avena fatua* populations are described to be resistant to members of three different herbicide mode-of-action families (Vila-Aiub et al. 2009).

Although there are no confirmed cases yet of glyphosate resistance in South Africa, circumstantial evidence point to possible development of glyphosate resistance in some wild oat populations in South Africa. The objectives of this research were (i) to screen seven wild oat populations from various localities in South Africa for possible loss of sensitivity to glyphosate and (ii) to confirm if resistance is present in two alleged resistant populations.

## 4.2. Material and methods

### 4.2.1. The experimental site

The experiments were conducted in a glasshouse at the Stellenbosch University Welgevallen experimental farm. The site is located at 33° 56´33" S and 18° 51´56" E at an altitude of 136 m above sea level.

### 4.2.2. The treatments and experimental design

#### 1. Screening seven wild oat populations for glyphosate resistance

Seeds from seven wild oat populations were selected. Selected populations had no history of suspected resistance to glyphosate. A randomized complete block design was arranged as a 7×4 factorial with 3 replications for the experiment. The experimental factors were seven wild oat populations: Lindley (27°52′S, 27°55′E), Eendekuil A and B (32°41′S, 18°53′E), Bethlehem A (28.224°S, 28.311°E), Bethlehem B (28.224°S, 28.311°E), Clarens (28°31′S, 28°25′E), and Prieska (29.7069° S, 22.7390° E) and four glyphosate dosage rates (0, 270, 540, 1080 g a.e $ha^{-1}$). The plants were sprayed 3-4 weeks after transplanting into pots.

#### 2. Screening two wild oat populations for glyphosate resistance

Two wild oat populations' seeds were selected withsuspected resistance to glyphosate. A complete randomized block design arranged as a 2×4 factorial with 6 replications was used for the experiment. The experimental factors were two wild oat seed populations Malmesbury (33.4655°′S 18.7185° E) and Eendekuil C (32°41′S 18°53′E) and four glyphosate dosage rates ( 0, 270, 540, 1080 g a.e $ha^{-1}$). The plants were sprayed 4 weeks after transplanting.

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**The trail establishment and management**

**Seedling preparations and germination process**

The nine wild oat population seeds were all germinated in a similar way.

**Figure 4.1:** Seven wild oat population seedlings germinated in petri dishes containing 1 Milli molar gibberellic acid.

**i. Gibberellic acid solution preparation**

Selected seeds were germinated in 6 ml of a prepared 1 mM of gibberellic acid solution (0.087 g of $GA_3$ mixed with 250 ml of distilled water), in 90 mm diameter plastic petri dishes containing two filter papers (Munktell Grade 391 Filter Paper, Vos Instruments) (Munktell 2018). The petri dishes were enclosed in a poly propylene bag to prevent evaporation of the germination solution and were kept in a growth chamber in the dark at a constant temperature of 20 $^oC$.

ii **Planting**

After the germination process of 1-3 weeks, seedlings were transferred to the glasshouse set at a night/day temperature of 20/25 $^oC$ inside the petri dishes where they germinated but the lids were removed (See Figure 4.1). Seedlings developed a green pigment colour 3-4 days after being placed in the glasshouse. Seedlings were then transplanted into 8 x 8 cm pots (four seedlings per pot) containing a coarse sand/gravel mix.

iii **Irrigation**

An automated irrigation system was used to water the plants at 08:00 am, 12:01 pm and 4:00 pm for approximately one minute with a balanced nutrient solution (Table 4.1).

**Table 4.1:** The nutrient content of the nutrient solution used to water the plants in the glasshouse

| EC = 2.0 | | | |
|---|---|---|---|
| Element (Macro) | Concentration mg $L^{-1}$ | Fertilizer | 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 |

**Pest and disease control**

No pests and diseases were experienced in the glasshouse.

**Herbicide application**

Glyphosate was applied to seedlings at the $4^{th}$ leaf growth stage, 3 – 4 weeks after transplant. The herbicide was applied by means of a pneumatic pot sprayer at a pressure of 2 bars in 100 L $ha^{-1}$ of water.

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## 4.3 Data collection

### Application of glyphosate

On the day of spraying extra control plants were harvested (This was done to compare difference of seedling sizes before glyphosate was applied) and the following variables were recorded:

#### i. Number of leaves per plant

The number of leaves per plant were counted and recorded and the mean number of leaves of the plants in the pot was calculated.

#### ii. **Leaf area (cm2)**

Leaf area per plant was measured using a leaf area meter (Campbell Scientific Africa (Pty) Ltd, LI-Cor3100C Area Meter) (Li-Cor 2016) (Figure 4.2) and the mean leaf area of the plants per pot was calculated.

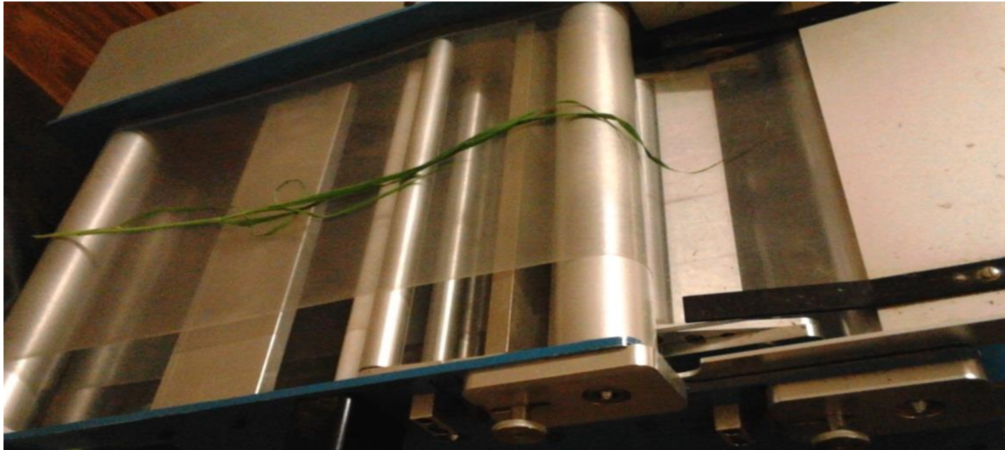

**Figure 4.2:** Leaf area per plant was measured using a leaf area meter (Li Cor).

#### iii. Plant height (cm)

A ruler was used to measure plant height of each plant. The height considered was of the stems and leaves above soil surface to the tip of the longest leaf (Figure 4.3). The mean plant height of the plants in the pot was calculated.

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**Figure 4.3:** Plant height was measured in cm using a ruler.

**iv. Fresh plant mass (g)**

From each pot, plants were harvested above soil surface by means of scissor and put into small brown paper bags. Fresh plant mass was measured using an electronic weighing scale andmean fresh mass per pot was calculated.

**v. Dry plant mass (g)**

After determining the fresh plant mass, the small brown paper bags with plants were placed into an oven and dried at 80ºC for 48 hours. The dry plants were weighed on an electronic weighing scale and mean dry mass per pot was calculated.

**Evaluation**

Six weeks after glyphosate application the following variables were recorded:

i. Survival percentage

Survival rate percentage was recorded six weeks after spraying (See Figure 4.4 and 4.5). The calculation was done using the following formulae:

$$\text{Percentage Survival \%} = \frac{\text{number of surviving plants per pot}}{4(\text{plants per pot})} \times 100\%$$

Plants that displayed any actively growing green leaves, no matter how small the leaves, six weeks after glyphosate application, were considered as surviving plants.

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**Figure 4.4:** Seven wild oat populations six weeks after glyphosate application, which revealed results shown in **Figure 4.6**.

**Figure 4.5:** Two wild oat populations six weeks after glyphosate application, which revealed results shown in **Figure 4.7**.

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### 4.4. Data analysis

Data was subjected to analysis of variance using the STATISTICA 12 program (Stastica 2012). Means of significant main effects and interactions in the experiments were separated using Turkey $HSD_{0.05.}$

## 4.5. Results

### i. Results of the tested seven wild oat populations

No significant interaction between the seven wild oat biotypes and glyphosate concentrations in terms of survival percentage were recorded (p=0.347). However, glyphosate dosage rate had a significant effect on survival percentage. Figure 4.6 shows that none of the seven populations are resistant to glyphosate. There were plants that survived in Lindley (16. 7%), Eendekuil A (25%), Bethlehem A (33%), Bethlehem B (8%), Clarens (8%) and Prieska (42%) populations, but no plants survived in the Eendekuil B population when 270 g a.e $ha^{-1}$ was applied. Prieska had the highest survival rate (42%), while Eendekuil B had the lowest survival rate (0%) at 270 g a.e $ha^{-1}$ glyphosate dosage rate. No plants survived at 540 g a.e $ha^{-1}$ and 1080 g a.e $ha^{-1}$ of glyphosate.

**Table 4.2:** Vegetative growth parameters for seven different wild oat populations growing in the glasshouse at the time of spraying

| Population | Seed germination process | Plant Height (cm) | Leaf Area ($cm^2$) | Dry plant mass (g) |
|---|---|---|---|---|
| Lindley | Gibberellic acid | $32.04^{a}$ | $23.92^{ab}$ | $0.40^{a}$ |
| Eendekuil A | Gibberellic acid | $20.19^{a}$ | $9.78^{a}$ | $0.11^{a}$ |
| Eendekuil B | Gibberellic acid | $29.99^{a}$ | $19.17^{ab}$ | $0.25^{a}$ |
| Bethlehem A | Gibberellic acid | $24.53^{a}$ | $12.88^{ab}$ | $0.31^{a}$ |
| Bethlehem B | Gibberellic acid | $24.13^{a}$ | $16.43^{ab}$ | $0.45^{a}$ |
| Clarens | Gibberellic acid | $32.23^{a}$ | $23.53^{ab}$ | $0.30^{a}$ |
| Prieska | Gibberellic acid | $30.2^{8a}$ | $28.47^{b}$ | $0.35^{a}$ |

Values in a row followed by the same letters indicate no significant differences between treatments at p = 0.05.

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**Table 4.2** shows the vegetative growth parameters of the different wild oat populations at the time of spraying. There were very little significant differences between the populations in terms of plant size at the time of spraying.

### ii. Results of the two wild oat populations tested

No significant interaction between the two populations and the four glyphosate dosage rates was recorded (p=0.917). Both populations from Eendekuil C and Malmesbury showed high susceptibility to glyphosate at different dosage rates (Figure 4.7). Malmesbury was discovered to be the most sensitive population though the survival rate percentage of Eendekuil C was not significantly different from Malmesbury population across all four dosage rates.

**Table 4.3:** Vegetative growth parameters for two different wild oat populations growing in the glasshouse at the time of spraying

| Population | Seed germination process | Plant Height (cm) | Leaf Area ($cm^2$) | Dry plant mass (g) |
|---|---|---|---|---|
| Malmesbury | Gibberellic acid | $46.09^a$ | $55.48^a$ | $0.83^a$ |
| Eendekuil | Gibberellic acid | $42.74^a$ | $54.01^a$ | $0.79^a$ |

Values in a row followed by the same letters indicate no significant differences between treatments at p = 0.05.

**Table 4.3** shows no significant differences in the vegetative growth parameters of the two wild oat populations at the time of spraying

In-terms of wet and dry mass parameters the results had a similar trend to the survival percentage results and therefore we did not include wet and dry mass results.

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## 4.6. Discussion

### i. Seven wild oat populations experiment

The Prieska population with 42% survival showed less sensitivity to glyphosate at 270 g a.e $ha^{-1}$ whereas the Eendekuil A and Bethlehem A populations revealed 25% and 33% survival percentages, respectively. However, survival percentages of the Eendekuil A and Bethlehem A populations did not differ significantly from the Prieska population. Survival at low glyphosate dosage rates is a result of low glyphosate concentrations, where surviving tissue succeeded to regrow under favourable conditions (Feng et al. 2003) but this is not and indication of resistance. The Prieska population is less sensitive to glyphosate at the lowest dosage rate applied in the experiment and when a farmer applies dosage rates lower than the recommended dosage rate, effectiove control will not be achieved (De Wet 2005).

The experiment revealed that the Eendekuil B population with 0% survival percentage showed the highest sensitivity to 270 g a.e $ha^{-1}$ dosage, although the Bethlehem B and Clarens populations, both with 8% survival percentage were not significantly different to Eendekuil B. A possible explanation for the higher sensitivity of Eendekuil B and lower sensitivity of Prieska at 270 g a.e $ha^{-1}$ is that most populations are diverse and when seed collecting takes place, it is possible to harvest only seed from more susceptible plants (De Wet 2005). It is however also possible that less sensitive plant have a higher rate of metabolising glyphosate applied at lower dosage rates (Simarmata and Penner 2008) and therefore it could be the onset of non-target site resistance. Non-target site resistance is described as resistance due to numerous mechanisms other than a target-site modification. This type of resistance can be supplied by mechanisms such as enhanced metabolism, low rates of herbicide translocation, sequestration and other mechanisms (Powles and Shaner 2001).

Application of sub-lethal dosage rates of herbicides, in general, creates and increases resistance problems. Prolonged use of sub-lethal rates add to weed management problems and again replenishing the weed seed bank (Doyle and Stypa 2004).

Furthermore, an increase in glyphosate dosage rates showed no significant differences in the survival rate percentage of seedlings in all populations. No plants survived when 540 g a.e $ha^{-1}$ and 1080 g a.e $ha^{-1}$ of glyphosate were applied. The recommended dosage rate of glyphosate is 540 g a.e $ha^{-1}$ to control weeds. Therefore at the recommended dosage rate, no indication of resistance was observed. According to De Wet (2005) farmers tend to suspect weeds of being resistant, after applying lower than recommended dosage rates and conclude that the herbicide

is ineffective, or resistance might be the problem. Suspected resistant weed samples are sent for resistance testing and it is usually discovered that resistance does not occur at the recommended rate. Glyphosate's efficacy is determined by its effective inhibition of EPSPS (Feng et al. 2003) and the recommended application rate should control plants effectively.

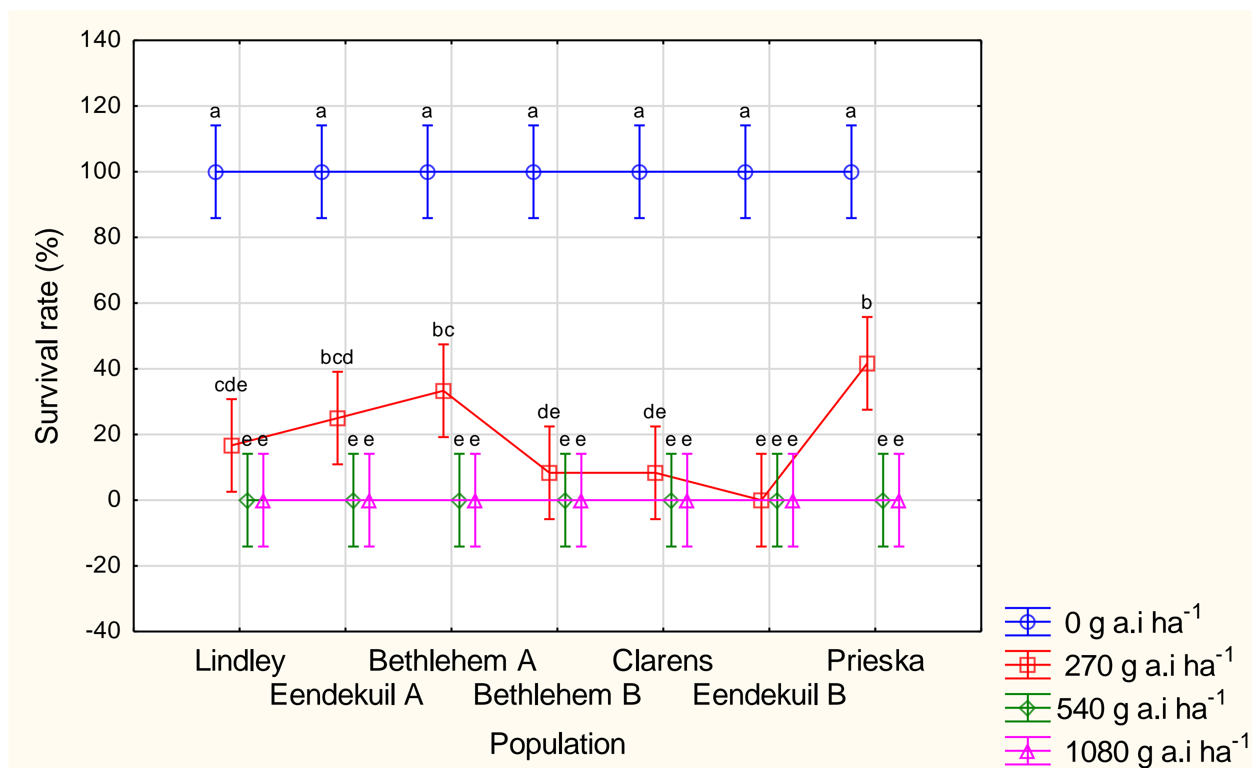


**Figure 4.6:** Interaction effects between seven wild oat populations and four glyphosate dosage rates on survival percentage. Where means showed significant difference at p=0.05 level it is indicated by different letters.

### ii. Two wild oat populations experiment

Gill et al. (1994) and Gressel (1988) stated that survival percentages ranging up to 25%, is usually caused by inadequate amounts of herbicide which result in poor control. Weed species are actually not resistant but application of low rates can deceive farmers when poor control is observed. Populations (Malmesbury and Eendekuil C) used in this experiment were the first progeny from previously survived parent plants where glyphosate was applied. It usually takes more than two consecutive years multiple applications of glyphosate before a weed species canshow resistance (Bradshaw et al. 1997). A widespread occurrence of resistant grass weed populations were found in Australia after ±15 years of successful use of glyphosate (Bradshaw et al. 1997). In addition the population used in the experiment conducted in Australia were

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discovered to be resistant when 720 to 1440 g a.e ha$^{-1}$ dose were applied (Bradshaw et al. 1997).

The Eendekuil C and Malmesbury populations showed 0% survival percentage when 540 and 1080 g a.e ha$^{-1}$ of glyphosate was applied. These wild oat populations can therefore not be considered to have resistance against glyphosate. Palmer amaranth was discovered and confirmed to be resistant to glyphosate when three times the prescribed dosage rate of 840 g a.e ha$^{-1}$ gave less than 18% control of the population. Glyphosate control certain Palmer amaranth biotype only at 75% when 12 times the recommended rate was applied (Culpepper et al. 2006).

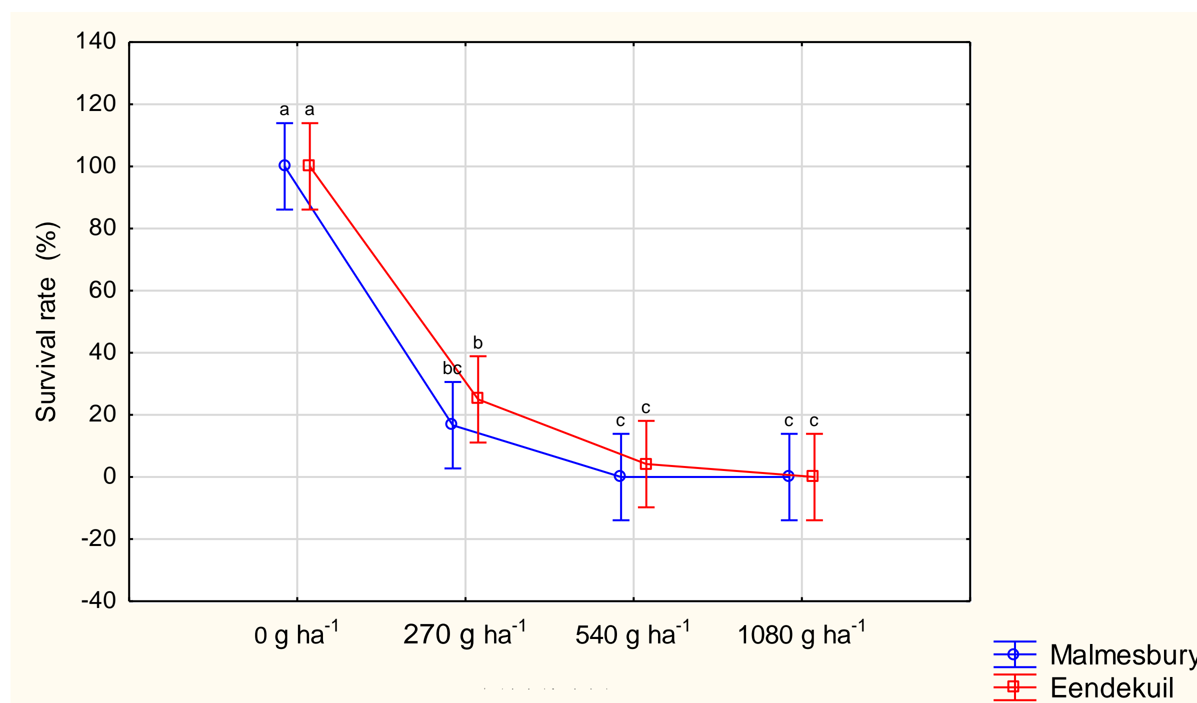


**Figure 4.7:** Interaction effects between two wild oat populations and four glyphosate dosage rates on survival percentage. Where means had significant differences at p=0.05 level it is indicated by different letters.

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## 4.7. Conclusion

None of the nine populations in both experiments that were tested were resistant to glyphosate since no ing plants were detected in any population at 540 g a.e $ha^{-1}$. According to the label specification of glyphosate 540 g a.e $ha^{-1}$ is the recommended rate for effective control of wild oats. In the first experiment the Prieska population showed less sensitivity to glyphosate at lower dosage rates of 270 g a.e $ha^{-1}$, but showed no resistance when the recommended rate of 540 g a.e $ha^{-1}$ was applied. Both populations from Malmesbury and Eendekuil C in experiment two showed high susceptibility to the lowest glyphosate dosage rate of 270 g a.e $ha^{-1}$. The results of these experiments proved susceptibility rather than resistance. Resistance is described as an inherited ability from parent plants and the populations tested in the second experiment were a progeny of survived plants. This is inexplicable because wild oats have self-pollinated reproductive behavior, i.e. pollen transfer from an anther to the stigma of the same plants (Auld and Medd 1992). The sensitivity of the plants to glyphosate could perhaps be influenced by the growing temperature and therefore the susceptibility to glyphosate should be tested at different temperature regimes.

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## References

Auld BA, Medd RW. 1992. Weeds. An illustrated botanical guide to the weeds of Australia. (Inkata Press, Melbourne) Photo. pp 37-38.

Bastiaans L. 2008. Focus on ecological weed management: What is hindering adoption*? Weed Research* 48: 481–491.

Beckie HI, Kirkland KJ. 2003. Implication of reduced herbicide rates on resistance enrichment in wild oat (*Avena fatua*). *Weed Technology* 17: 138-148.

Bradshaw LD, Padgette SR, Kimball SL, Wells BH.1997. Perspective on glyphosate resistance. *Weed Technology* 11: 189-198.

Cairns AL, Laubsher EW. 1986. Differential tolerance of Western Cape wild oat biotypes to diclofop-methyl and mixtures containing diclofop-methyl. Final report, Department of Agronomy and Pastures, University of Stellenbosch, Stellenbosch.

Culpepper AS, Grey LT, Vencill KM, Kilter JM. 2006. Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. *Weed Science* 54: 620–626.

De Wet H. 2005. Paraquat and glyphosate resistance in *Conyza bonariensis* in the Western Cape in the Republic of South Africa. MSc Agriculture thesis, Stellenbosch University, South Africa.

Doyle P, Stypa M. 2004. Reduced herbicide rates. A Canadian perspective 1. *Weed Technology* 18: 1157-1165.

Feng PCC, Chiu T, Sammons RD. 2003. Glyphosate efficacy is contributed by its tissue concentration and sensitivity in velvetleaf (*Abutilon theophrasti*). *Pest Biochemistry and Physiology* 77: 83-91.

Gill G, Holmes J, Kelley R. 1994. Herbicide resistance: A reference manual. Department of Agriculture, Western Australia, *Miscellaneous publication*. pp 1-53.

Gressel J.1988. Multiple resistances to wheat selective herbicides: new challenges for molecular biology. *Oxford surveys* of *Plant Molecular and Cell Biology* 5: 195-203.

Gressel J. 1991. Why get resistance? It can be prevented or delayed. In: Gaseley JG, Gussans GW, Atkin RK (eds). *Herbicide resistance in weeds and crops*. Butterworth-Heinemann Ltd, Oxford, Unite Kingdom. pp 1-26.

Stellenbosch University https://scholar.sun.ac.za

Gressel J. 2002. *Molecular Biology of Weed Control*. Taylor and Francis, London.

Gressel J, Ammon HU, Folgelfors H, Gasquez J, Kay QON, Kees H. 1982. Discovery and distribution of herbicide-resistant weeds outside North America. In: LeBaron HM, Gressel J (eds). Herbicide resistance in plants. John Wiley & Sons, New York. pp 31-56.

Heap IM, Murray BG, Loeppky HA, Morrison IN. 1993. Resistance to Aryloxyphenoxypropionate and Cyclohexanedione Herbicides in Wild Oat (*Avena fatua*). *Weed Science* 41: 232-238.

Heap I. 2009. International survey of herbicide resistant weeds. http://www.weedscience.com. [accessed 28 March 2017].

Heap I. 2011. The international survey of herbicide resistant weeds. http://www.weedscience.com. [accessed 28 March 2017]

Heap I. 2014. Global perspective of herbicide-resistant weeds. *Pest Management Science* 70: 1306–1315.

Heap I. 2017. The International Survey of Herbicide Resistant Weeds. Available www.weedscience.org [accessed 21 October 2017].

Jasieniuk M, Brûlé–babel AL, Morrison IN. 1996. The evolution and genetics of herbicide resistance in weeds. *Weed Science* 44: 176-193.

Li-Cor. 2016. LI-3100C Area Meter. Campbell Scientific Africa (Pty) Ltd https://licor.co.za/?portfolio=li-3100c

Morrison IN, Bourgeois L. 1995. Approaches to managing ACCase inhibitor resistance in wild oat on the Canadian prairies. Proceedings of the British Crop Protection Conference Weeds. pp 567-576.

Munktell. 2018. Munktell Grade 391 Filter Paper Vos Instruments. https://www.vosinstrumenten.nl/onderwijs/disposables-filters/filters/rondfilters/munktell-grade-391.html

Oerke EC. 2006. Crop losses to pests. *The Journal of Agricultural Science* 144: 31–43.

Stellenbosch University https://scholar.sun.ac.za

Owen MJ, Powles SB. 2010. Glyphosate-resistant rigid ryegrass (*Lolium rigidum*) populations in the Western Australian grain belt. *Weed Technology* 24: 44–49.

Perez A, Kogan M. 2003. Glyphosate-resistant *Lolium multiflorum* in Chilean orchards. *Weed Research* 42: 12–19.

Perez-Jones A, Park KW, Colquhoun J, Mallory-Smith CA, Shaner D. 2005. Identification of glyphosate-resistant ryegrass (*Lolium multiflorum*) in Oregon. *Weed Science* 53: 775–779.

Pieterse PJ. 2010. Herbicide resistance in weeds: a threat to effective chemical weed control in South Africa. *South African Journal of Plant and Soil* 27: 66-73.

Pieterse PJ, Cairns ALP. 2008. Current status of herbicide resistance in South Africa. Combined Congress of the Soil Science Society of South Africa, South African Society of Crop Production, Southern African Society for Horticultural Sciences and Southern African Weed Science Society, Grahamstown, January, 2008.

Powles SB, Shaner DL. 2001. *Herbicide resistance and world grains*. CRC Press, Florida.

Pratley J, Baines P, Eberbach P, Incerti M, Broster J. 1996. Glyphosate resistance in annual ryegrass. In: Virgona J, Michalk D (eds), Proceedings of the 11th Annual Conference of the Grasslands Society of New South Wales, Australia. p 126.

Preston C, Wakelin AM, Dolman FC, Bostamam Y, Boutsalis P. 2009. A decade of glyphosate-resistant *Lolium* around the world: Mechanisms, genes, fitness, and agronomic management. *Weed Science* 57: 435–441.

Simarmata M, Bughrara S, Penner D. 2005. Inheritance of glyphosate resistance in rigid ryegrass (*Lolium rigidum*) from California. *Weed Science* 53: 615–619.

Simarmata M, Penner D. 2008. The basis for glyphosate resistance in rigid ryegrass (*Lolium rigidum*) from California. *Weed Science* 56: 181–188.

Statistica. 2012. Data analysis software system Version 12, Statsoft Inc. www.statsoft.com

Vila-Aiub MM, Neve P, Powles SB. 2009. Fitness costs associated with evolved herbicide resistance alleles in plants. *New Phytologist* 184: 751- 767.

Stellenbosch University https://scholar.sun.ac.za

Wakelin AM, Preston C. 2006. Inheritance of glyphosate resistance in several populations of rigid ryegrass (*Lolium rigidum*) from Australia. *Weed Science* 54: 212–219.

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# CHAPTER 5
# EFFECT OF TEMPERATURE ON THE EFFICACY OF GLYPHOSATE ON WILD OATS

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## Abstract
**Temperature is one of the most crucial environmental factors to consider when spraying herbicides to control weed species. Several studies reported on poor controlof weeds in uncontrolled environments due to variable climatic conditions. A glasshouse experiment was conducted at Welgevallen experimental farm to investigate the response of wild oats exposed to different glyphosate dosage levels when grown under different temperature levels. Six wild oat populations investigated in Chapter 4 were selected *viz*. Prieska, Bethlehem A, Malmesbury, Eendekuil A, B and C, and were exposed to five glyphosate dosage rates; 0, 180, 360, 540 and 720 g a.e ha$^{-1}$. Plants were sprayed at 5-6 weeks after transplanting into pots. Plants were grown at four different temperature levels of 10/15 ºC, 15/20 ºC, 20/25 ºC and 25/30 ºC, night/day. A significant interaction between population, glyphosate dosage rate and temperature were recorded for the survival percentage, fresh plant mass pot$^{-1}$ and dry plant mass pot$^{-1}$. The Bethlehem A and Prieska populations showed the highest survival percentage when 180 g a.e ha$^{-1}$ of glyphosate was applied at the 20/25 ºC (Bethlehem A), 15/20 ºC (Prieska) and 25/30 ºC (Prieska) glasshouse temperature regimes. The lowest survival percentage resulted when all the population were sprayed with 360, 540 and 720 g a.e ha$^{-1}$ at all four temperatures in each glasshouse. Based on the results found wild oats did not show any signs of herbicide resistance.**

**Keywords**: Glyphosate, resistance, temperature, survival percentage, wild oats.

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## 5.1. Introduction

Environmental factors may vary on how they influence herbicide efficacy. In most summer seasons, the common conditions early in the day are reduced temperature, low light and high relative humidity, when compared to the conditions during the afternoon (Cieslik et al. 2013). Different temperatures as a single environment factor alone can cause the physiological condition of weeds to change, which then affect the herbicide efficacy due to the natural presence of enzymes responsible for removal of herbicide toxic compounds by detoxification metabolism in plants, which can compromise the agronomic performance of the products. Environmental factors can cause a large increase or decrease in the effectiveness of herbicides (Matzenbacher et al. 2014). The efficacy of most post-emergence herbicides may be influenced by temperature, relative humidity and precipitation (Gerber et al. 1983). These climatic conditions may influence processes such as herbicide absorption, translocation and plant metabolism which affects herbicide efficacy on controlling weed species (Waltz et al. 2004).

Temperature is one of the most crucial environmental factors to consider when spraying herbicides to control weed species. Several cases where herbicides fail to control weeds in an uncontrolled environment due to climatic conditions exist. Suitable confirmation tests are important to clarify whether symptoms of resistance are caused by environmental conditions (Kraehmer et al. 2014). Temperature influence both plant metabolism and herbicide efficacy. Mahan et al. (2006) noticed that thermal reliance of herbicide activity restricts their actions and this is supported with reaction rates. According to Mahan et al. (2006), this acquired new information and knowledge was useful in determining ideal weed control temperatures.

Penner (2015) also reported that within temperatures ranging from 10 ºC to 30 ºC, an increase in temperature will improve the phytotoxity of herbicides. In a trial conducted by Smeda and Putnam (2010), control of green foxtail decreased as temperature increased from low (18 °C) to high (30 °C) while no temperature influence was detected on Japanese millet. Penner (2015) attributes improved efficacy of herbicides at higher temperatures to the increased herbicide uptake and translocation in plants but decreased efficacy can be attributed to volatilization of the herbicide at higher temperatures. Conflicting responses might be triggered by differences in metabolism of plants that are grown under different temperatures (Kumaratilake and Preston 2005).

In a trial conducted to survey the influence of temperature on herbicide efficacy in wild radish, it was concluded that herbicide efficacy on wild radish was improved under higher temperatures

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(Kumaratilake and Preston 2005). At an active high dosage rate of 600 g $ha^{-1}$ herbicide, 100% control of wild radish was observed at high temperature of 20/25 °C, 70% was observed at low to medium temperature of 15/20 °C and 20% was observed at low temperature of 5/10 °C. The study concluded that absorption is not significantly affected by temperature but in contrast translocation is highly depended on temperature (Kumaratilake and Preston 2005). In the very same trial, herbicide efficacy was also reliant on relative humidity. Low relative humidity decreased herbicide efficacy.

Where glyphosate was applied at 840 g a.e. $ha^{-1}$ to plants early morning before sunrise and to plants right after midday, 54% and 100% control was achieved respectively. In another trial glyphosate applied at 840 g a.e. $ha^{-1}$ early morning, midday and at the end of day resulted in 69, 100, and 37% of velvetleaf control, respectively (Waltz et al. 2004). The efficacy of glyphosate also increased at high temperatures compared to low temperatures for control of weed species such as giant ragweed (Zahoor et al. 2017). The temperature influence on herbicidal activity is supported by the fact that temperature is commonly related with several chemical reaction rates in plants (Zanatta et al. 2008).

The overall objective of this study was to determine the most effective dosage rate of glyphosate on wild oats grown under different temperatures. Specific objectives were, (i) to observe the trend in which temperature influences glyphosate efficacy, (ii) to determine the effective dosage of glyphosate at different temperatures, and (iii) to assess response of wild oats from six different populations to glyphosate, and (iv), to determine an ideal useful weed control temperatures.

## 5.2. Material and methods

### 5.2.1. The experimental site

The experiment was conducted in a glasshouse at the Stellenbosch University Welgevallen experimental farm. The site is located at 33° 56´33" S and 18° 51´56" E at an altitude of 136 m above sea level.

### 5.2.2. The treatments and experimental design

Seeds from six wild oat populations were selected. Selected populations were chosen from the experiments in Chapter 4. A randomized complete block design was arranged as a 6×5×4 factorial with 6 replications for the experiment. The experimental factors were six wild oat populations: Prieska (29.7069° S, 22.7390° E), Bethlehem A (28.224°S, 28.311°E), Malmesbury (33.4655°′S, 18.7185° E), Eendekuil A, B and C (32°41′S, 18°53′E) exposed to five glyphosate

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dosage rates of 0, 180, 360, 540 and 720 g a.e ha$^{-1}$. Plants were sprayed 5-6 weeks after transplanting into pots. Plants were grown at four different temperature levels of 10/15 ºC, 15/20 ºC, 20/25 ºC and 25/30 ºC night/day.

### 5.2.3. The trail establishment and management

**Seedling preparations and germination process**

Seeds of six wild oat populations were all germinated in a similar way in petri dishes as shown in Figure 5.1.

**Figure 5.1:** Seeds of six selected wild oat populations.

**i. Gibberellic acid solution preparation**

The selected seeds were germinated in 6 ml of a prepared 1 mM of gibberellic acid solution (0.087 g of $GA_3$ was mixed with 250 ml of distilled water), in 90 mm diameter plastic petri dishes containing two filter papers (Munktell Grade 391 Filter Paper, Vos Instruments) (Munktell 2018). Petri dishes were enclosed in a poly propylene bag to prevent evaporation of the germination solution and were kept in a growth chamber in the dark at a constant temperature of 20 ºC.

**ii Planting**

After the germination process that lasted for about 1-3 weeks, seedlings were transferred to the glasshouse and placed inside the petri dishes where they germinated with lids removed. Seedlings developed a green pigment colour 3 days after being placed in the glasshouse.

Seedlings were then transplanted into 8 cm x 8 cm pots (four seedlings per pot) containing a coarse sand/gravel mix in a glasshouse.

### iii Irrigation

An automated irrigation system was used to water the plants. Plants were irrigated at 08:00 am, 12:00 pm and 4:00 pm for approximately 1 minuteusing a balanced nutrient solution (Table 5.1).

**Table 5.1:** The nutrient content of the nutrient solution used to water the plants in the glasshouse

| **EC = 2.0** | | | |
|---|---|---|---|
| Element (Macro) | Concentration mg $L^{-1}$ | Fertilizer | 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 |

### Pest and disease control

No pests and diseases were experienced in the glasshouse.

### Herbicide application

After the transplanted seedlings developed into the four leaf stage in the glasshouses running at 10/15 ºC and 15/20 ºC; seedlings developed into the 5-10 leaf stage in the glasshouses running at 20/25 ºC and 25/30 ºC (about 5-6 weeks) glyphosate was sprayed. The herbicide was applied by means of a pneumatic pot sprayer at a pressure of 2 bars in 100 L $ha^{-1}$ of water.

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## 5.3. Data collection

### Application of glyphosate

On the day of spraying extra control plants (This was done to compare difference of seedling sizes before glyphosate was applied) was harvested and the following variables were recorded:

**i. Number of leaves per plant**

The number of leaves per plant were counted and recorded and the mean number of leaves of plants per pot was calculated.

ii. **Leaf area (cm2)**

Leaf area per plant was measured using a leaf area meter (Campbell Scientific Africa (Pty) Ltd, LI-Cor3100C Area Meter) (Li-Cor 2016) and the mean leaf area of plants per pot was calculated.

**iii. Plant height (cm)**

A ruler was used to measure plant height of each plant. The height considered was of the stems and leaves above soil surface to the tip of the longest leaf. The mean plant height of the plants in the pot was calculated.

**iv. Fresh plant mass (g)**

Plants from each pot were harvested above soil surface by means of scissor and put into small brown paper bags. Fresh plant mass was measured using an electronic weighing scaleand mean fresh mass per pot was calculated.

**v. Dry plant mass (g)**

After determining the fresh plant mass, the paper bags with plants were placed into an oven and dried at 80ºC for 48 hours. The dry plants were weighed on an electronic weighing scale and the mean dry mass per pot was calculated.

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**Evaluation**

Six weeks after glyphosate application the following variables were recorded:

**Figure 5.2:** Six wild oat populations before glyphosate were sprayed, grown under 10/15 ºC.

**Figure 5.3:** Six wild oat populations after glyphosate were sprayed, grown under 10/15 ºC.

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**Figure 5.4:** Six wild oat populations before glyphosate were sprayed, grown under 20/25 ºC.

**Figure 5.5:** Six wild oat populations after glyphosate were sprayed, grown under 20/25 ºC.

**i. Survival**

Survival percentage was recorded six weeks after spraying (Figure 5.2 to 5.5). The calculation was done using the following formulae:

Percentage Survival = $\frac{\text{number of surviving plants per pot}}{\text{4(plants per pot)}} \times 100\%$

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Plants that displayed any actively growing green leaves, no matter how small the leaves, six weeks after glyphosate application, were considered as surviving plants. All surviving fresh plant mass and dry plant mass was weighed (g).

## 5.4. Data analysis

Data was subjected to analysis of variance using the STATISTICA 12 program (Stastica 2012). Means of significant main effects and interactions in the experiments were separated using Tukey $HSD_{0.05.}$

## 5.5. Results

A significant interaction ($p<0.05$) was recorded between population, glyphosate dosage rates and temperature in terms of the survival percentage, fresh plant mass $pot^{-1}$ and dry plant mass $pot^{-1}$.

Results showed that the Bethlehem A population had a significantly higher ($p<0.05$) survival percentage when 180 g a.e $ha^{-1}$ was applied at 20/25 ºC glasshouse temperatures but there was no significant difference between the Prieska and Bethlehem A populations at 180 g a.e $ha^{-1}$ at 15/20 ºC and 25/30 ºC glasshouse temperatures. The lowest survival percentage appeared when all the population were sprayed with 360, 540 and 720 g a.e $ha^{-1}$ under all four temperatures in each glasshouse (Figure 5.6). However, at 540 g a.e $ha^{-1}$, the recommended application rate, Bethlehem A population showed less sensitivity in the 25/30 ºC glasshouse. This was not significantly different from the other treatments in terms of survival percentage, but do indicate survival of some plants at the recommended dosage rate.

Results of fresh plant mass $pot^{-1}$ showed major differences between populations and temperature regimes in the control treatments. No significant differences between the treated plants at any of the populations or dosage rates or temperatures were observed. The variation in the control treatments probably caused the significant three-way interaction between the three factors (Figure 5.7).

Results of dry plant mass $pot^{-1}$ (Figure 5.8) revealed exactly the same trends as observed in fresh mass $pot^{-1}$.

.

**Table 5.2** shows the vegetative growth parameters of the different wild oat populations at the time of glyphosate application. Results showed a slight trend of plants being bigger at the

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higher temperatures at the time of spraying. Generally it appears as if the Malmesbury and Eendekuil C populations resulted in smaller plants compared to the other populations.

**Table 5.2:** Vegetative growth parameters for six different wild oat populations growing in the glasshouse at the time of glyphosate application

| Population | Temperature | Plant Height (cm) | Leaf Area ($cm^2$) | Dry plant mass (g) |
|---|---|---|---|---|
| Prieska | 10/15°C | $38.30^{abcd}$ | $33.74^{abc}$ | $0.36^{ab}$ |
| Bethlehem A | 10/15°C | $37.10^{abcd}$ | $33.33^{abcde}$ | $0.54^{abc}$ |
| Malmesbury | 10/15°C | $27.00^{a}$ | $10.17^{a}$ | $0.13^{a}$ |
| Eendekuil A | 10/15°C | $42.43^{abcde}$ | $42.19^{abcd}$ | $0.50^{abc}$ |
| Eendekuil B | 10/15°C | $32.92^{ab}$ | $18.50^{ab}$ | $0.36^{abc}$ |
| Eendekuil C | 10/15°C | $28.19^{ab}$ | $11.84^{a}$ | $0.09^{a}$ |
| Prieska | 15/20°C | $55.33^{cde}$ | $81.53^{efg}$ | $0.65^{abc}$ |
| Bethlehem A | 15/20°C | $51.03^{abcde}$ | $76.12^{cdefg}$ | $0.64^{abc}$ |
| Malmesbury | 15/20°C | $33.45^{abcd}$ | $35.29^{abcde}$ | $0.24^{ab}$ |
| Eendekuil A | 15/20°C | $48.49^{abcde}$ | $62.32^{bcdef}$ | $0.68^{abc}$ |
| Eendekuil B | 15/20°C | $49.55^{abcde}$ | $76.85^{cdefg}$ | $0.78^{bc}$ |
| Eendekuil C | 15/20°C | $42.51^{abcde}$ | $55.72^{abcdef}$ | $0.60^{abc}$ |
| Prieska | 20/25°C | $63.91^{e}$ | $116.46^{g}$ | $0.86^{bc}$ |
| Bethlehem A | 20/25°C | 50.29de | $51.85^{abcdef}$ | $0.70^{abc}$ |
| Malmesbury | 20/25°C | $53.10^{cde}$ | $48.14^{abcdef}$ | $0.69^{abc}$ |
| Eendekuil A | 20/25°C | $57.31^{cde}$ | $70.00^{cdefg}$ | $0.56^{abc}$ |
| Eendekuil B | 20/25°C | $54.60^{cde}$ | $64.92^{bcdef}$ | $0.63^{abc}$ |
| Eendekuil C | 20/25°C | $51.03^{abcde}$ | $32.05^{abcd}$ | $0.34^{abc}$ |
| Prieska | 25/30°C | $40.00^{abcd}$ | $72.53^{cdefg}$ | $062^{abc}$ |
| Bethlehem A | 25/30°C | $58.00^{de}$ | $96.62^{ef}$ | $0.77^{bc}$ |
| Malmesbury | 25/30°C | $52.33^{cde}$ | $55.39^{bc}$ | $0.55^{abc}$ |
| Eendekuil A | 25/30°C | $52.88^{cde}$ | $78.41^{defg}$ | $0.95^{c}$ |
| Eendekuil B | 25/30°C | $54.60^{cde}$ | $68.67^{bcdefg}$ | $0.71^{abc}$ |
| Eendekuil C | 25/30°C | $51.03^{abcde}$ | $64.43^{bcdef}$ | $0.50^{abc}$ |

. (Means within columns or rows followed by the same letter(s) do not differ significantly at p=0.05).

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## 5.6. Discussion

### Survival percentage

Prieska and Bethlehem populations appear to have lower levels of susceptibility when 180 g a.e. $ha^{-1}$ of glyphosate was sprayed, although this did not appear under the same temperature regimes. Less sensitivity at the lowest dosage rate does not actually confirm herbicide resistance but do indicate variable sensitivity to glyphosate. There was no clear confirmation of resistance in the six wild oats populations at any of the four controlled temperature regimes. However, the Bethlehem A and Prieska populations showed increased tolerance to a glyphosate dosage rate at the 15/20 and 20/25 ºC temperature regimes (Figure 5.6). Another interesting observation was that the survival rate of all, but particularly the Bethlehem and Prieska populations, was lower at 10/15 ºC than at the higher temperatures at the 180 g a.e $ha^{-1}$ dosage rates. This is contradictory to what Mahana et al. (2006) discoveredwhere herbicide activity depends on warm temperatures and cold temperatures restricts actions. The fact that some plants of the Bethlehem A and Eendekuil B populations survived the 540 g a.e. $ha^{-1}$ dosage rate, although statistically insignificant, may indicate very low levels of resistance to glyphosate.

There is no clear trend which indicated improvement of phytotoxicity of glyphosate with increasing temperature regimes in this trial. This contradicts with Penner (2015) who reported that between temperatures ranging from 10 ºC to 30 ºC, an increase in temperature resulted in an increase in efficacy. Penner (2015) reported that efficacy of herbicide activity at warm temperatures increases herbicidal uptake and translocation and cold temperature does the opposite. Kumaratilake and Preston 2005 reported that herbicide efficacy was greater on wild radish under higher temperatures. Zahoor et al. (2017) also reported a higher mortality rate of Giant Ragweed plants in high temperatures (mid-day) than in cooler temperatures (morning and evening times).

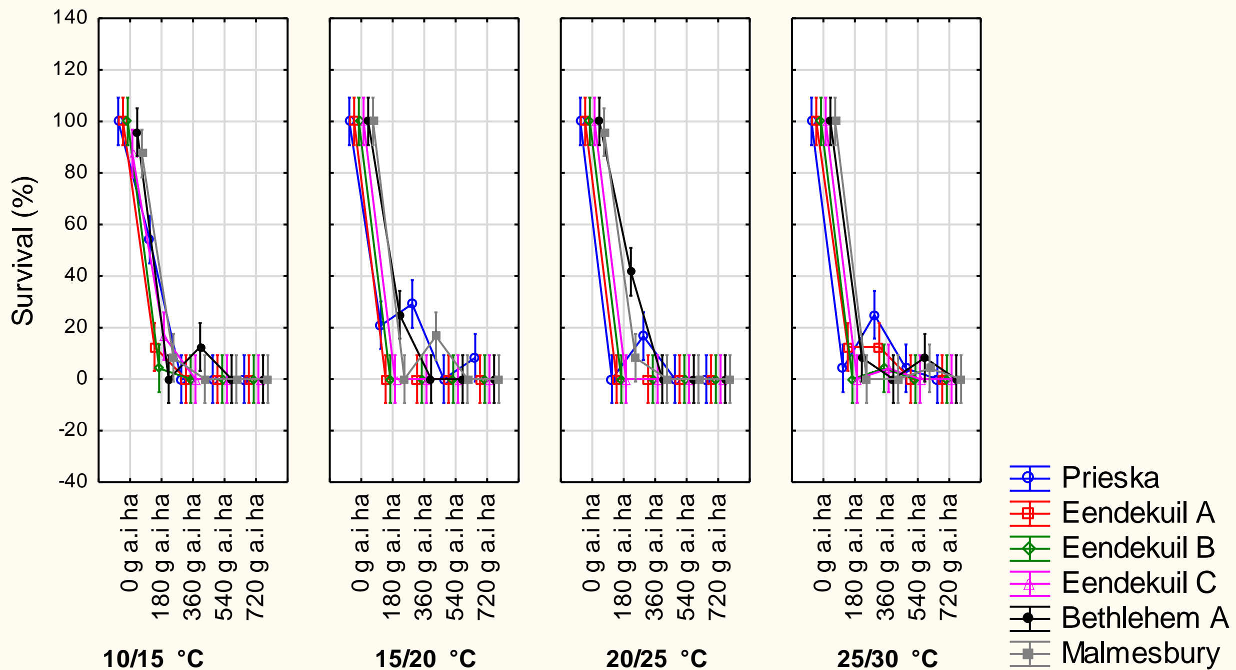


**Figure 5.6:** Interaction effect between six wild oat populations, five glyphosate dosage rates and four temperature regimes on survival rate.Bars represent the standard errors of the mean.

**Fresh mass pot$^{-1}$**

The Prieska population had the highest fresh plant mass pot$^{-1}$ of all treated plants growing under the highest controlled temperature of 25/30 ºC. Temperature has a great influence on plant growth as photosynthesis, plant metabolism, plant growth, and plant development are reliant on temperature. Temperature also influences evapotranspiration, thus it affects the water condition of the plants, cuticle hydration, and mineral absorption (Zanatta et al. 2008). This however did not seem to influence the effect of glyphosate on the growth of treated plants.

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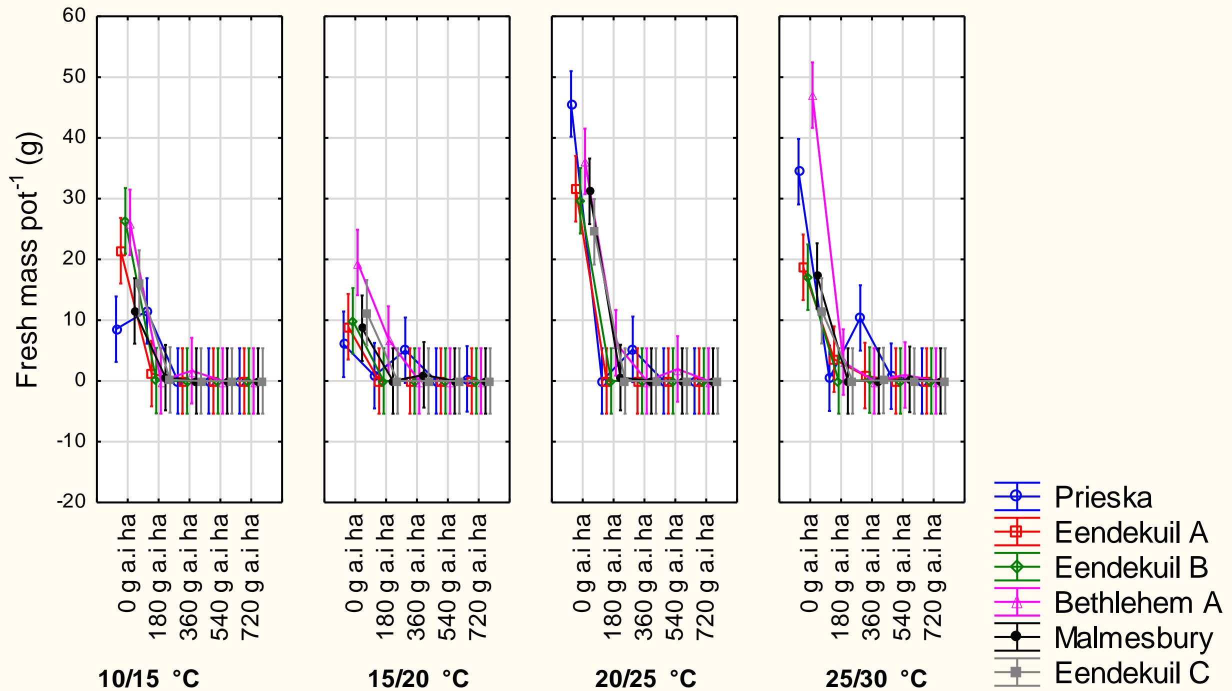


**Figure 5.7:** Interaction effect between six wild oat populations, five glyphosate dosage rates and four temperature regimes on fresh mass pot$^{-1}$

**Dry mass pot$^{-1}$**

Dry mass production pot$^{-1}$ did not react differently to the treatments than fresh mass pot$^{-1}$. High control rate was obtained at all dosage rates and temperature regimes. High temperatures during application are usually beneficial to herbicide efficacy (Caseley and Coupland 1985). Kumaratilake and Preston (2005) discovered that different metabolism of plants that are grown under different temperature results in variable control levels but this was not evident in this study.

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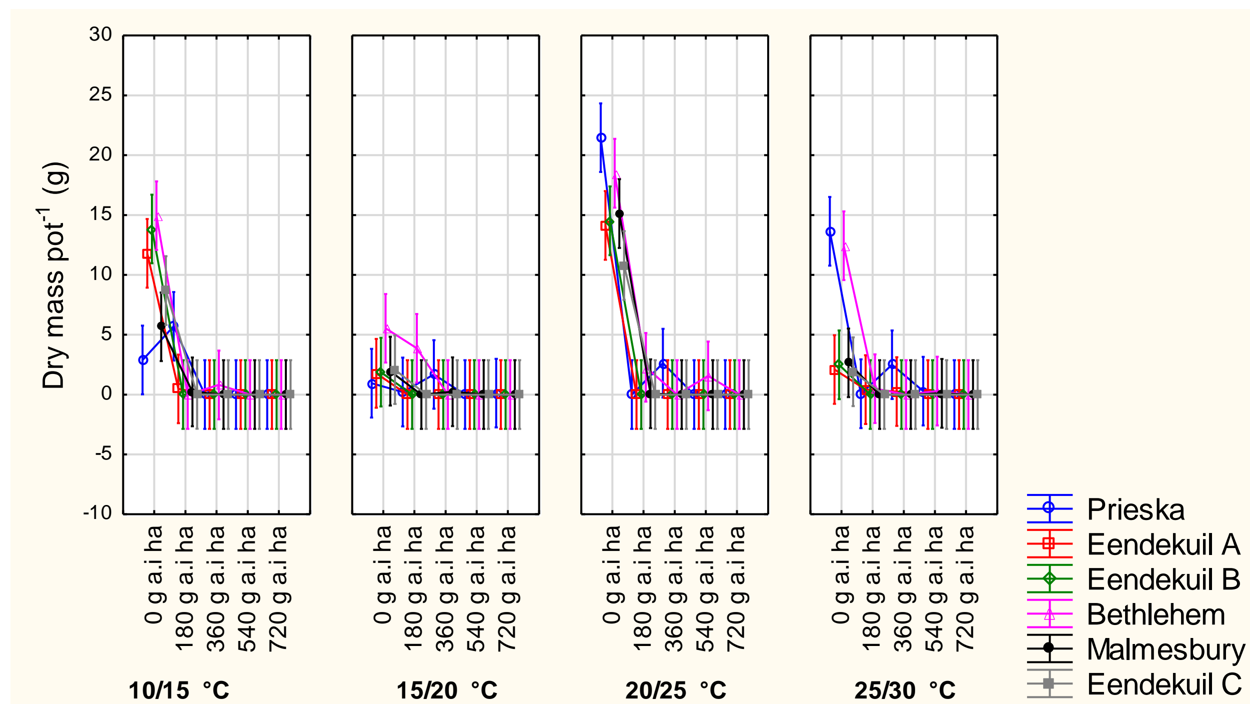


**Figure 5.8:** Interaction effect between six wild oat population, five glyphosate dosage rates and four temperature regimes on dry mass pot$^{-1}$. Bars represent the standard errors of the mean.

## 5.7. Conclusion

It appears as if the Prieska and Bethlehem A populations are less sensitive to glyphosate at 180 g a.e ha$^{-1}$compared to the other populations. Temperature had no effect on herbicide activity when 360, 540 and 720 g a.e ha$^{-1}$ of glyphosate was applied to all the populations. The wild oat populations tested in this study does not clearly show resistance to glyphosate, however some plants from the Bethlehem A and Eendekuil B populations survived the recommended dosage rate of glyphosate and may indicate low levels of resistance to glyphosate. This is commonly found when a population is in the early stages of developing resistance. Future research might include the effect of higher temperaturer such as 30/35 ºC on glyphosate efficacy on wild oats.

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## Reference


Caseley JC, Coupland D. 1985. Environmental and plant factors affecting glyphosate uptake, movement and activity. In: Grossbard E, Atkinson D (eds). *The herbicide glyphosate*. Butterworths, London.

Cieslik LF, Vidal RA, Trezzi MM. 2013. Environmental factors affecting the effectiveness of herbicides inhibiting ACCase: a review. *Planta Daninha*, 31: 483-489.

Gerber HR, Nyffeler A, Green DH. 1983. The influence of rainfall, temperature, humidity and light on soil- and foliar-applied herbicides. *Journal of Applied Biology and Biotechnology* 4: 1–14.

Kraehmer H, Van Almsick A, Beffa R, Dietrich H, Eckes P, Hacker E, Ruediger H, Strek HJ, Stuebler H, Wilms L. 2014. Herbicides as weed control agents: State of the art: II. Recent achievements. *Plant Physiology*. 1132-1148.

Kumaratilake AR, Preston C. 2005. Low temperature reduces glufosinate activity and translocation in wild radish (*Raphanus raphanistrum*). *Weed Science* 53: 10–16.

Li-Cor. 2016. LI-3100C Area Meter. Campbell Scientific Africa (Pty) Ltd https://licor.co.za/?portfolio=li-3100c

Mahan JR, Dotray P, Light GG, Dawson KR. 2006. Thermal dependence of bioengineered glufosinate tolerance in cotton. *Weed Science* 54: 1–5.

Matzenbacher FO, Vidal RA, Merotto JR, Trezzi MM. 2014 Environmental and physiological factors that affect the efficacy of herbicides that inhibit the enzyme protoporphyrinogen oxidase: a literature review. *Planta Daninha*, 32: 457-463.

Munktell. 2018. Munktell Grade 391 Filter Paper Vos Instruments. https://www.vosinstrumenten.nl/onderwijs/disposables-filters/filters/rondfilters/munktell-grade-391.html

Penner D. 2015. Effect of temperature on phytotoxicity and root uptake of several herbicides. *Weed Technology 19*: 571–576.

Smeda R J, Putnam AR. 2010. Influence of temperature, rainfall, grass species, and growth stage on efficacy of fluazifop-P-butyl. *Weed Technology* 4: 349–355.

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Statistica. 2012. Data analysis software system Version 12, Statsoft Inc. www.statsoft.com

Waltz AL, Martin AR, Roeth FW, Lindquist L. 2004. Glyphosate efficacy on velvetleaf varies with application time of day. Agronomy and Horticulture, Faculty Publications. P 419. http://digitalcommons.unl.edu/agronomyfacpub/419 [accessed 12 November 2017].

Zahoor AG, Jugulam M, Jhala AJ. 2017. Temperature influences efficacy, absorption, and translocation of 2,4-Dor glyphosate in glyphosate-resistant and glyphosate-susceptible common ragweed (*Ambrosia artemisiifolia*) and giant ragweed (*Ambrosia trifida*). *Weed Science* 65: 588–602.

Zanatta AL, Rosso P, Folador MSRB, Figueiredo MG, Pizzolatti LD, Leite FRMB. 2008. *Silva Insulino mimetic* effect of kaempferol 3-neohesperidoside on the rat soleus muscle. *Journal of Natural Products* 71: 532-535.

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# CHAPTER 6
# EFFECT OF VARYING TEMPERATURE BEFORE AND AFTER APPLICATION ON THE EFFICACY OF GLYPHOSATE ON WILD OATS

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## Abstract

**Temperature has a major influence on post-emergence herbicide efficacy. Two wild oat populations which previously showed signs of resistance to glyphosate, Bethlehem A and Malmesbury, were selected. Seeds from these populations were germinated in 1Milli molar of gibberellic acid solution. At four leaf stage the seedlings were treated with four glyphosate dosage rates (0, 180, 360 and 540 g a.e ha$^{-1}$). The plants were grown at two different temperature regimes of 15/20 $^{0}$C and 25/30 $^{0}$C night/day. After glyphosate application half of the plants grown in each glasshouse were switched from 15/20 $^{0}$C to 25/30 $^{0}$C and *vice versa*. Significant two-way interactions between temperature and dosage rate and population and temperature were recorded for survival percentage when the temperatures were kept constant before and after glyphosate application. It appears as if the efficacy of glyphosate was slightly lower at the higher temperature when sub-lethal doses were applied. Similarly the Bethlehem A population appeared to be slightly less sensitive to glyphosate at the same dosage rates. Interactions in terms of fresh and dry mass did not show consistent trends. When plants were switched between temperatures after application of glyphosate, a three-way interactions for survival percentage and fresh mass and a two-way interaction between temperature and dosage rate for dry mass were recorded. No consistent trends were noted in terms of the three-way interactions and in the two-way interaction the different temperature regimes had no effect on the efficacy of glyphosate. The two wild oat populations did not show resistance characteristics based on these results obtained.**

**Keywords:** Glyphosate, resistance, survival rate, temperature wild oats

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### 6.1 Introduction

Environmental factors such as temperature has a major influence on post-emergence herbicide efficacy (Gerber et al. 1983; Price 1983). Temperature may influence processes such as absorbtion and translocation of herbicide which influence herbicide activity. Numerous studies found that temperature increases improve efficacy of glyphosate on wild oat (*Avena fatua* L.) (Adkins et al. 1998). Similarly glyphosate activity on jungle rice (*Echinochloa colona* (L.) Link) increased as temperature increased from 20 to 35 ˚C (Tanpipat et al. 1997). Higher temperature boosts the effectiveness of $^{14}$C-glyphosate absorption by cultivated velvetleaf cells (Royneberg et al. 1992). Approximately double the amount of glyphosate was absorbed at 28 ˚C as compared to 16˚C or 4˚C.

In general high temperature increase plant vulnerability to post-emergence herbicides (Hammerton 1967). Glyphosate has been used with variable levels of success; perhaps because its efficacy may be less effective at unfavourable environmental conditions such as high temperature (Caseley 1972). Temperature has a huge impact on controlling and determining glyphosate or other herbicide application programmes.

In most cases glyphosate may be applied early morning, before noon and right after sunset. Herbicide application at different times of the day brought awareness that time of application may influence efficacy. Level of temperature conditions before, during and after application influence herbicide activity within the weed species. This can critically affect the level of weed management achieved from an herbicide (Celestine 2018).

The longer time in which plants develop under high temperatures reduces herbicide penetration by the thickness of the leaf cuticle and the opening and closing of stomata. Extremely high and low temperature conditions slow down plant metabolism and can decrease the activity of herbicides. Optimum temperatures to successfully manage weeds are between 18 and 29 ˚C (Celestine 2018). When a farmer has a strong dependence on glyphosate for weed management, understanding the environmental effects on glyphosate activity will aid in ideal efficacy from the day of application (Waltz et al. 2004).

The overall objective of this study was to determine the effective dosage rate of glyphosate on wild oats grown under two temperatures. Specific objectives were (i) to observe how temperature influences glyphosate efficacy, (ii) to determine the effective dosage rate of glyphosate at two different temperatures, and (iii) to assess response of two wild oat

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populations when moved from warmer to cooler temperatures and *vice versa* after glyphosate application.

## 6.2 Material and methods

### 6.2.1 The experimental site

The experiment was conducted in a glasshouse at the Stellenbosch University Welgevallen experimental farm. The site is located at 33° 56´33" S and 18° 51´56" E at an altitude of 136 m above sea level.

### 6.2.2 The treatments and experimental design

Seeds from two wild oat populations were selected. Selected populations were chosen from the previous experiment described in Chapter 5. A randomized complete block design was arranged as a 2×4×2 factorial with 6 replications for the experiment. The experimental factors were two wild oat populations: Bethlehem A (28.224°S 28.311°E) and Malmesbury (33.4655°'S18.7185° E) exposed to four glyphosate dosage rates 0, 180, 360 and 540 g a.e $ha^{-1}$ at two different temperature levels 15/20 ⁰C and 25/30 ⁰C. The plants were sprayed at 5-6 weeks after transplanting into the pots.

In a second trial, the same procedure as above were applied but in this case the plants growing in the 15/20 ⁰C glasshouse were moved to the 25/30 ⁰C glasshouse immediately after spraying and similarly the plants growing in the 25/30 ⁰C glasshouse were moved to the 15/20 ⁰C glasshouse immediately after spraying.

### 6.2.3 The trail establishment and management

**Seedling preparations and germination process**

The selected two wild oat population seeds were all germinated in a similar way.

**i. Gibberellic acid solution preparation**

The selected seeds were germinated in 6 ml of a prepared 1 Milli-molar of gibberellic acid solution (0.087 g of $GA_3$ was weighed and mixed with 250 ml of distilled water), in 90 mm diameter plastic petri dishes containing two filter papers (Munktell Grade 391 Filter Paper, Vos Instruments) (Munktell 2018). The petri dishes were enclosed in a poly propylene bag to prevent evaporation of the germination solution and were kept in a growth chamber in the dark at a constant temperature of 20 ⁰C.

### ii Planting

After the germination process that lasted for about 1-3 weeks, seedlings were equally divided and transferred to glasshouses that was set at night/day temperature of 15/20 ºC and 25/30 ºC respectively inside the petri dishes where they germinated with lids removed. Seedlings developed a green pigment colour 3-4 days after being placed in the glasshouse. Seedlings were then transplanted into 8 cm x 8 cm pots (four seedlings per pot) containing a coarse sand/gravel mix in a glasshouse.

### iii Irrigation

An automated irrigation system was used to water the plants at 08:00 am, 12:00 pm and 4:00 pm for close to one minute. The plants were irrigated with a balanced nutrient solution (Table 6.1).

**Table 6.1:** The nutrient content of the nutrient solution used to water the plants in the glasshouse

| EC = 2.0 | | | |
|---|---|---|---|
| Element (Macro) | Concentration mg $L^{-1}$ | Fertilizer | 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 |

### Pest and disease control

No pests and diseases were experienced in the glasshouse.

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**Herbicide application**

After the transplanted seedlings developed into the four leaf stage in the glasshouses running at 15/20 ºC and 25/30 ºC (about 5-6 weeks) glyphosate was applied. The herbicide was applied by means of a pneumatic pot sprayer at a pressure of 2 bars in 100 L ha$^{-1}$ of water.

## 6.3 Data collection

On the day of spraying extra control plants were harvested and the following variables were recorded:

**i. Number of leaves per plant**

The number of leaves per plant were counted and recorded and the mean number of leaves of the plants in the pot was calculated.

**ii. Leaf area (cm2)**

Leaf area per plant was measured using a leaf area meter (Campbell Scientific Africa (Pty) Ltd, LI-Cor3100C Area Meter) (Li-Cor 2016) and the mean leaf area of the plants per pot was calculated.

**iii. Plant height (cm)**

A ruler was used to measure plant height of each plant. The height considered was of the stems and leaves above soil surface to the tip of the longest leaf. The mean plant height of the plants in the pot was calculated.

**iv. Fresh plant mass (g)**

From each pot, plants were harvested above soil surface by means of scissors and put into small brown paper bags and the fresh plant mass was then measured using an electronic weighing scale. The mean fresh mass per pot was then calculated.

**v. Dry plant mass (g)**

After determining the fresh plant mass, the small brown paper bags with plants were placed into an oven and dried at 80ºC for 48 hours. The dry plants were then weighed on an electronic weighing scale and the mean dry mass per pot was then calculated.

**Evaluation**

Six weeks after glyphosate application the following variables were recorded

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### i. Survival percentage

Survival percentage was recorded six weeks after spraying. The calculation was done using the following formulae:

$$\text{Percentage Survival} = \frac{\text{number of surviving plants per pot}}{4(\text{plants per pot})} \times 100\%$$

Plants that displayed any actively growing green leaves, no matter how small the leaves, six weeks after glyphosate application, were considered as surviving plants.

## 6.4 Data analysis

Data was subjected to analysis of variance using the STATISTICA 12 program (Stastica 2012). Means of significant main effects and interactions in the experiments were separated using Turkey $HSD_{0.05.}$

## 6.5 Results

### TEMPERATURE NOT SWITCHED

There were no significant three-way interactions between temperature, dosage rate and population for survival percentage. Significant two-way interactions ($p<0.05$) between temperature and dosage rate and between population and temperature were however recorded for survival percentage.

The highest survival percentages, occurred at 25/30 $^0$C controlled temperature treatment where glyphosate was applied at 180 g a.e $ha^{-1}$ and 360 g a.e $ha^{-1}$ respectively (Figure 6.1).). There was a significant ($p<0.05$) dosage rate between temperature interaction was recorded. The efficacy of glyphosate tends to be lower at the higher temperatures.

The survival percentages of the two wild oat populations at 180 g a.e. $ha^{-1}$ and 360 g a.e. $ha^{-1}$ did not differ significantly ($p>0.05$) from each other, but a significant interaction between dosage rate and population was recorded (Figure 6.2).

There were significant ($p<0.05$) three-way interactions between temperature, dosage rate and wild oat populations in terms of both fresh- and dry mass per pot (Figures 6.3 and 6.4). The Bethlehem A population produced more fresh mass at 180 and 360 g a.e $ha^{-1}$ compared to the Malmesbury population (Figure 6.3). There was also an inexplicable significant drop in fresh mass production of the Bethlehem A population in the control treatment at the 25/30 $^0$C temperature.

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The same trends, although less pronounced than in the fresh mass parameter, were observed in the dry mass parameter (Figure 6.4).

**SWITCHEDTEMPERATURES**

The results revealed a significant three-way interaction between population, dosage rate and temperature for survival percentage. The survival percentages of the two populations showed contrasting trends at the 180 g a.e. $ha^{-1}$ dosage rate (Figure 6.5). The Bethlehem A population had a better survival rate when plants were moved from the low to the high temperatures than *vice versa*. In contrast, the Malmesbury population showed the opposite trend. None of the differences were however significant at the $p=0.05$ level.

Fresh mass production per pot also showed a significant ($p<0.05$) three-way interaction. Fresh mass production at the 180 g a.e. $ha^{-1}$ dosage rate (Figure 6.6) reflected the same trend as the survival percentage at the same dosage rate (Figure 6.5). None of the differences between temperature treatments or populations were, however, significant.

In terms of dry mass production per pot, no significant differences between populations were evident but a significant ($p<0.05$) two-way interaction between dosage rate and temperature occurred. Figure 6.7 showed no obvious trends regarding temperature and the dry mass production at the treatments where glyphosate was applied. There was a relatively large difference between the dry mass produced at the two temperature treatments where no glyphosate was applied and that probably caused the significant interaction.

Table 6.2 indicates that at time of glyphosate application, the Bethlehem A population plants were much bigger compared to the Malmesbury plants, consequently plants growing at the higher temperatures were much bigger compared to plants growing at the lower temperatures.

## 6.6. Discussion

**TEMPERATURE NOT SWITCHED**

A trend of higher survival percentages with an increase of temperature at low dosage rates was observed for both wild oat populations. Environmental factors such as temperature have a huge influence on the effect of herbicide activity (Heap 2005). In some similar cases temperature has a great influence of increasing metabolism which reduces herbicide effect and leads to increased survival percentages (Johnson and Young 2002). Similar results were found, where survival percentage increased with an increase of temperature (Devine et al.1993; Godar et al. 2015). High temperatures are most likely favourable for absorbing and translocating glyphosate,

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which have been witnessed in *Cynodon dactylon* (Bermuda grass) (Jordan 1977). This theory was also confirmed by testing *Avena fatua* with difenzoquat (Sharma et al. 1976).

The opposite can also be true. Herbicide has a direct impact of inhibiting enzymes and killing the plant (Powles and Preston 2006; Duke and Powles 2009 and Yu et al 2007). Higher temperatures reduce the herbicidal activity in plants by speeding up the metabolism as well as evapotranspiration which dries up droplets applied as foliar herbicides (Devine et al. 1993; Johnson and Young 2002; Godar et al. 2015).

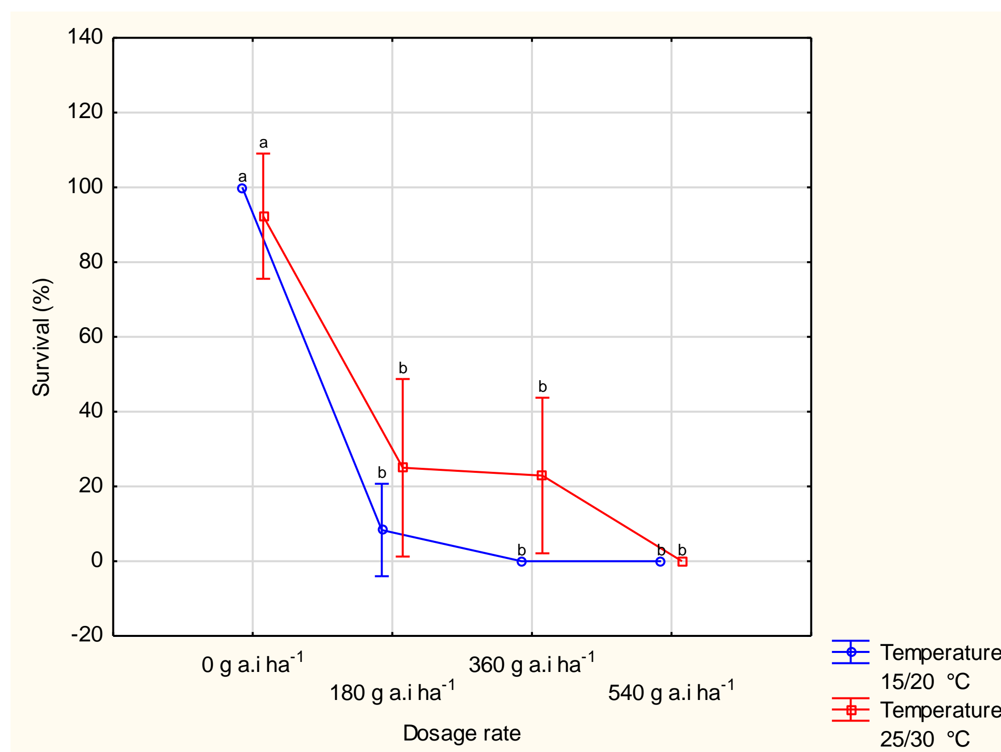


**Figure 6.1: Effect of** temperature and glyphosate dosage rate interaction on survival percentage of wild oat populations. Where means were significantly different at the p=0.05 level it is indicated by different letters.

As expected, survival percentage decreased with increased dosage rates to 100% control at the recommended dosage rate of 540 g a.e. $ha^{-1}$. Smeda and Putnam (2010) reported that an increase of dosage rate from the control dosage to near the recommended dosage rate, should control weed population.

The Bethlehem A population showed a higher survival rate than the Malmesbury population at sub-lethal dosage rates, although the differences were not significant. Two populations from

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the same species may have different responses to herbicide doses applied and the levels of sensitivity to herbicide between some of the populations can be completely different (Heap 2005).

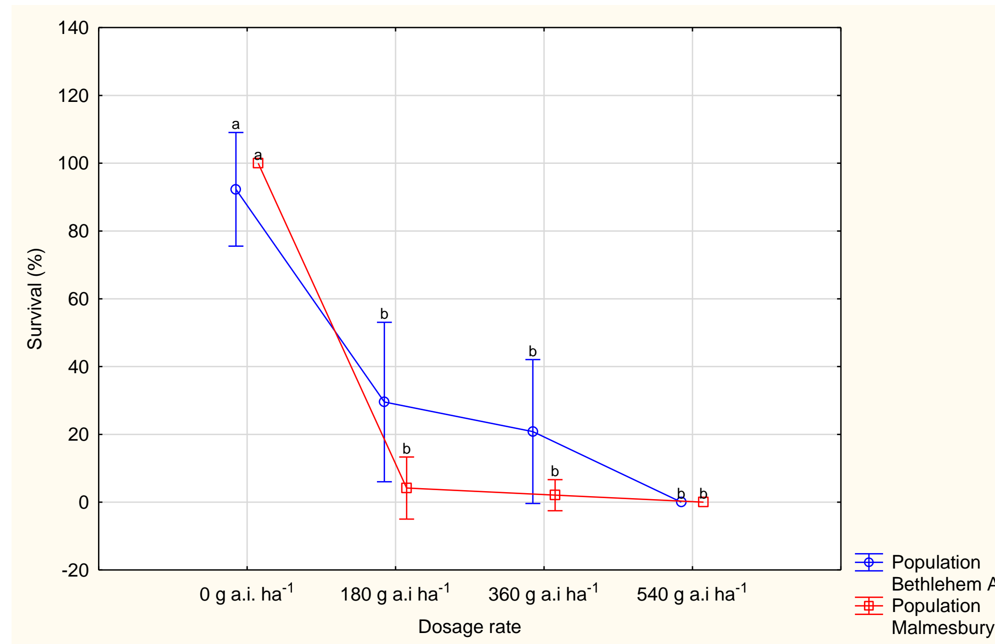


**Figure 6.2:** Glyphosate dosage rate and population interaction effects on survival percentage of wild oat populations. Where means were significantly different at the p=0.05 level it is indicated by different letters

This can possibly be further explained by the higher survival percentage that the Bethlehem population displayed at the lower dosage rates. The better growth at the higher temperatures is also displayed by the size of the plants at the time of spraying (Table 6.2). The exceptional low fresh mass produced by the Bethlehem A population at the high temperature in the control treatment (Fig 6.3) is inexplicable. It is possible that one or two pots in that specific treatment combination could have suffered from an unknown stress factor and it was not noted during the growing period.

**Table 6.2:** Vegetative growth parameters of two different wild oat populations at the time of glyphosate application at 4 to 10 leaf growth stage.

| Plant Parameters | Population | | Temperature | |
|---|---|---|---|---|
| | Malmesbury | Bethlehem A | 15/20 | 25/30 |
| Plant length | 39.66[a*] | 47.55[b] | 32.05[a] | 55.16[b] |
| Leaf area | 32.78[a] | 64.97[b] | 21.75[a] | 76.00[b] |
| Dry mass/pot$^{-1}$ | 0.34[a] | 0.65[b] | 0.33[a] | 0.66[b] |

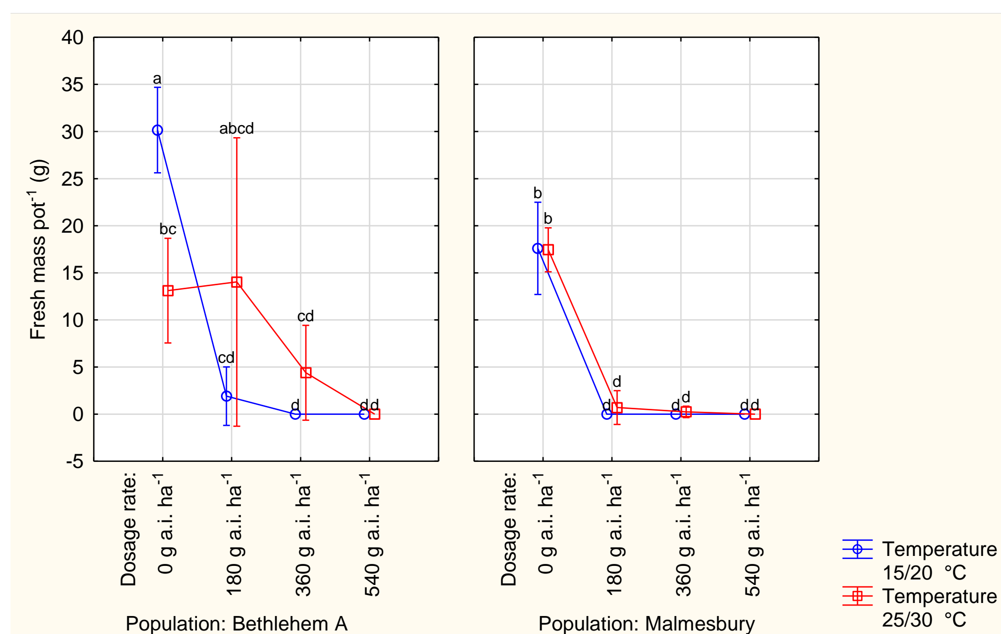


**Figure 6.3:** Interaction effects between wild oat populations, dosages rate and two temperatures on fresh mass pot$^{-1}$. Where means were significantly different at the p=0.05 level it is indicated by different letters.

Glyphosate stunts and terminates growth (Schuette 1998), therefore the amount of dry mass produced after glyphosate application is expected to be very small. Since plants contain a high percentage of moisture, dry mass values are generaly much smaller than fresh mass values (Wood and Roper 2000).

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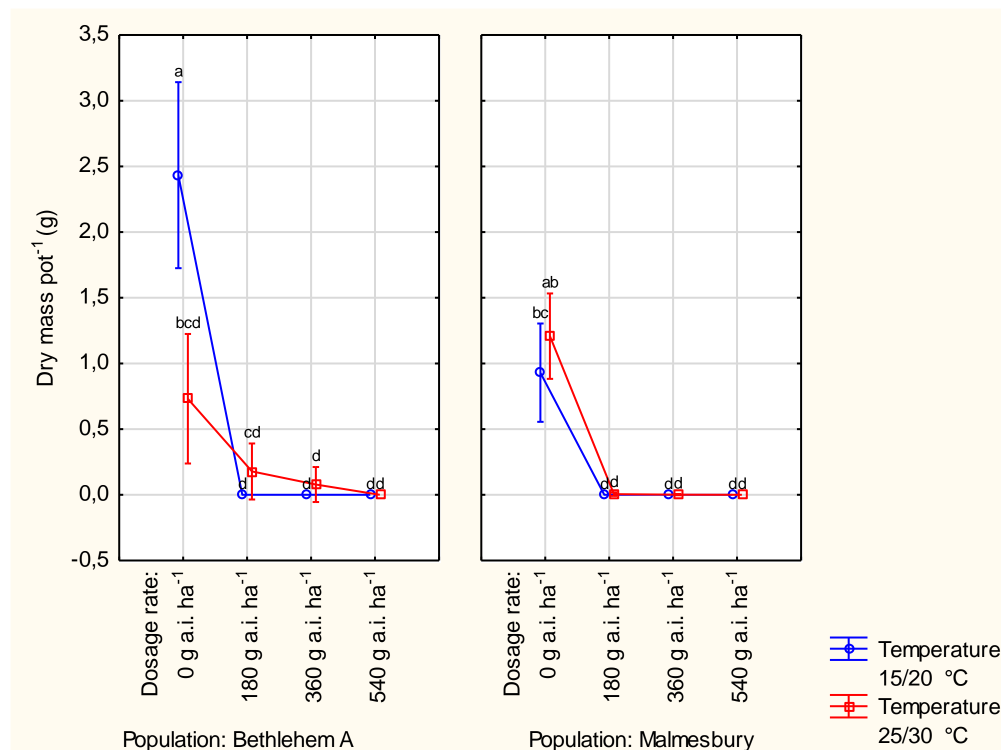


**Figure 6.4:** Interaction effects between wild oat populations, dosages rate and two temperatures on dry mass pot$^{-1}$.Where means were significantly different at the p=0.05 level it is indicated by different letters.

**SWITCHED TEMPERATURES**

Figure 6.5 shows that the Bethlehem A population showed less sensitivity to glyphosate when the plants were moved from the low temperature to the higher temperature after spraying. Ramsey et al. (2002) discovered that wild oats which grew under low temperatures demonstrated significant decrease in survival and when sprayed plants were placed in a high temperature, level of survival increased.

Surprisingly, the Malmesbury population showed less sensitivity to glyphosate when plants were moved from high to low temperatures. This contradicts the Bethehem A population results as well as the findings of Ramsey et al. (2005), who observed high level of uptake and efficacy of herbicides where plants were exposed to low temperatures after spraying. Low temperature generally slows down the process of drying foliar applied droplets which could be the mechanism of higher efficacy.

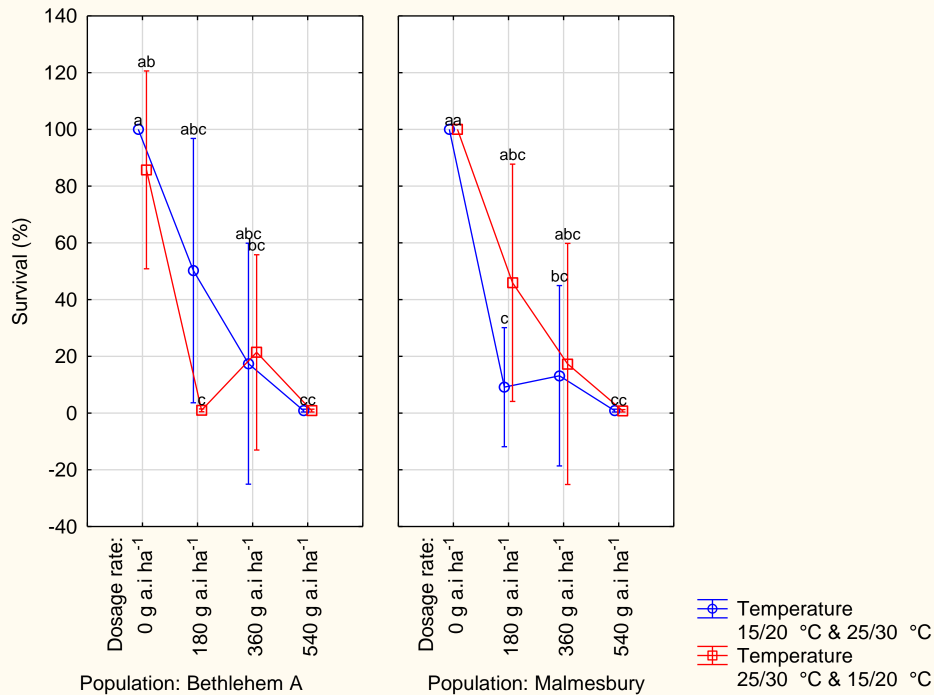


**Figure 6.5:** Interaction effects between wild oat populations, dosage rates and switched temperatures on survival percentage. Where means were significantly different at the p=0.05 level it is indicated by different letters

Both population's fresh mass production pot$^{-1}$ decreased with more than 50% compared to the untreated survived plants. Heap (1994 and 2005) reported that when glyphosate application at the recommended rate reduces fresh mass pot$^{-1}$ by 50% compared to untreated plants, plants are considered as susceptible .

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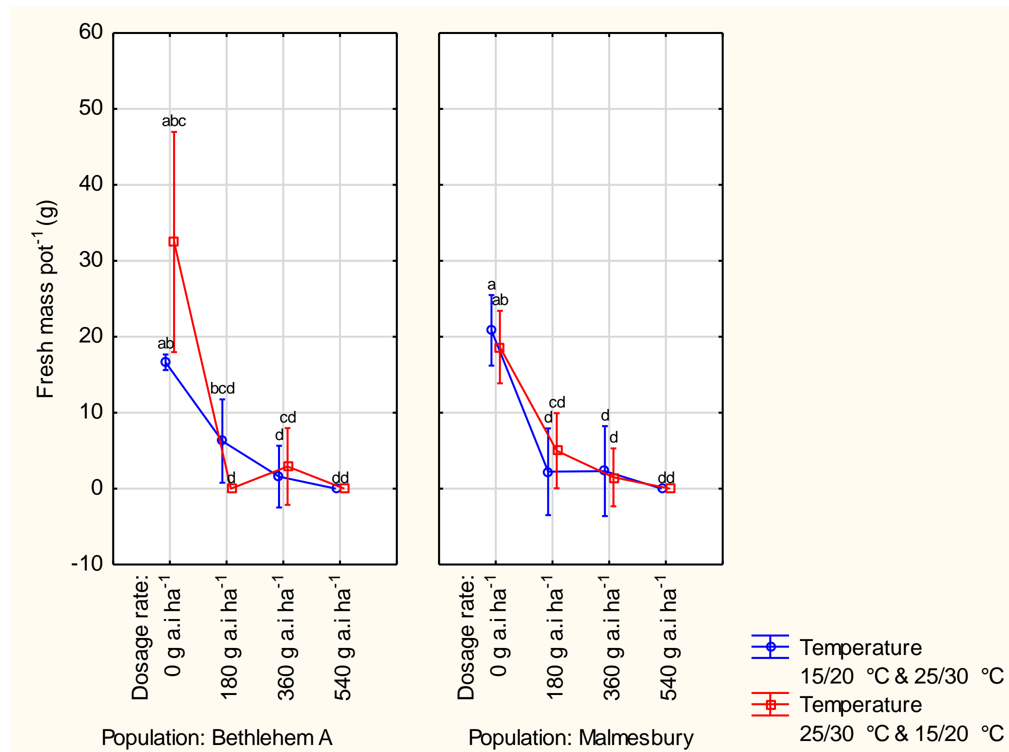


**Figure 6.6:** Interaction effects between wild oat populations, dosage rates and switched temperatures on fresh mass pot$^{-1}$. Where means were significantly different at the p=0.05 level it is indicated by different letters.

Plants growing under high temperatures first before glyphosate application produced more dry mass compared to plants growing inlower temperatures first.

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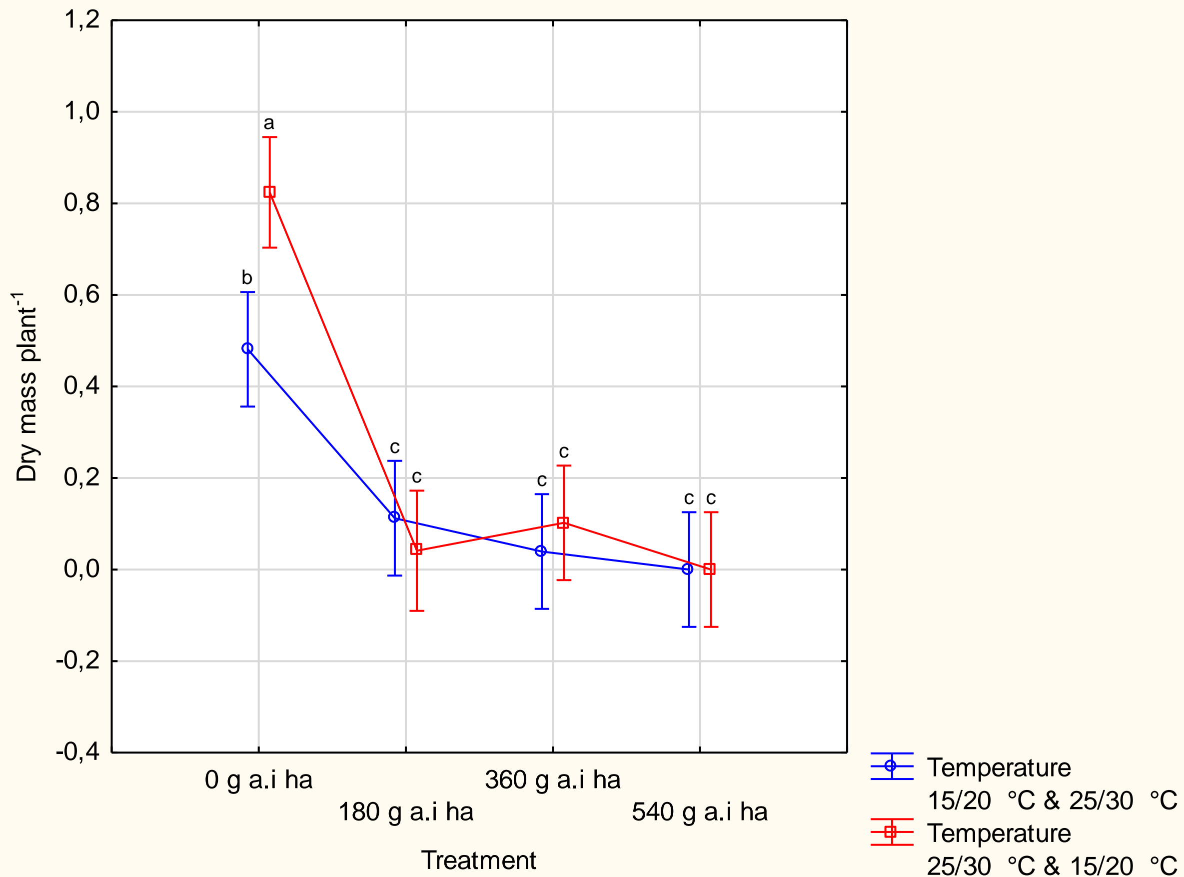


**Figure 6.7:** Interaction effects of glyphosate dosage rates and switched temperatures on dry mass plant$^{-1}$. Where means were significantly different at the p=0.05 level it is indicated by different letters.

## 6.7 Conclusion

### Temperatures not switched

The Bethlehem A population showed higher survival percentages at low glyphosate dosage applications (180 g a.e. ha$^{-1}$) but at the highest dosage rate (540 g a.e. ha$^{-1}$) applied, no plants survived.

It might be safe to conclude that the Bethlehem A wild oat population shows some tolerance to glyphosate compared to the Malmesbury population that did not show any sign of tolerance at low dosage rates of glyphosate (180 g a.e. ha$^{-1}$).

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**Switched temperatures**

Contradicting results were found with the Bethlehem A and Malmesbury populations when changing temperature regimes. This however prevented the forming of a clear conclusion on the effect of varying temperatures before and after glyphosate application. No plants in any of the two trials described in this chapter survived the recommended dosage rate of 540 g a.e. $ha^{-1}$ of glyphosate under any temperature regimes. This is a good indication that no resistance to glyphosate is evident in these two wild oat populations.

**Reference**

Adkins SW, Tanpipat S, Swarbrick JT Boersma M. 1998. Influence of environmental factors on glyphosate when applied to *Avena fatua* or *Urochloa panicoides*. *Weed Research* 38:129–138.

Caseley JC. 1972. The effect of environmental factors on the performance of glyphosate against *Agropyron repens*. In: Proceedings 11th British Weed Control Conference*, Brighton*, 64: 1–7.

Celestine D. 2018. Factors Affecting Herbicide Performance. Techline Invasive Plant NEWS. https://www.techlinenews.com/herbicides/herbicideperformance2014 [accessed 06 August 2018].

Devine D, Duke SO, Fedtke C, Huber LA, Bernhaut K. 1993. Foliar Absorption of Herbicides. In: Devine M, Duke SO, Fedtke LA (eds). *Physiology of herbicide action*. PTR Prentice-Hall, Englewood Cliffs pp 29–52.

Duke SB, Powles SO. 2009. Glyphosate-resistant crops and weeds. *Journal of Agro biotechnology Management and Economics* 12:346-457.

Gerber HR, Nyffeler A, Green DH. 1983. The influence of rainfall, temperature, humidity and light on soil- and foliar-applied herbicides. *Aspects of Applied Biology* 4:1–14.

Godar AS, Varanasi VK, Nakka S, Prasad PV, Thompson CR, Mithila J. 2015. Physiological and molecular mechanisms of differential sensitivity of palmer amaranth (*Amaranthus palmeri*) to mesotrione at varying growth temperatures. PLoS One 10 (5) e0126731.https://doi.org/10.1371/journal.pone.0126731 [accessed 22 February 2018].

Stellenbosch University https://scholar.sun.ac.za

Hammerton JL. 1967. Environmental factors and susceptibility to herbicides. *Weeds* 15:330–335.

Heap IM. 2005. Criteria for confirmation of herbicide-resistant weeds - with specific emphasis on confirming low-level resistance. International Survey of Herbicide Resistant Weeds. pp 1-4

Heap IM. 1994. Identification and documentation of herbicide resistance. *Phytoprotection* 75: 85-90.

Johnson BC, Young BG. 2002. Influence of temperature and relative humidity on the foliar activity of mesotrione. *Weed Science* 50: 157–161.

Jordan TN. 1977. Effects of temperature and relative humidity on the toxicity of glyphosate to bermudagrass (*Cynodon dactylon*). *Weed Science* 25: 448–451.

Li-Cor. 2016. LI-3100C Area Meter. Campbell Scientific Africa (Pty) Ltd https://licor.co.za/?portfolio=li-3100c

Munktell. 2018. Munktell Grade 391 Filter Paper Vos Instruments. https://www.vosinstrumenten.nl/onderwijs/disposables-filters/filters/rondfilters/munktell-grade-391.html

Powles SB, Preston C. 2006. Evolved glyphosate resistance in plants: Biochemical and genetic basis of resistance. *Weed Technology* 20: 282–289.

Price CE. 1983. The effect of environment on foliage uptake and translocation of herbicides. *Annals of Applied Biology* 4:157–169.

Ramsey RJL, Stephenson GR, Hall JC. 2002. Effect of relative humidity on the uptake, translocation, and efficacy of glufosinate ammonium in wild oat (*Avena fatua*). *Pesticides and Biochemistry Physiology* 73: 1–8.

Ramsey RJL, Stephenson GR, Hall JC. 2005. A review of the effects of humidity, humectants, and surfactant composition on the absorption and efficacy of highly water soluble herbicides. *Pesticide and Biochemistry Physiology* 82:162–175.

Royneberg T, Balke NE, Lund-Hoie K. 1992. Effects of adjuvants and temperature on glyphosate absorption by cultured cells of velvetleaf (*Abutilon theophrasti Medic.*). *Weed Research* 32:419–428.

Stellenbosch University https://scholar.sun.ac.za

Schuette J. 1998. Environmental fate of glyphosate. *Environmental Monitoring and Pest Management.* pp 1-13

Sharma MP, Vandenborn WH, Friesen HA, MCBeath DK.1976. Penetration, translocation, and metabolism of $^{14}$C-difenzoquat in wild oat and barley. *Weed Science* 24: 379-84.

Smeda RJ, Putnam AR. 2010. Influence of temperature, rainfall, grass species, and growth stage on efficacy of fluazifop. *Weed Technology* 4: 349–355.

Statistica. 2012. Data analysis software system Version 12, Statsoft Inc. www.statsoft.com

Tanpipat S. Adkins SW, Swarbrick JT, Boersma M. 1997. Influence of selected environmental factors on glyphosate efficacy when applied to awnless barnyard grass (*Echinochloa colona* (L.) Link). *Australian Journal of Agricultural Research*. 48: 695–702.

Waltz AL, Martin AR, Roeth FW, Lindquist JL. 2004. Glyphosate efficacy on velvetleaf varies with application time of day. Agronomy and Horticulture. Faculty Publications. P 419. http://digitalcommons.unl.edu/agronomyfacpub/419 [accessed on 20 December 2017].

Wood AJ, Roper J. 2000. A simple and non-destructive technique for measuring plant growth and development. *American Biology Teacher Journal*. p 215

Yu Q, Cairns ALP, Powles S. 2007. Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. *Journal of Plant Biology* 225: 499–513.

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# CHAPTER 7

## General conclusions and recommendations

### Seed treatment for wild oats

This study confirmed that pre-germination treatment with ammonia gas and using $GA_3$ as germination solution improved the germination of the two wild oat populations (Malmesbury and Prieska) tested. Gibberellic acid produced long, thin seedlings, but had no influence on the survival percentage of wild oat seedlings treated with glyphosate. Therefore, $GA_3$ treatment was used throughout the entire study, as it was a quick and effective germination method. It would be advisable to use $GA_3$ as a pre-germination process to break dormancy of wild oats during experiments. It saves times, labour and water as compared to the ammonia treatment.

### Glyphosate treatments

The Prieska wild oat population showed less sensitivity to glyphosate at 270 g a.e $ha^{-1}$ with a survival percentage of 42% (Chapter 4). Low levels of susceptibility was recorded for the Prieska and Bethlehem A populations, sprayed with 180 g a.e $ha^{-1}$ (Chapter 5). Lower levels of susceptibility were noticed again in the Bethlehem A and Prieska populations at 180 g a.e $ha^{-1}$ with survival percentages of 50% and 46%, respectively when switched between 25/30 ºC and 15/20 ºC (Chapter 6). There was no indication of significant resistance to glyphosate in all populations tested when the recommended rate (540 g a.e $ha^{-1}$) or any other rate was applied to the plants.

Heap (2005) mentioned that a farmer may consider the recommended rate of herbicide as a benchmark. The issue is that recommended rates are based on subjective rate determinations that may differ from location to location depending on the conditions that the weed species is subjected to. Concluding that there is herbicide resistance using recommended dosage rates leads to incompetent, misleading and unreliable classification of resistance. Some plants may survive when four times the recommended dosage rate is applied under certain conditions.

It would be advisable that farmers must use the recommended glyphosate dosage rate to control wild oats and avoid low dosage rates which might initiate resistant wild oat populations. Weed scienists should test 4 to 10 times the recommended glyphosate dosage rates. Heap (2005) and Bradshaw et al. (1997) reported that documentation or listing of resistant weeds able

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to survive greater than 10 fold levels of the recommended rate makes it relatively straight-forward or easy to prove resistance.

## Population

Through the entire study the Prieska and Bethlehem A populations showed low level of sensitivity to low rates of glyphosate applied. However, all tested populations. (Malmesbury, Lindley, Eendekuil A, B and C, Bethlehem A and B, Clarens and Prieska) were susceptible to glyphosate herbicides since no plants survived at 540 g a.e. $ha^{-1}$ in most experiments of the study. Resistance is an inherited characteristics or ability from parent plants and the populations used for these experiments were progeny of surviving plants from fields where other herbicides were applied. The tested plants were the first generation of these populations exposed to glyphosate under experimental conditions.

The wild oat populations tested in this study does not clearly show resistance to glyphosate but the fact that some plants from the Bethlehem A and Eendekuil B populations did survive the recommended dosage rate of 540 g a.e. $ha^{-1}$ of glyphosate while all the other plants were controlled at that dosage rate, may indicate low levels of resistance to glyphosate (Chapter 5). This is commonly found when a population is in the early stages of developing resistance.

To further investigate the possibility of glyphosate resistance, it would be preferable to use a larger sample of seeds specifically from the Bethlehem A, Eendekuil B and Prieska populations. The Malmesbury and Eendekuil C populations may be used as susceptible populations. Susceptible population such as Eendekuil C and Malmesbury must be included in the experiments for comparison with possible resistant plants to avoid biased results. HRAC (1999) reported that when comparing resistant and susceptible plants of the same species, it is important to conduct replicated and scientifically sound trials. Heap and Le Baron (2001) stated that weed populations with genetically inherited resistant genes survives a dosage rate of herbicide at which the weed species normally is controlled. If there is an agricultural situation where a previously susceptible weed population thrives after herbicide was applied, populations should be tested again to confirm that herbicide resistance and not unfavourable spraying conditions caused the failure of the herbicide.

## Temperature

The Prieska population revealed low levels of susceptibility when glyphosate at 180 g a.e. $ha^{-1}$ was sprayed at 15/20 ºC and 25/30 ºC glasshouse temperatures. The Bethlehem A population

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had the highest survival percentage when 180 g a.e. $ha^{-1}$ was applied under 20/25 ºC glasshouse temperatures in Chapter 5. Temperature (10/15 ºC, 15/20 ºC, 20/25 ºC and 25/30 ºC) had no effect on glyphosate activity when 360, 540 or 720 g a.e. $ha^{-1}$ was applied to the Prieska, Bethlehem A, Malmesbury and Eendekuil A, B and C populations in Chapter 5. The Bethlehem A population had the highest survival percentage (50%) when switched from 15/20 ºC to 25/30 ºC temperature at 180 g a.e. $ha^{-1}$ dosage rate. The Malmesbury population had a 46% survival percentage when switched from 25/30 ºC to 15/20 ºC after spraying 180 g a.e. $ha^{-1}$. Low survival percentages occurred at 360 g a.e. $ha^{-1}$ and no plants survived at 540 g a.e. $ha^{-1}$.

Higher temperature appeared to negatively influence the efficacy of glyphosate. The highest survival was discovered after switching the temperatures, but the contradictory responses of the two populations make a definitive conclusion impossible (Chapter 6).

It is suggested that more experiments are to be conducted on the Malmesbury, Prieska, Eendekuil B and Bethlehem A populations. Temperature studies should be expanded to elucidate the contradiction in response to fluctuating temperature regimes in the Malmesbury and Bethlehem A populations. Higher temperature fluctuations such as 30/35 ºC can be included to simulate prevalent temperatures under field conditions.

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## References

Bradshaw LD, Padgette SR, Kimball SL, Wells BH.1997. Perspective on glyphosate resistance. *Weed Technology* 11: 189-198.

Heap IM 2005. Criteria for confirmation of herbicide-resistant weeds - with specific emphasis on confirming low level resistance. pp 1-4.

The Herbicide Resistance Action Committee (HRAC). 1999. Detecting herbicide resistance. Guidelines for conducting diagnostic tests and interpreting results. http://www.plantprotection.org/hrac/ [accessed 22 January 2018].

Heap I, LeBaron H. 2001. In: Powles SB, Shaner DL (eds). Herbicide Resistance and World Grains. pp 1-22.

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# Appendices

**Appendix 1**: Analysis of variance of interaction effects between seed treatment and three wild oat populations on germination percentage

| Effect | Fixed Effect Test for germination percentage % | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Seed Treatment | 2 | 24.968 | 0.000 |
| Population | 2 | 1.434 | 0.249 |
| Seed Treatment* Population | 4 | 6.0883 | 0.00055 |

**Appendix 2**: Analysis of variance of interaction effects between seed treatment and three wild oat populations on germination rate

| Effect | Fixed Effect Test for germination rate percentage % | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Seed Treatment | 2 | 28.967 | 0.000 |
| Population | 2 | 0.540 | 0.465 |
| Seed Treatment* Population | 4 | 3.62198 | 0.01234 |

**Appendix 3**: Analysis of variance of interaction effects between seed treatment, dosage rate and population on survival percentage

| Effect | Fixed Effect Test for survival rate percentage % | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |

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| | | | |
|---|---|---|---|
| Seed Treatment | 1 | 0.731 | 0.394 |
| Dosage Rate | 3 | 285.955 | 0.000 |
| Population | 2 | 7.368 | 0.000 |
| Seed Treatment* Dosage | 3 | 1.199 | 0.313 |
| Seed Treatment* Population | 2 | 5.731 | 0.004 |
| Dosage* Population | 6 | 5.809 | 0.000 |
| Seed Treatment* Dosage* Population | 6 | 2.924 | 0.01069 |

**Appendix 4**: Analysis of variance of interaction effects between seed treatment, dosage rate and population on fresh mass pot$^{-1}$

| Effect | Fixed Effect Test for survival rate % | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Seed Treatment | 1 | 5.4822 | 0.019566 |
| Dosage Rate | 3 | 137.9095 | 0.000000 |
| Population | 2 | 4.4533 | 0.012062 |
| Seed Treatment* Dosage | 3 | 3.4480 | 0.016500 |
| Seed Treatment* Population | 2 | 6.8366 | 0.001167 |
| Dosage* Population | 6 | 3.4832 | 0.002176 |
| Seed Treatment* Dosage* Population | 6 | 5.2437 | 0.000029 |

**Appendix 5**: Analysis of variance of interaction effects between seed treatment, dosage rate and population on dry mass pot$^{-1}$

| | Fixed Effect Test for dry plant mass % |
|---|---|

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| Effect | Degree of freedom | F-value | p-value |
|---|---|---|---|
| Seed Treatment | 1 | 4.95062 | 7.9566 |
| Dosage Rate | 3 | 82.56717 | 132.6923 |
| Population | 2 | 4.25970 | 6.8651 |
| Seed Treatment* Dosage | 3 | 4.27150 | 6.8651 |
| Seed Treatment* Population | 2 | 4.22974 | 6.7980 |
| Dosage* Population | 6 | 3.7100 | 5.9643 |
| Seed Treatment* Dosage* Pop | 6 | 6.0862 | 0.000003 |

**Appendix 6**: Analysis of variance of interaction effects between seven wild oat populations and four-dosage rates on survival percentage

| Effect | Fixed Effect Test for Survival rate percentage % | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Seed Treatment | 6 | 1.133 | 0.355 |
| Population | 3 | 320.800 | 0.00 |
| Seed Treatment* Population | 18 | 1.1333 | 0.03470 |

**Appendix 7**: Analysis of variance of interaction effects between two wild oat populations and four dosage rates on survival percentage

| Effect | Fixed Effect Test for Survival rate percentage % | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |

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| Seed Treatment | 1 | 0.413 | 0.524 |
|---|---|---|---|
| Population | 3 | 93.867 | 0.000 |
| Seed Treatment* Population | 3 | 0.1688 | 0.9172 |

**Appendix 8:** Analysis of variance of interaction effects between six wild oat populations, five dosage rates and four different temperatures on survival percentage

| Effect | Fixed Effect Test for survival percentage | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Temperature | 3 | 0.371 | 0.774 |
| Treatment | 4 | 1953.706 | 0.000 |
| Population | 5 | 8.417 | 0.000 |
| Temperature*Treatment | 12 | 2.722 | 0.001 |
| Temperature*Population | 15 | 1.375 | 0.154 |
| Treatment*Population | 20 | 2.871 | 0.000 |
| Temperature*Treatment*Population | 60 | 2.5557 | 0.0000 |

**Appendix 9:** Analysis of variance of interaction effects between six wild oat populations, five dosage rates and four different temperatures on fresh mass pot $^{-1}$

| Effect | Fixed Effect Test for fresh mas pot$^{-1}$ | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Temperature | 3 | 14.570 | 0.000 |
| Treatment | 4 | 280.994 | 0.000 |
| Population | 5 | 7.823 | 0.000 |

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| | | | |
|---|---|---|---|
| Temperature*Treatment | 12 | 14.388 | 0.000 |
| Temperature*Population | 15 | 1.415 | 0.134 |
| Treatment*Population | 20 | 3.571 | 0.000 |
| Temperature*Treatment*Population | 60 | 1.9729 | 0.00004 |

**Appendix 10:** Analysis of variance of interaction effects between six wild oat populations, five dosage rates and four different temperatures on dry mass pot $^{-1}$

| Effect | Fixed Effect Test for dry mass pot $^{-1}$ | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Temperature | 3 | 19.025 | 0.000 |
| Treatment | 4 | 150.036 | 0.000 |
| Population | 5 | 5.378 | 0.000 |
| Temperature*Treatment | 12 | 18.445 | 0.000 |
| Temperature*Population | 15 | 1.074 | 0.378 |
| Treatment*Population | 20 | 2.283 | 0.001 |
| Temperature*Treatment*Population | 60 | 1.7150 | 0.00103 |

**Appendix 11:** Analysis of variance of interaction effects between two wild oat populations, five dosages rates and two temperature treatments on survival percentage

| Effect | Fixed Effect Test for survival percentage | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Temperature | 1 | 4.9078 | 0.029578 |

| | | | |
|---|---|---|---|
| Treatment | 3 | 134.3786 | 0.0000 |
| Population | 1 | 6.1802 | 0.015002 |
| Temperature*Treatment | 3 | 3.5324 | 0.018457 |
| Temperature*Population | 1 | 1.9697 | 0.164353 |
| Treatment*Population | 3 | 4.1966 | 0.0008237 |
| Temperature*Treatment*Population | 3 | 2.2049 | 0.093912 |

**Appendix 12**: Analysis of variance of interaction effects between two wild oat populations, five dosages rates and two temperature treatments on fresh mass pot$^{-1}$

| Effect | Fixed Effect Test for fresh mass pot$^{-1}$ | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Temperature | 1 | 0.0015 | 0.968926 |
| Treatment | 3 | 132.1422 | 0.000000 |
| Population | 1 | 18.5853 | 0.000046 |
| Temperature*Treatment | 3 | 15.5025 | 0.000000 |
| Temperature*Population | 1 | 0.0418 | 0.00000 |
| Treatment*Population | 3 | 4.0164 | 0.010244 |
| Temperature*Treatment*Population | 3 | 14.2095 | 0.00000 |

**Appendix 13**: Analysis of variance of interaction effects between two wild oat populations, five dosages rates and two temperature treatments on dry mass pot$^{-1}$

| Effect | Fixed Effect Test for dry mass pot$^{-1}$ | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |

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| | | | |
|---|---|---|---|
| Temperature | 1 | 7.6054 | 0.007208 |
| Treatment | 3 | 157.9435 | 0.000000 |
| Population | 1 | 9.1949 | 0.00327 |
| Temperature*Treatment | 3 | 13.3401 | 0.00000 |
| Temperature*Population | 1 | 16.9106 | 0.00000 |
| Treatment*Population | 3 | 5.2851 | 0.002241 |
| Temperature*Treatment*Population | 3 | 24.7858 | 0.00000 |

**Switched Temperature**

**Appendix 14**: Analysis of variance of interaction effects between two wild oat populations, five dosages rates and two switched temperature treatments on survival percentage

| Effect | Fixed Effect Test for survival percentage | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Temperature | 1 | 0.1888 | 0.665122 |
| Treatment | 3 | 63.8373 | 0.00000 |
| Population | 1 | 0.0520 | 0.820269 |
| Temperature*Treatment | 3 | 0.2480 | 0.862502 |
| Temperature*Population | 1 | 5.5115 | 0.221363 |
| Treatment*Population | 3 | 0.1966 | 0.898430 |
| Temperature*Treatment*Population | 3 | 3.5912 | 0.017178 |

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**Appendix 15**: Analysis of variance of interaction effects between two wild oat populations, five dosages rates and two temperature treatments on fresh mass pot$^{-1}$

| Effect | Fixed Effect Test for fresh mass pot$^{-1}$ | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Temperature | 1 | 83.0695 | 0.00000 |
| Treatment | 3 | 1.395 | 0.307521 |
| Population | 1 | 1.0547 | 0.0307521 |
| Temperature*Treatment | 3 | 2.7695 | 0.046981 |
| Temperature*Population | 1 | 1.1785 | 0.323223 |
| Treatment*Population | 3 | 1.4892 | 0.225920 |
| Temperature*Treatment*Population | 3 | 6.197 | 0.000842 |

**Appendix 16:** Analysis of variance of interaction effects between two wild oat populations, five dosages rates and two temperature treatments on dry mass pot$^{-1}$

| Effect | Fixed Effect Test for dry plant mass | | |
|---|---|---|---|
| | Degree of freedom | F-value | p-value |
| Temperature | 1 | 3.51786 | 0.064359 |
| Treatment | 3 | 47.73016 | 0.00000 |
| Population | 1 | 2.08321 | 0.152831 |
| Temperature*Treatment | 3 | 4.19948 | 0.008208 |
| Temperature*Population | 1 | 1.14879 | 0.287025 |

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| | | | |
|---|---|---|---|
| Treatment*Population | 3 | 0.80004 | 0.497465 |
| Temperature*Treatment*Population | 3 | 1.73217 | 0.167038 |