Germination of the grass weed *Eleusine indica* (L.) Gaertn. population as affected by temperature, light and its response to glyphosate

by

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TABLE OF CONTENTS

DECLARATION

I, the undersigned, hereby declare that the dissertation submitted herewith for the degree MSc (Agric) Agronomy to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other university.

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LIST OF ABBREVIATIONS

ANOVA: Analysis of variance

CABI: Centre for Agriculture and Biosciences International

DAE: Days after emergence

DAS: Days after sowing

DAT: Days after treatment

EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase

GR: Glyphosate-resistant

LSD: Least significant difference

R: Resistant

a.e: Acid equivalent

ha: Hectares

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The grass weed *Eleusine indica* is listed as one of the most problematic weeds species in the world. It is characterized by vigorous growth and an extended root system that contributes to making this weed highly competitive. The discovery of glyphosate-resistant (GR) biotypes across the world, which currently places *E. indica* in the top five in the list of GR weeds, has only exacerbated the problem status of the weed. Glyphosate is still regarded as the most important herbicide and has become the most historically successful herbicide throughout the world. *Eleusine indica* populations that proved to be resistant to glyphosate have been identified under various cropping situations and are now amongst the most widely distributed herbicide resistant species, causing great economic impact worldwide. This has triggered interest on the biology of this species and its response to glyphosate in order to improve its control. Several studies have been conducted on this species across the world but no studies have been conducted in South Africa. Therefore, there is a need for a better understanding of the biology of the weed and information on effective control of this cosmopolitan weed in South Africa as well as globally. The objectives of the present study were to determine the effects of temperature and light on seed germination of *E. indica* as well as to investigate the response of *E. indica* to glyphosate. Growth chamber and glasshouse experiments were conducted on a population of *Eleusine indica* obtained from Hatfield experimental farm of the University of Pretoria (location: 25°45'7.08"S, 28°15'33.12"E). All experiments were conducted at phytotron facilities on the experimental farm. In the growth chamber, experiments were conducted to assess the germination behaviour of *E. indica* under different temperature and light regimes with different germination media. The lowest germination rate was recorded in H_2O medium, under light/dark conditions (82.42%). Seeds germinated best in KNO_3 medium regardless of alternating temperature regimes or light conditions. The highest final germination was recorded in KNO_3 medium in the dark (99.17%). Germination of *E. indica* was high (>80%) at all three alternating day/night temperature regimes and light interactions. The response of *E. indica* to glyphosate was investigated in a growth chamber with a rapid *in vitro* experiment and in the glasshouse by means of a dose-response method. Two *in vitro*

methods were tested with un-germinated and pre-germinated seeds placed on filter papers in Petri dishes to which solutions with glyphosate concentrations of 0 and 900 g a.e. ha^{-1} were added. After seven days, root and shoot lengths of the seedlings were measured. The shoot and root development of both the un-germinated and pre-germinated seeds were significantly reduced by glyphosate. In the glasshouse, a dose-response experiment was conducted to determine the response of *E. indica* to a range of glyphosate dosages. Glyphosate dosage rates were 0, 0.25x, 0.5x, 1x, 2x and 4x the registered label recommended rate of Roundup Turbo$^{®}$ (1x = 900 g a.e. ha^{-1}). Biomass was measured 28 days after treatment. All glyphosate doses examined in the dose-response experiment significantly reduced the biomass of *E. indica*. The findings of both these experiments reveal that the *E. indica* population examined in this study is sensitive to glyphosate. Finally, the effect of growth stage on the response of *E. indica* to glyphosate was investigated in the glasshouse. Plants were treated with glyphosate at the field recommended dose of Roundup Turbo$^{®}$, 900 g a.e. ha^{-1} at four different growth stages: 21, 35, 49, and 63 days after emergence (DAE). Control treatments were maintained for comparison for each growth stage. Biomass was measured 28 days after treatment. While plant biomass was significantly reduced by glyphosate, older plants (63 DAE) were visually less affected by glyphosate. Despite glyphosate treatment, these plants continued to grow and produce seeds. Therefore, growth stage had an effect on the response of *E. indica* to glyphosate. The results of this study demonstrate the importance of growth stage on glyphosate application even where plants are sensitive to the herbicide. Growers should therefore consider and be aware of the importance of timing glyphosate application. There is a need to intensify this study to examine populations from various geographic locations. Germination characteristics and weed response methods for weeds from different locations might differ as a result of different climatic conditions and weed control practices. This information is essential for improving methods currently available for control of this weed in the country, and perhaps even in other parts of the world.

INTRODUCTION

Weed infestation has always been an issue in agricultural crop production systems (Johnson et al. 2009). Weeds are a serious threat to crop growth and the expected crop yield due to interference with the crop through the phenomena of allelopathy and competition. According to Talaka and Rajab (2013), for every unit of weed growth, there will be one less unit of crop growth. The impact of weed infestation on yield loss is influenced by the type of infesting weeds, density of the weeds, and duration of the infestation (Hakim et al. 2013). Other important factors governing weed impact on crop yield are site-specific, such as type of crop, soil type and climatic factors. On average, weeds cause 34% of crop yield losses, globally, posing a serious threat to food security (Délye et al. 2013). Weed management practices in agriculture are conducted mainly for reducing yield losses resulting from weed infestations (Lundkvist and Verwijst 2011). Yield loss resulting from weed infestations does not only result from competition but also from reduced harvesting efficiency and crop quality (Shuma et al. 1995).

Eleusine indica is regarded as one of the world's worst weed (Chen et al. 2015b). It is a major competitor in a wide variety of ecosystems worldwide. In agro-ecosystems it infests a wide range of crops including fruit orchards (Jalaludin et al. 2014). It can also be found in sports turf as well as along roadsides (Chen et al. 2015a). *Eleusine indica* causes a major reduction in both crop quality and quantity in agro-ecosystems (Jalaludin et al. 2010). According to Alcantara et al. (2016), the biomass of a maize plant competing with 10 to 16 *E. indica* seedlings can be reduced by nearly 52% compared to a non-competing maize plant. Furthermore, *E. indica* is a secondary host to diseases, nematodes, and viruses that affect various crops including maize (Chauhan and Johnson 2008). *Eleusine indica* is therefore a serious threat to cash crops and its control is essential especially in agro-ecosystems (Jalaludin et al. 2010).

The management and control of *E. indica* is conducted mainly with the use of herbicides. The heavy dependence on herbicides has led to the development of herbicide resistance in the species (Takano et al. 2016). Herbicide resistance in weeds

is an evolutionary process that occurs as a result of the selection pressure caused by the regular use of herbicides with the same mode of action (Jalaludin et al. 2010). Several cases have been reported on *E. indica* biotypes found to be resistant to herbicides with different modes of action (Takano et al. 2016). *Eleusine indica* is regarded as one of the ten most important herbicide resistant species in the world, and it has evolved resistance to a number of herbicides including glyphosate (Nandula et al. 2005, Heap 2018).

Glyphosate, described as a "once in a century" herbicide (Duke and Powles 2008), is a systemic, non-selective herbicide that is used for controlling annual and perennial weed species as well as volunteer crops under various situations in both crop and non-crop lands (Nandula 2010). Glyphosate has become the most historically successful herbicide throughout the world (Duke 2018). This has been attributed to some of its attractive characteristics which include low acute and chronic toxicity, lower costs relative to other herbicides and its broad spectrum effectiveness (Johnson et al. 2009, Duke 2018). In South Africa, glyphosate is the most popular and widely used herbicide (Gouse 2014). It is among the leading products used to control weeds and invading alien plant species in the country (Mensah et al. 2013).

Initially, glyphosate use was restricted to pre-planting because of its non-selective nature. This constraint was, however, defeated by the introduction of genetically modified (GM) glyphosate resistant (GR) crops (Johnson et al. 2009, Zhang et al. 2015b). Before the advent of GR crops, famers controlled weeds by applying selective herbicides. When GR crops came on the market they were rapidly adopted by farmers (Green 2012). The technology enabled farmers to use glyphosate without restrictions, making it the most successful herbicide ever (Zhang et al. 2015b, Duke 2018). As is the case with many other herbicides, the repeated use of glyphosate in high volumes on a broad front over a number of years has resulted in the evolution of 41 glyphosate-resistant weed species including *E. indica* (Alcantara et al. 2016, Heap 2018).

Eleusine indica is a worldwide weed problem and the development of herbicide resistance in this species has increased problems associated with controlling it (Takano et al. 2016). The development of herbicide resistance to glyphosate is a cause for great concern; it poses a great threat to the sustainable use of this herbicide (Jalaludin et al. 2014). As one of the weed species implicated, control of *E. indica* has to contend with the new reality of reduced herbicide options, which has caused great problems for growers (Ng et al. 2004).

Several factors influence the efficacy of herbicides by influencing herbicide uptake by target plants and their subsequent fate within the plant system. Growth stage of weeds is one of the essential factors that determine herbicide efficacy (Khan et al. 2011). According to Singh et al. (2005), growth stage has an influence on the efficacy of glyphosate – younger plants were more easily controlled compared to those at an advanced growth stage. Anecdotal evidence obtained from farmers is that grass weeds, including *E. indica*, are sometimes inadequately controlled with glyphosate. One reason is that in the field, at a particular point in time, a particular weed occurs at different growth stages ranging from very young (3 to 6 leaves) to older stages that are not ideal for control. This is particularly influenced by the variability in the germination behaviour of the species in a particular field. According to Singh and Singh (2009), germination requirements of weed seeds vary even within a species. Temperature, light and seed burial depth are some of the important factors that influence germination in *E. indica* (Chauhan and Johnson 2008). Differences in the response of the species to these factors results in seeds germinating at different times throughout the season, therefore resulting in size variation. Understanding of optimum conditions for germination of *E. indica* can assist with timing glyphosate application in such a way that during glyphosate application the majority of the seedlings are at the ideal growth stage for effective control.

The objectives of this study were:

- To determine the effects of temperature and light on seed germination of *E. indica*.
- To investigate the response of *E. indica* to different doses of glyphosate.
- To assess the influence of growth stage on the response of *E. indica* to glyphosate.

CHAPTER 1

Literature review

1.1 *Eleusine indica* species

The genus *Eleusine* consists of 9 species: *E. africana, E. coracana, E. kigeziensis, E. indica, E. floccifolia, E. intermedia, E. multiflora*, *E. jaegeri,* and *E. tristachya* (CABI 2015, Waterhouse 1994). *E. coracana* and *E. indica* are closely related and it is believed that *E. coracana* developed from *E. indica* (Dida et al. 2007, Waterhouse 1994).

Eleusine indica, commonly known as goosegrass, is a member of the *Poaceae* family (Zhang et al. 2015b). It is an annual, self-pollinating grass (Chen et al. 2015b) that is able to survive under a wide range of environmental conditions (Nandula et al. 2005). The exact origin of *E. indica* is unclear (Baerson et al. 2002), however, it is considered native to Africa and temperate and tropical Asia (CABI 2015). It is widely distributed in many parts of the world across tropical and subtropical regions. Its existence has been documented in South Asia, Southeast Asia, the Pacific, Eastern and Southern Africa, as well as North America (Chauhan and Johnson 2008, Chen et al. 2015a).

1.1.1 Biology, ecology and control of *Eleusine indica*

Eleusine indica is characterized by its prostrate and spreading or erect growth (Steed et al. 2016). It can grow to nearly 40 cm in height. The leaves are green in colour, flat and can grow up to a width of 8 mm and a length of 15 cm (CABI 2015). *Eleusine indica* consists of flattened branching stems with little to no hairs along the edges. The stem bases and stem sheaths exhibit a whitish colour with a flattened rosette-forming growth (Figure 1.1). *Eleusine indica* possesses a tough well-developed fibrous root system. Its inflorescence is made up of 2 to 13 digitately arranged spikes composed of rows of spikelets containing the seeds (Steed et al. 2016) (Figure 1.2).

Figure 1.1: *Eleusine indica* rosette stem base (Steed et al. 2016)

Figure 1.2: Inflorescence of *E. indica* (ZipcodeZoo 2013)

Eleusine indica is a highly fertile grass with a propagation that depends mainly on seeds (Chauhan and Johnson 2008, CABI 2015). The seeds are reddish-brown to black in colour surrounded by a thin membranous layer (Figure 1.3) (Steed et al. 2016). A single plant is able to produce approximately 140 000 seeds (Jalaludin et al. 2010). According to Chauhan and Johnson (2008), *E. indica* seeds exhibit some level of dormancy which is mostly present in freshly shed seeds while older seeds have no deep dormancy. Seed germination in *E. indica* is controlled by several factors including temperature, light, seed burial depth and pH (Chauhan and Johnson 2008). *Eleusine indica* displays vigorous growth and development that is highly accelerated in higher temperature conditions because of its C4 photosynthetic mechanism (Takano et al. 2016).

1 mm

Figure 1.3: *Eleusine indica* seeds (Weeds of Australia 2016)

As a result of its vigorous growth and copious seed production, *E. indica* is regarded as an aggressively invasive weed, therefore, the control and management of such species is important, especially in agronomic systems (Steed et al. 2016). Various control

methods are available for the management of *E. indica*. Methods range from mechanical to chemical (herbicide) weed control. Mechanical weed control manages weeds directly through physical force aimed at causing damage to or physically removing the weeds. This involves different hoeing, tillage and soil cultivation methods used in agriculture to prepare fields (Lundkvist and Verwijst 2011). Small plants of *E. indica* can be easily removed by hand while for well-developed plants that have grown to 6 to 10 cm in diameter a hoeing tool is required (Breeden and Brosnan 2016). Because of the prostrate growing nature of some *E. indica* plants, mowing is not recommended for control as this will not eradicate the plants (Steed et al. 2016). Mechanical weed control is, however, a labor-intensive process and chemical weed control has been adopted as an alternative (Breeden and Brosnan 2016).

Chemical control, which involves the use of herbicides to suppress or kill undesired plants, is a commonly used method for weed control. A wide variety of pre- and post-emergence herbicides is available for the control of *E. indica* (Table 1.1). Pre-emergence herbicides (graminicides) provide effective control of this weed species (Breeden and Brosnan 2016, Steed et al. 2016). These herbicides can be applied alone or in combination with a different herbicide(s) for a broader spectrum of weed control (Steed et al. 2016). Post-emergence herbicides should be applied after seedlings have emerged above-ground. Caution should be exercised when choosing post-emergence herbicides for *E. indica* in order to avoid selection of herbicides that may injure non-target species such as a grass crop (Breeden and Brosnan 2016). Many graminicides have excellent selectivity in grass crops but crop safety can never be taken for granted – herbicide selectivity is relative and not absolute.

Table 1.1: Some of the herbicides registered to control *E. indica* (Croplife SA 2017)

Active ingredient	Trade name	Crop(s)
2,4-D	2,4-D Amine 480 SL	Barley; grain sorghum; grass pastures; lawns; maize; potatoes; rye; sugarcane; wheat
Acetochlor	Acetochlor 900 EC	Afforestation; groundnuts; maize; potatoes; sugarcane
Ametryn	Ametrex 500 SC	Bananas; pineapples; sugarcane
Atrazine	Agrizine 500 SC	Grain sorghum; maize; pineapples; sugarcane
Atrazine/terbuthylazine	Terbucide Plus 900 WDG	Maize
Bromacil	Bromacil 800 WP	Citrus; industrial; pineapples; sisal
Diuron	Develop 800 WDG	Avocadoes; bananas; citrus; coffee; macadamias; mangoes; pecans; sugarcane
EPTC	EPTC 720 EC	Dry beans; kidney beans; potatoes; sunflowers
Fluazifop-p-butyl	Fluent 125 EC	Bananas; clover; dry beans; green beans; groundnuts; mangoes; medics; onions; potatoes; soy beans; sugarcane eradication
Flumetsulam	Flumetsulam 800 WDG	Dry beans; groundnuts; maize; soy beans; soy beans (glyphosate tolerant)
Glufosinate-ammonium	Bound 200 SL	Apples; apricots; cherries; grapes; industrial; nectarines; peaches; pears; plums & prunes; sugarcane
Glyphosate/mesotrione	Canon Smart 500 SC	Maize (glyphosate tolerant)
Glyphosate/mesotrione/S-metolachlor	Halex GT	Maize (glyphosate tolerant)
MCPA	MCPA 400 SL	Apples; barley; grapes; grass pastures; lawns; maize; oats; peaches; pears; potatoes; rye; grain sorghum; sugarcane; wheat
Mesotrione	Cantron 480 SC	Maize; sugarcane
Metolachlor	Platinum Plus 915 EC	Dry beans; groundnuts; lupins; maize; potatoes; soy beans; sunflowers

1.2 Herbicides

The first synthetic herbicides with the ability to select between crop and weed were discovered in the early 1940's when weed-killing properties were discovered for phenoxyacetic herbicides, namely MCPA (monochlorophenoxyacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) (Dill 2005, Johnson et al. 2009). These two herbicides were effective for controlling broadleaf (dicot) weeds in grass (monocot) crops such as maize, small grains and sugarcane. Since then a variety of herbicides have been introduced to the markets, gradually replacing most tillage practices used for weed control (Johnson et al. 2009). For almost 70 years, herbicides have been the most effective and economic means for weed control ever developed (Heap and Duke 2018). Herbicides can kill 90 to 99 % of the target species while other weed control methods would require multiple labor-intensive techniques to achieve similar efficacies (Délye et al. 2013). Farmers have been relying heavily on herbicides for controlling weeds since the commercialization of the first herbicide (Green 2014). Herbicides have made a huge contribution towards increasing food production (Nandula 2010). Unfortunately, excessive use of certain herbicides has resulted in weed shifts and the development of herbicide resistance in weeds (Johnson et al. 2009).

1.2.1 Herbicide resistance

Herbicide resistance is defined as the ability of a plant to grow and reproduce after exposure to a herbicide field rate which would under normal conditions result in the death of the biotype (Johnson et al. 2009). This is an evolutionary process that results from a selection pressure influenced by the frequency of herbicide use, herbicide efficiency, and the duration of herbicide efficiency (Ng et al. 2004, Pieterse 2010). There have been increasing reports of herbicide-resistant weeds worldwide (Masni et al. 2008). Cases of multiple resistance have also been reported in various weed species (Heap 2018). Multiple resistance is when a biotype is found to be resistant to more than one mechanism of action simultaneously (Jalaludin et al. 2014), e.g. resistance to both EPSPs and ACCase inhibitors (Takano et al. 2016).

The first case of herbicide resistance was reported in 1957 and numerous weed species have since developed resistance to a wide range of herbicides (Shrestha and Hemree 2007, Délye et al. 2013). The steady increase in cases of herbicide-resistant weed species has accumulated to 254 confirmed species worldwide (Heap 2018). In South Africa, Cairns and Laubscher reported the first case of herbicide resistance in 1986 in a biotype of wild oats (*Avena fatua*) which showed resistance to diclofop-methyl in the Western Cape Province (Pieterse 2010, Ferreira et al. 2015). Since then, more cases of weed species resistant to herbicides with various modes of action have been documented in the country. In some situations (herbicide/weed species combinations), cases of multiple resistance have also been reported in South Africa (Table 1.2) (Heap 2018).

Table 1.2: Weed species and herbicide mode of action groups confirmed in cases of herbicide resistance in South Africa (Heap 2018)

Species	Common name	First year	Herbicide mode of action
Avena fatua	Wild oat	1986	Multiple resistance: 2 Sites of action ACCase inhibitors ALS inhibitors
Lolium rigidum	Rigid ryegrass	1993	Multiple resistance: 2 Sites of action ACCase inhibitors ALS inhibitors
Amaranthus hybridus (syn: quitensis)	Smooth pigweed	1993	Photosystem II inhibitors
Raphanus raphanistrum	Wild radish	1997	ALS inhibitors
Phalaris minor	Little seed canary grass	1999	Multiple resistance: 2 Sites of action ACCase inhibitors ALS inhibitors
Lolium rigidum	Rigid ryegrass	2001	EPSP synthase inhibitors
Stellaria media	Common chickweed	2002	ALS inhibitors
Conyza bonariensis	Hairy fleabane	2003	PSI Electron Diverter
Conyza bonariensis	Hairy fleabane	2003	EPSP synthase inhibitors
Plantago lanceolata	Buckhorn plantain	2003	EPSP synthase inhibitors
Lolium rigidum	Rigid ryegrass	2003	Multiple resistance: 3 Sites of action ACCase inhibitors PSI Electron Diverter EPSP synthase inhibitors

Herbicide-resistant weeds survive herbicide treatments through various resistance mechanisms. Mechanisms of herbicide resistance are classified as target-site resistance mechanisms and non-target-site resistance mechanisms (Délye et al. 2013). Target-site resistance mechanisms include herbicide target site mutations while non-target-site include any other mechanism such as reduced absorption and translocation

of herbicides as well as rapid metabolic herbicide detoxification (Koger and Reddy 2005, Délye et al. 2013). Reduced translocation is however the most uncommon herbicide resistance mechanism because of the excellent mobility of herbicides (Shaner 2009). The incidence of herbicide resistance in weeds can increase production costs, reduce crop yield and in some instances reduce the value of the land (Zhou et al. 2016). Understanding herbicide resistance and the mechanisms of resistance is an essential key towards developing effective strategies for managing herbicide resistance (Nandula 2010).

1.3 The herbicide glyphosate

Glyphosate [N-(phosphonomethyl) glycine] is a modified phosphonomethyl derivative of the amino acid glycine (Nandula 2010, Sammons and Gaines 2014). It is an odourless, white crystalline solid with a chemical structure that comprises a single amino function and three ionisable acidic locations (Figure 1.4). This herbicide was developed at Monsanto in 1970 and was commercialized under the trade name Roundup in 1974 (Nandula et al. 2005, Nandula 2010). Glyphosate consists of a strong intermolecular hydrogen bonding which makes its volatility minimal. This suggests that glyphosate is unlikely to evaporate from treated surfaces. Glyphosate is readily dissolved in dilute aqueous bases and acids, producing anionic and cationic salts, respectively (Nandula 2010). It is available in various formulations which include isopropylamine, sodium, ammonium, potassium and trimethyl-sulfonium (Franz et al. 1997). These are known as monobasic salts which help enhance the solubility of glyphosate in aqueous solutions (Nandula 2010). The main producer of glyphosate is Monsanto, its founding company, however, a large number of companies capable of producing glyphosate are operating globally (Woodburn 2000).
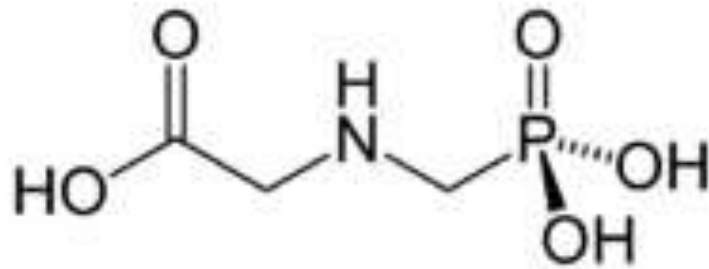
Figure 1.4: Chemical structure of glyphosate (Franz 1997)

Glyphosate is regarded as the world's most important herbicide (Zhang et al. 2015a) and is now registered for use in over 130 countries around the world (Nandula 2010). In South Africa, glyphosate was registered in the 1970s, since then it has been an essential weed control instrument and is now available in more than 20 trade names in the country's pesticide market (Gouse 2014). The global market for glyphosate consists of its use as both an agricultural and non-agricultural herbicide (Woodburn 2000). Maize, wheat and soybean farmers are the main users of glyphosate in SA (Gouse 2014). It is used for pre-plant, post-emergence, post-harvest and post-direct applications. The use of glyphosate has dramatically increased with a drastic decrease in the use of other herbicides (Nandula et al. 2005). Its popularity in glyphosate-tolerant crops, which came on the market in 1996, has boosted glyphosate use. Glyphosate is also a key tool, and often the only means of weed control, in zero-tillage systems. Some of the attributes that contribute to the success of this herbicide include its unique mode of action, rapid uptake and excellent translocation, low toxicity to mammals, and its friendliness to the environment (Duke and Powles 2008). These are some of the reasons for the widespread adoption of glyphosate. Because of these attributes, it is anticipated that glyphosate will remain as the prevailing herbicide in agriculture for some time yet (Nandula 2010).

1.3.1 Mode of action

The primary mode of action for glyphosate is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Shrestha and Hemree 2007, Duke and Powles 2008) (Figure 1.5). The EPSPS gene is a component of the shikimate pathway (Chen et al. 2015b) which is located in the chloroplast (Sammons and Gaines 2014). About 20% of the total fixed carbon in plants moves through the shikimic pathway resulting in the biosynthesis of the three essential aromatic amino acids, tryptophan, tyrosine, and phenylalanine (Baerson et al. 2002). These aromatic amino acids play a role in the formation of certain proteins and other essential secondary metabolites (Zhang et al. 2015b), including phenolics, lignins, tannins, and other phenylpropanoids (Green 2009). The inhibition of the activity of the enzyme EPSPS by glyphosate causes an accumulation of shikimic acid in the shikimate pathway (Chen et al. 2015b). Glyphosate causes a blockage in the pathway resulting in a high flow of carbon which is then converted into high levels of shikimic acid in the pathway (Duke and Powles 2008, Zhang et al. 2015b). This prevents the production of chorismate which is required for the biosynthesis of the essential aromatic amino acids (Schuette 1998), thus resulting in the inhibition of the biosynthesis of three essential aromatic amino acids (Chen et al. 2015b).

The inhibition of the EPSPS causes a reduction in transpiration and photosynthesis, a decline in chlorophyll content and, subsequently, the death of the plant (Nandula 2010). The visible injury symptoms are chlorosis and gradual wilting of the plant, followed by browning of the entire above-ground biomass and weakening of underground plant organs (Schuette 1998). It can take 10 to 20 days after treatment for these injury symptoms to be fully visible before plant death sets in (Ismail et al. 2004).
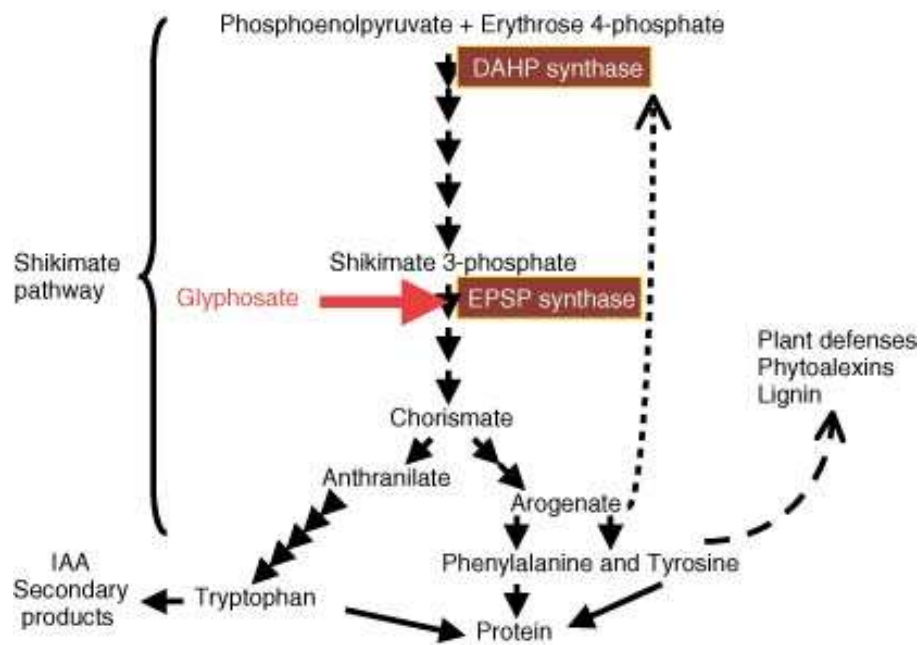
Figure 1.5: The shikimate pathway and the inhibition of the EPSPS by glyphosate (Duke and Powles 2008)

1.3.2 Absorption and translocation of glyphosate

Glyphosate is a foliar-applied herbicide (Johnson et al. 2009). It is taken up through the green leaves and shoots of the plant (Vereecken 2005, Duke and Powles 2008). The translocation of glyphosate within the plant resembles the source-to-sink movement of photo-assimilates (Nandula 2010). In order for glyphosate to reach its target site, the EPSPS gene, once it gets to the cell, it must enter the chloroplast (Nandula 2010). The phloem mobility of glyphosate is due to its unique chemical composition of a single basic and three acidic functions (Shaner 2009). The plant absorbs the herbicide which is then distributed through it in the xylem and phloem penetrating the actively growing vegetative and reproductive tissues which are the primary sinks for glyphosate (Johnson et al. 2009). Phytotoxic levels of glyphosate accumulate in the meristematic zones of the shoots and roots, as well as underground roots and reproductive organs (Shaner 2009). This means that glyphosate controls underground meristems, corms, rhizomes and other plant organs which may possibly regenerate when only the aboveground vegetative material is killed (Nandula 2010). Excellent absorption and

translocation of glyphosate are the main reasons for its outstanding efficacy. As a result even small amounts of glyphosate that come into contact with plant foliage can cause injury to the plant (Duke and Powles 2008).

The efficacy of glyphosate depends strictly on the amount distributed to the symplastic or living portion of the plant (Nandula 2010). The ability of glyphosate to effectively control the plant is determined by its rapid diffusion through the plant cuticle and its widespread translocation throughout the plant (Powles and Preston 2006). However, the absorption and translocation of glyphosate are dependent on a number of interdependent factors. These include plant species, plant health, growth stage, environmental conditions, cuticle composition and thickness, droplet size and droplet spread as well as the concentrations of glyphosate and added surfactants (Schuette 1998, Nandula 2010).

1.4 Glyphosate resistance

1.4.1 Glyphosate resistance in weeds

Glyphosate is distinguished by its distinct mode of action, chemical structure, limited metabolism in plants and the lack of residual effect (Pieterse 2010). The intensive use of glyphosate for weed control has led to the development of weed biotypes that are resistant to it (Vargas et al. 2013). This process is influenced by a number of factors including target site mutation, amplified expression of EPSPS, and altered translocation (Zhang et al. 2015a). Some of these conditions cause changes to the genetic composition of the weed populations, of which the one of greatest concern is evolvement of glyphosate-resistant (GR) weeds (Masni et al. 2008).

In 1996, rigid ryegrass (*Lolium rigidum*) was the first weed species for which a case of a GR biotype was reported (Chen et al. 2015a). This discovery was made in Australia and other biotypes were later identified in United States, South Africa and France (Johnson et al. 2009). Since then, the number of GR weed species has increased worldwide (Figure 1.6) and currently the average rate of new GR discoveries is more than two

species per year (Duke 2018). Currently, 41 weed species had been reported to have evolved resistance to glyphosate placing it in the top five on the list of herbicides with herbicide-resistant species (Heap 2018). Some of the weed species that were identified as GR include: *Eleusine indica,* Malaysia (1996) (Chen et al. 2015a), *Conyza canadensis*, United States (2000) (VanGessel 2001), *Conyza bonariensis,* South Africa (2003) (Nandula et al. 2005), *Plantago lanceolata,* South Africa (2003) and *Amaranthus palmeri,* United States (2005) (Heap 2018). The evolution of GR in a number of weed species poses a great threat to the sustainability of the herbicide as the most important herbicide (Zhang et al. 2015a).
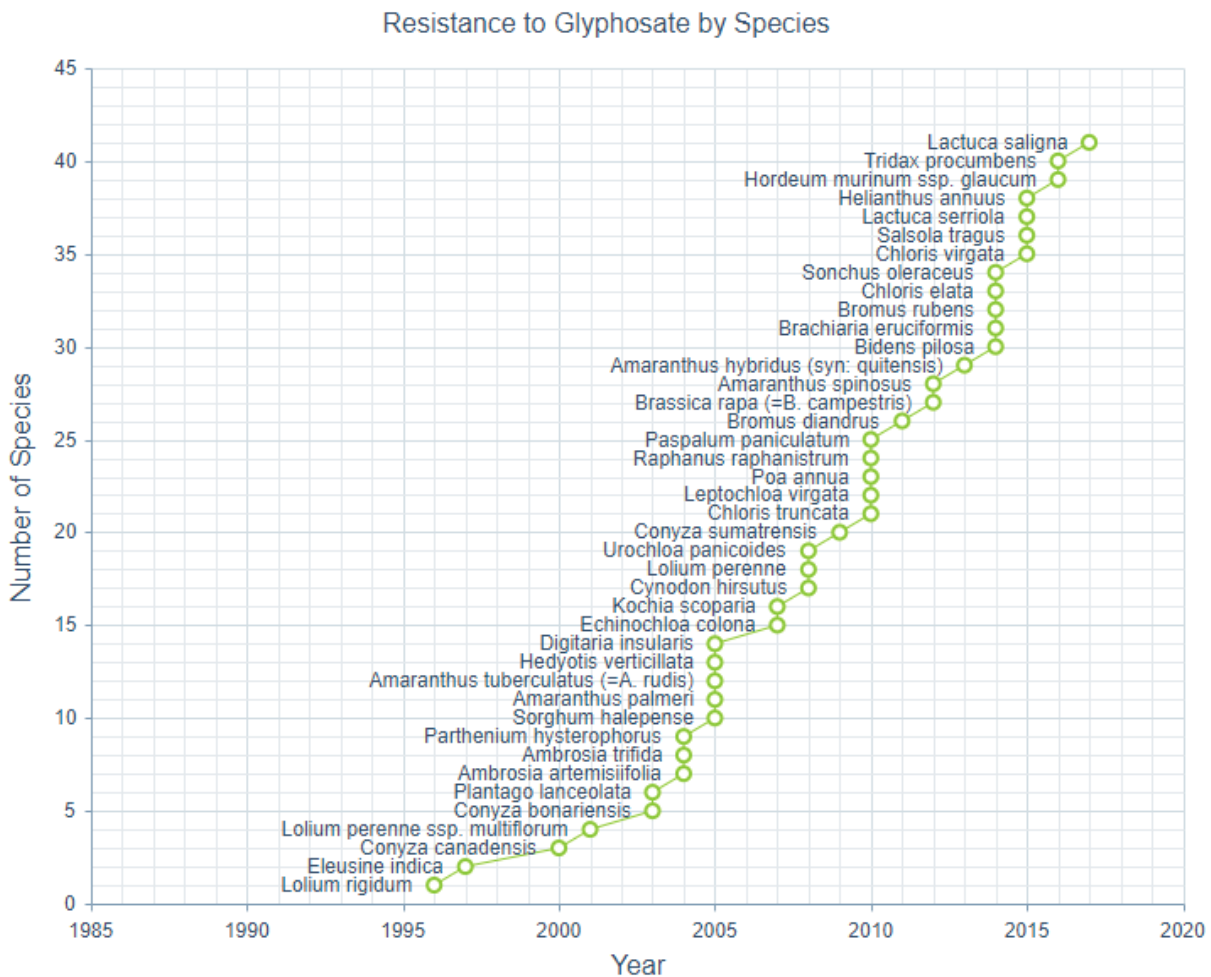
Figure 1.6: Increase in glyphosate resistant weeds worldwide (Heap 2018)

The spread of GR weeds, amongst other resistance cases involving many herbicides, forces farmers to look at alternative methods for weed control. Some crop producers often rely on herbicide practices that were available before GR crops were introduced (Green 2014). Because of limited herbicide options for GR weeds some farmers resort to mechanical weed control and other labour intensive and high cost practices. Some farmers even returned to planting non-GR crops making it possible to use older herbicide options. Appropriate and effective weed management methods need to be developed hurriedly to curtail the rapid spread of GR weeds (Zhou et al. 2016).

The resistance of weeds to glyphosate occurs as a result of various mechanisms (Zhang et al. 2015a). These include target site mutations, amplification of the EPSPS gene, reduced translocation (Nandula et al. 2005, Zhang et al. 2015a), and vacuole sequestration (Chen et al. 2015b, Yu et al. 2015). Target site mutation as well as reduced translocation are the two major mechanisms that play a significant role in glyphosate resistance (Nandula et al. 2005, Ghanizadeh et al. 2016). Glyphosate resistance in some weed species can be a result of one or more of these mechanisms operating in unison (Koger and Reddy 2005).

1.4.2 Glyphosate resistance in crops

Glyphosate resistance in transgenic crops with glyphosate tolerance was first reported in soybean. The first GR soybean crops were created in 1996 by isolating a CP4 strain of an *Agrobacterium* species, *Agrobacterium tumifacien* (Nandula et al. 2005). The CP4-EPSPS proved to be highly tolerant to glyphosate and identical to the sensitive EPSPS found in most plant species (Dill 2005). The CP4-EPSPS gene was cloned and inserted in the germplasm of soybean resulting in plants with tolerance to high levels of glyphosate (Johnson et al. 2009). The insertion of the CP4-EPSPS resulted in normal functioning of the shikimate pathway by bypassing the endogenous EPSPS (Figure 1.7) (Dill 2005). This technology was patented as the Roundup Ready[®] brand. GR soybean [*Glycine max* (L.) Merr.] was released on the world market by Monsanto in 1996 (Johnson et al. 2009).
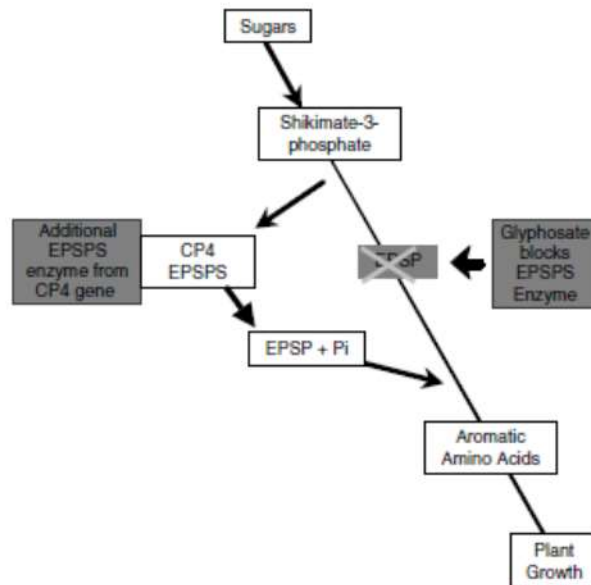
Figure 1.7: The role of CP4 EPSPS gene in the normal functioning of the shikimate pathway (Dill 2005)

The success of the development of GR soybean was followed by the release of more GR crops to the markets. These include canola (*Brassica napa* L.) (1996), cotton (*Gossypium hirsutum* L.) (1997), maize (*Zea mays* L.) (1998), sugarbeet (*Beta vulgaris* L.) (1999) and alfalfa (*Medicago sativa* L.) (2005) (Duke and Powles 2008, Green 2012). The adoption of these crops by farmers has been remarkable throughout the world because their introduction made this technology the most rapidly adopted technology in the history of agriculture (Green 2018). In the world, almost 90% of the genetically modified crops grown by farmers are GR crops. In USA, the adoption of GR soybean increased to greater than 90% within 10 years of its introduction since 1996 (Duke 2018). In South Africa, GR or Roundup Ready maize was introduced in 2003/04. Maize is the most important field crop in the country and since the introduction of this technology its adoption increased significantly (Gouse 2014). GR crops changed the way in which farmers managed weeds, providing relatively simpler and more effective control practices (Green 2009). The new development allowed farmers to use glyphosate even post-planting, without worrying about crop injury (Nandula et al. 2005). This was a great development since the use of glyphosate after planting was restricted

due to its great risk of causing injury to the crops (Zhang et al. 2015b). Now farmers could adopt newly developed cropping systems, such as conservation agriculture and no-tillage farming, which significantly changed practices in crop production and weed management (Johnson et al. 2009).

The high adoption rate of GR crops is attributed to the reduced production costs and labour inputs as well as the efficient and simple weed management solutions provided by the use of glyphosate (Zhou et al. 2016). With GR crops, farmers were now able to control weeds on larger areas of land by employing a weed control program involving a single herbicide (Johnson et al. 2009). Consequently, glyphosate became the herbicide of choice and its use increased worldwide (Zhang et al. 2015b). In South Africa, glyphosate has been the most used herbicide in crop production as a result of the GR maize, soybean and cotton varieties introduced to the markets (Gouse 2014). The intensified use of GR crops and the subsequent increase in the use of glyphosate for weed control led to the evolution of natural resistance in a wide range of weed biotypes (Vargas et al. 2013). However, this did not have any negative impact on the adoption of the technology. Farmers continue using GR crops and relying on glyphosate for weed control in these crops as most of the weed species are still susceptible to it (Duke 2018).

1.5 Glyphosate resistance in *Eleusine indica*

Several cases of herbicide resistance in *E. indica* have been reported worldwide (Takano et al. 2016). Resistance to acetyl coA carboxylase (ACCase) inhibitors, acetolactate synthase (ALS) inhibitors, EPSPS inhibitors, photosystem II inhibitors, and glutamine synthetase inhibitors have also been reported (Takano et al. 2016). Cases of multiple resistance have been reported in some biotypes. A study by Jalaludin et al. (2014) confirmed multiple resistance in *E. indica* across essential non-selective herbicides, namely glyphosate, glufosinate and paraquat. Glyphosate is the world's most important herbicide while glufosinate and paraquat are commonly used as its alternatives (Jalaludin et al. 2014).

The first case of glyphosate resistance in *E. indica* was reported in a guava orchard in Malaysia in 1997, making it the second weed found to be GR worldwide (Lee and Ngim 2000, Chen et al. 2015a). This case was confirmed after dose-response experiments were conducted in an orchard in Teluk Intan, Perak. This experiment confirmed a high level of glyphosate resistance in a biotype of *E. indica* (Lee and Ngim 2000). Since then, glyphosate resistance in *E. indica* has been reported in several other countries under various cropping situations (Table 1.2) (Heap 2018). Glyphosate resistant *E. indica* is now amongst the most widely distributed GR weed species with the highest economic impact (Heap and Duke 2018).

Table 1.3: Glyphosate-resistant *E. indica* globally (Heap 2018)

Country	Year	Situation
Malaysia	1997	Orchards
Colombia	2006	Coffee
Bolivia	2007	Soybean
Costa Rica	2010	Pejibaye palm
United States (Mississippi)	2010	Cotton
Argentina	2012	Maize, fallow and soybean
Indonesia	2012	Oil palm nursery
Japan	2013	Rice paddy levee
Brazil	2016	Maize, soybean and wheat
Brazil	2017	Beans, maize, cotton, and soybean

The mechanism of GR in *E. indica* is due to EPSPS mutations, the target site for glyphosate (Chen et al. 2015b, Johnson et al. 2009). Target site mutation involves substitution of a proline (CCA) to serine (TCA) at amino acid 106 (Pro106 to Ser106), or a proline to threonine substitution at amino acid 106 (Pro106 to Thr106) (Powles and Preston 2006, Zhang et al. 2015a). These two different gene mutations of the EPSPS in *E. indica* are the known promoters of glyphosate resistance in this weed species

(Powles and Preston 2006). The concentration of shikimic acid in the resistant biotypes of *E. indica* is less compared to that of susceptible biotypes due to the reduced inhibition of the EPSPS enzyme in the shikimic pathway (Ismail et al. 2004).

Several studies have been conduct on *Eleusine indica* across the world but no studies have been conducted in South Africa. Better understanding of the biology of the weed and information on its response to herbicides is needed for effective control of this cosmopolitan weed.

CHAPTER 2

Germination of *Eleusine indica* as affected by temperature and light

2.1 Introduction

Seed germination is an essential process in determining the success and competitiveness of a weed in an agronomic cropping system (Chauhan et al. 2006). Weed seed germination is influenced by specific requirements that often differ even across related weed species (Hugo et al. 2014). These factors include factors such as moisture, temperature, light, nitrogen, soil salinity, pH, burial depth and mulching (Sweeney et al. 2008, Chauhan and Johnson 2010, Honarmand et al. 2016). According to Burgos (2015), temperature and light conditions are the most important factors determining optimum seed germination.

Seed germination of annual weed species is significantly affected by temperature and light. Both temperature and light play a major role in regulating secondary dormancy (Nishimoto and McCarty 1997). Temperature influences germination by breaking dormancy and determining the germination rate of the seeds. Temperature requirements for breaking dormancy vary among weed species. Some species require specific temperatures to break dormancy and germinate while others can germinate over a wide range of temperatures (Singh and Singh 2009). Germination of several major weed species is highly stimulated by fluctuating temperatures (Nishimoto and McCarty 1997). Furthermore, temperature plays a significant role in the adaptability of weed species in different geographic locations (Singh and Singh 2009).

Light plays a significant role in initiating germination of many weed seeds. Light requirements for germination vary significantly with weed species (Singh and Singh 2009). Various weed species respond differently to both light and darkness. Germination in some species is equal in both light and dark while some species essentially require light for germination. In many species, mostly small-seeded species, light breaks dormancy and stimulates germination (Chauhan and Johnson 2010).

Species with light-driven germination are more likely to prevail in continuous no-till agriculture systems (Chauhan and Johnson 2010), because burying of weed seeds is not achieved through tillage of soil, and hence, seeds tend to accumulate near or on the soil surface. Limiting light exposure of the seeds of weed species with light-dependent germination can assist with the control of such weeds (Singh and Singh 2009). Primary and secondary tillage operations can bury and thus prevent germination of seeds with light-dependency. For certain weed species, however, light is not a primary requirement for germination. As a result, these species have a higher germination potential even when buried under the soil or light not reaching the soil surface as a result of canopy closure (Chauhan and Johnson 2010).

As a result of the increase in evolution of herbicide-resistant weed biotypes, understanding the biology and ecology of weeds is essential in developing effective weed management strategies (Singh and Singh 2009). Several studies conducted on germination of *E. indica* highlight the importance of temperature and light in seed dormancy breaking and germination in the species (Lim et al. 2015, Takano et al. 2016). Furthermore, several studies report that an interaction between fluctuating temperatures and light significantly affects germination of *E. indica* seeds (Nishimoto and McCarty 1997, Ismail et al. 2002, Chauhan and Johnson 2008).

Eleusine indica is rated a serious weed species on a global scale because of high seed production and vigorous growth, as well as resistance to various herbicide modes of action (Takano et al. 2017). Its germination and survival characteristics are important areas to targets in management strategies. Information on optimum conditions under which a specific weed species germinates can assist in predicting periods of significant emergence flushes. With this information available, more control methods can be applied proactively when the best efficacies can be achieved (Hugo et al. 2014). This includes implementing weed control strategies that would either suppress germination or encourage it where means of controlling seedlings, including herbicide use, are readily available (Chauhan and Johnson 2010). The objective of this component of the

study was to determine the effect of temperature and light on germination of *E. indica*, and to consider whether such information could aid control efforts.

2.2 Materials and methods

2.2.1 *Eleusine indica* plant material used in the study

Eleusine indica plants were collected from the University of Pretoria's Hatfield experimental farm, GPS coordinates 25°45'0.48"S 28°15'37.13"E (Figure 2.1). Inflorescences containing the seeds were removed and air-dried. After drying the inflorescences were placed in brown paper bags and shaken to separate seeds from inflorescence. The seeds were cleaned manually by rubbing between the fingers to remove the thin husk covering them. The seeds were stored in brown paper bags at room temperature until they were used for the experiments. Before the experiments commenced seeds were sterilized in 5% (v/v) solution sodium hypochlorite (NaOCl) solution for 10 minutes, followed by rinsing three times with distilled water. Seeds were placed on a paper towel to remove all surface moisture before use in experiments.

Figure 2.1: Location on the University of Pretoria experimental farm where plant material for the study were collected.

2.2.2 Germination test: Temperature and light effect

To investigate the optimum temperature and light conditions for the germination of the *E. indica* population occurring on Hatfield Experimental Farm (25°45'7.08"S, 28°15'33.12"E), 100 seeds were placed in 90-mm-diameter Petri dishes on filter paper (Whatman No. 1). The filter paper was wrapped around rectangular pieces of glass to prevent the seeds from being immersed in the solution added to the Petri dish. The seeds were germinated in 10 ml of 0.2% potassium nitrate (KNO_3) and 10 ml distilled water (H_2O) under both light/dark and complete darkness. Potassium nitrate is widely used for stimulating germination of many species. It was used according to recommendations made by the International Seed Testing Association (ISTA). The experiment was replicated four times with each replicate having five Petri dishes with 20 seeds in each. The Petri dishes were placed in a growth chamber for 14 days at three alternating day/night temperature regimes of 20/10, 30/20 and 35/20 °C. The photoperiod in each growth chamber was set at 12/12 hours, day/night intervals. Petri dishes were sealed with parafilm to prevent moisture loss. For germination under complete darkness, Petri dishes were covered with two layers of aluminum foil to ensure that no light penetrates. Germinated seeds were counted and removed from the Petri dishes at seven and 14 days from the day of incubation. Seeds were considered germinated as soon as there was visible protrusion of the radicle. The experiment was terminated after 14 days.

2.2.3 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS software version 9.3. A completely randomized design was used in all experiments. Mean separation was done with Tukey's Studentized Range least significant difference (LSD) test.

2.3 Results

2.3.1 Germination of *E. indica* seeds after seven days in a growth chamber

The 3-factor interaction of temperature, light and germination medium, i.e. H_2O or KNO_3, significantly influenced germination of *E. indica* after seven days in the growth

chamber (F=7.03, p=0.0026). Under alternating light/dark conditions, germination increased with an increase in temperature from 20/10 to 30/20 ^{o}C where H_2O was the medium in which germination occurred (Table 2.1). A further temperature increase to 35/20 ^{o}C did not have a significant effect. Under complete darkness in H_2O medium, at both the 20/10 and 30/20 ^{o}C temperature regimes, germination was significantly higher compared to germination under light/dark conditions at these two temperature regimes. At the highest temperature (35/20 ^{o}C), in H_2O medium, seed germination was not affected by light conditions. In the KNO_3 medium, irrespective of light and temperature conditions, there were no significant differences in germination between any of the treatment combinations. The minimum germination percentage attained at those treatments was 92%, with 99% the maximum. In this experiment the highest germination (99%) was reached at the highest alternating temperature (35/20 ^{o}C), under complete darkness in KNO_3, and the lowest germination (64%) under light/dark in H_2O at 20/10 ^{o}C (Table 2.1).

Table 2.1: Effect of temperature and light on germination of *E. indica* seeds for 7-day incubation period

| Medium | Temperature | | | | | |
| | 20/10 ^{o}C | | 30/20 ^{o}C | | 35/20 ^{o}C | |
	Light/dark	Dark	Light/dark	Dark	Light/dark	Dark
H_2O	64.75d	89.50abc	84.25c	96.00ab	88.25bc	91.00abc
KNO_3	92.00abc	96.75ab	91.75abc	98.25a	96.00ab	99.00a
LSD = 9.75						

(Means within columns or rows followed by the same letter(s) are not significantly different from each other at p=0.05; ANOVA presented in Appendix A, Table A1)

2.3.2 Germination of *E. indica* seeds after 14 days in a growth chamber

At final germination count the 3-factor interaction of temperature, light and germination medium, i.e. H_2O or KNO_3, was not significant (F=0.85, p=0.4362). However, all other possible interactions between temperature, light and germination medium were

significant. Germination was lowest (84.88%) at the lowest temperature regime of 20/10 ^oC under light/dark (Table 2.2). Under light/dark conditions no significant difference was observed between germination at 20/10 ^oC and 30/20 ^oC. Under the same light/dark conditions, germination at 35/20 ^oC was significantly higher (93.50%) than at 20/10 ^oC (84.88%), but not significantly different from germination attained at 30/20 ^oC (89.25%). Germination was above 95% under dark conditions across all three temperature regimes. Temperature had no significant effect on germination in complete darkness. However, at 20/10 ^oC and 30/20 ^oC there was a significant difference in germination between ligh/dark and dark conditions. At higher alternating temperatures (35/20 ^oC), no significant differences were obtained on germination under different light conditions (Table 2.2).

Table 2.2: Effect of temperature and light on germination of *E. indica* for 14-day incubation period

Illumination	Temperature		
	20/10 ^oC	30/20 ^oC	35/20 ^oC
Light/dark	84.88c	89.25bc	93.50ab
Dark	96.13a	95.50a	97.63a
LSD = 5.72			

(Means within columns or rows followed by the same letter(s) are not significantly different from each other at p=0.05; ANOVA presented in Appendix A, Table A2)

There was a significant interaction between temperature and germination medium at the end of the the 14-day incubation period (F=3.31, p=0.0477). Lowest germination (84.13%) was recorded at 20/10 ^oC in H_2O medium (Table 2.3). In the same medium there was no significant difference between germination at 20/10 ^oC and 30/20 ^oC (87.38%), but germination at the lowest temperature regime (84.13%) was significantly lower than at the highest temperature regime of 35/20 ^oC (92.63%). At all three temperature regimes, KNO_3 medium significantly improved germination compared to H_2O. At 30/20 and 35/20 ^oC, significant differences were observed in final germination

percentage reached after 14 days between KNO_3 and H_2O media. The highest germination was attained in KNO_3 at 35/20 ^{o}C (98.50%). In KNO_3 medium there were no significant differences in germination between the three temperature regimes (Table 2.3).

Table 2.3: Effect of temperature and germination medium on germination of *E. indica* after 14-day incubation period

Medium	Temperature		
	20/10 ^{o}C	**30/20 ^{o}C**	**35/20 ^{o}C**
H$_2$O	84.13d	87.38cd	92.63bc
KNO$_3$	96.88ab	97.38ab	98.50a
LSD = 5.72			

(Means within columns or rows followed by the same letter(s) are not significantly different from each other at p=0.05; ANOVA presented in Appendix A, Table A2)

The interaction between factors light and germination medium (F=13.56, p=0.0008) was also significant at final germination measurement for *E. indica*. In H_2O medium there was a significant difference in germination under light/dark and complete darkness (Table 2.4). Lowest germination (82.42%) was recorded in H_2O under light/dark. No significant difference was recorded in KNO_3 medium under different light conditions. Under light/dark conditions, there was a significant difference in germination between H_2O (82.42%) and KNO_3 (86%). Similar observation was made under complete darkness. The best germination (99.17%) was recorded in KNO_3 medium under complete darkness (Table 2.4).

Table 2.4: Effect of light and germination treatments on germination of *E. indica* after 14-day incubation period

Treatment	Illumination	
	Light/dark	Dark
H_2O	82.42c	93.67b
KNO_3	96.00ab	99.17a
LSD = 4.18		

(Means within columns or rows followed by the same letter(s) are not significantly different from each other at p=0.05; ANOVA presented in Appendix A, Table A2)

2.4 Discussion

Weed seed germination is an important process regulated by various environmental factors (Chauhan and Johnson 2008). Reproduction in *E. indica* is mainly through seeds and the species is able to produce a high number of seeds with prolonged viability after dispersion (Takano et al. 2016). *Eleusine indica* seeds are able to germinate under various environmental conditions (Chauhan and Johnson 2008). In the present study, germination of *E. indica* was influenced by interactions between temperature and light. In this study, alternating temperature regimes had an effect on the final germination achieved. According to Nishimoto and McCarty (1997), alternating temperatures are an essential requirement for the successful germination of *E. indica*. It is also important to note that where germination is enhanced by alternating temperatures, none of the single constant temperatures involved in the alternation is solely responsible for the germination but the alternation itself (Masin et al. 2017).

Highest germination (>90%) was recorded under dark conditions after both seven and 14 days in the growth chamber. According to Nishimoto and McCarty (1997), light is not essential for germination of *E. indica*, however, under some conditions, it can stimulate germination. In the present study, seeds reached higher germination irrespective of light

conditions. In this study, light or the absence thereof had an effect in stimulating germination as significant differences occurred between light/dark and dark conditions. In both H_2O and KNO_3, as well as across all alternating temperature regimes, highest germination percentages were achieved under complete darkness. Previous studies suggest that the response of seed germination to light may vary within a species (Ismail et al. 2002, Ohadi et al. 2011). In the population used in the present study, the light response might have been influenced by the after-ripening of the seeds that occurred during the period of four months' storage before conducting the experiments. De Casas et al. (2012) describe after-ripening as a process whereby physiological dormancy decreases with time in seeds stored under dry conditions. Chauhan and Johnson (2008) suggest that seeds of *E. indica* lose sensitivity to light after three months of after-ripening. Kołodziejek and Patykowski (2015) also suggest that the exposure of seeds to light during counting may influence the effect of light on the germination. Even a short exposure of the seeds to light can break dormancy which subsequently affects seed germination (Singh and Singh 2009). Furthermore, the environmental conditions experienced by mother plants during its seed production stage and subsequent maturation of the seed also affect germination of the produced seeds (Bhatt et al. 2016, Penfield and MacGregor 2017). Therefore, all those factors identified in the above-mentioned studies might be true for the germination response of seeds tested in the present study. The seed germination response of the seeds tested in the present study cannot be linked to any one particular factor tested as all these factors were important determinants of seed germination.

In the present study, germination of *E. indica* seeds under different light and temperature conditions, in a medium containing KNO_3, significantly increased germination. Although KNO_3 is often used for stimulating seed germination and breaking dormancy, no clear explanation has been provided for its mechanism of action (Çetinbaş and Koyuncu 2006). According to Millaku et al. (2012), the effect of KNO_3 on germination was discovered when Knop's solution proved to be effective in stimulating germination of some plant species. According to Ali (2017), the response of seeds to KNO_3 is, however, influenced by the sensitivity of the seed. In the present study, the

highest final germination was recorded in KNO_3 under darkness. Similar observations were made by Kołodziejek and Patykowski (2015) in *Rumex confertus* which displayed improved germination by KNO_3 under darkness. Similarly, in *Potentilla norvegica*, KNO_3 treatment improved germination and resulted in reduced light requirements in its germination (Fawcett and Slife 1978). In contrast, Ali (2017) reported reduced germination of *Thymus transcaspicus* seeds by KNO_3. In the present study, the combination of alternating temperatures and KNO_3 also played a significant role in promoting germination of *E. indica* seeds. This is in agreement with Nishimoto and McCarty (1997) who observed that incubation of *E. indica* seeds in KNO_3 under alternating temperatures resulted in a germination rate greater that 90%.

The combination of temperature, light and KNO_3 is effective in promoting germination in various species (Millaku et al. 2012). However, the effect of these factors is reduced when involved solely (Thanos and Rundel 1995). In soils, temperature fluctuation is higher on the soil surface and decreases with increasing depth in the soil profile (Nishimoto and McCarty 1997). Furthermore, Singh and Singh (2009) suggest that the main environmental factor that influences germination in weed seeds is soil temperature. In addition, application of nitrogen fertilizer, especially by surface broadcast, results in availability of nitrogen to both crops and weeds (Blackshaw 2005). It is not known whether nitrogen application in the form of KNO_3 could be of practical relevance through stimulation of weed seed germination. A combination of these factor in areas with high amounts of *E. indica* seeds can result in high infestations, considering that germination of this weed, and grass species in general, is greater at or near the surface (Chauhan and Johnson 2008). Any conditions, including tillage operations that bring the seeds to the surface, would promote germination and subsequent infestations by *E. indica*.

2.5 Conclusions

The germination experiment of the present study shows that the *E. indica* population under investigation can germinate under various temperature and light conditions. Germination percentage of *E. indica* in this study was different between temperature

regimes and light conditions. This variation in germination is likely an essential contributor to survival of the weed under varying environmental conditions. This suggests that from early to late season until harvest, *E. indica* populations can continue to germinate and emerge resulting in high weed infestation throughout the crop-growing season. Because *E. indica* is a problematic weed with a high competitive ability, it is important to ensure that the crop field is kept as free as possible of such weeds throughout the season. Information on *E. indica* germination is therefore essential for applying effective control methods at the right time, i.e. at a growth stage during which the weed is most susceptible. For grass weeds in general the optimal timing of herbicide application is pre-emergence, therefore, it is important to have reliable knowledge on the environmental conditions under which seed germination is most likely to occur.

CHAPTER 3

Response of an *Eleusine indica* population to glyphosate herbicide

3.1 Introduction

Glyphosate being the most widely used herbicide globally, the response of various problematic weed species to the herbicide has been examined worldwide (Nandula et al. 2005). The first case of glyphosate resistance was reported in 1996 for a *Lolium rigidum* population in Australia. Resistance to glyphosate has been confirmed in 41 weed species around the world (Heap 2018). *Eleusine indica* is one of the most problematic grass weed species in the world, and the first occurrence of resistance was reported in this species in 1997 (Lee and Ngim 2000). As a result of this discovery, there has been increasing interest in the response of various *E. indica* populations occurring in fields where glyphosate has been the most used herbicide in various cropping systems. The number of reports on cases of glyphosate resistance in the species has steadily increased since it was first reported in 1997 (Heap 2018). The discovery that certain biotypes of *E. indica* are resistant to glyphosate in many parts of the world causes a serious threat to the continued successful management of this weed. This has been exacerbated by the discovery of multiple resistance in some *E. indica* biotypes (Heap 2018). Multiple resistance to more than one herbicide mechanism-of-action results in reduced herbicide options available for farmers to control problematic weed species (Molin et al. 2017).

The standard practice for investigating the response of a weed species to a herbicide involves the evaluation of the suspected resistant (R) biotype through a detailed dose-response experiment (Vargas et al. 2013). The premise is that dose significantly influences the efficiency of herbicides on weeds (Khan et al. 2007). This procedure involves exposing the weed population of interest to a number of herbicide doses, normally ranging from six to eight doses including an untreated control. The response of the treated plants is monitored based on visual assessment compared to the untreated

control plants. Resistance is confirmed when the suspected R biotype survives the doses that control the susceptible biotype (Beckie et al. 2000, Nandula 2016). Such pot experiments take about eight weeks to be completed and are thus time consuming and relatively expensive. Therefore, a quicker test for detecting herbicide response, the Petri dish assay, has been used by several researchers for glyphosate investigations of this kind. This involves germinating seeds of the species of interest in a Petri dish containing a solution with a known concentration of glyphosate. This method is a relatively affordable alternative which can be completed in a week (Perez and Kogan 2003, Neve et al. 2004, Perez-Jones et al. 2007, Ghanizadeh et al. 2015).

According to our knowledge, investigations on the response of *E. indica* to glyphosate in South Africa have never been conducted. The objective of this investigation was to determine the response of an *E. indica* population from the Hatfield experimental farm to glyphosate treatment. This was accomplished by means of a rapid *in vitro* experiment followed by a detailed dose-response pot experiment. It is hoped that methodology developed during this research could be used in more detailed work of this nature in future.

3.2 Materials and methods

3.2.1 Petri dish rapid test

To determine the response of *E. indica* to glyphosate, two rapid *in vitro* methods were tested in a growth chamber on Hatfield Experimental Farm. Un-germinated and pre-germinated seeds of the Hatfield *E. indica* population were assessed in these experiments.

3.2.1.1 Un-germinated seeds

Seeds were soaked in 0.2% KNO_3 for one hour to stimulate germination and thereafter placed on a paper towel to remove surface moisture. One disc of Whatman no. 1 filter paper was wrapped around rectangular pieces of glass to prevent the seeds from becoming immersed in the solution added to the Petri dish. Ten seeds were placed in the Petri dish on the filter paper and 6 ml of a glyphosate stock solution was applied to

each dish. Seeds were treated with commercial glyphosate formulation (potassium salt), Roundup Turbo®, at rates of 0 and the label recommended rate (900 g a.e. ha^{-1} or 2 L ha^{-1}). Control seeds (0 glyphosate) were treated with 6 ml of distilled H_2O. Each treatment was replicated eight times. The Petri dishes were sealed with parafilm and placed in a growth chamber at 35/25 °C with a 12 hr photoperiod. Root and shoot lengths were measured after seven days.

3.2.1.2 Pre-germinated seeds

Pre-germinated seed was treated in the same manner as un-germinated seed, undergoing surface sterilization before being transferred to Petri dishes containing filter paper wrapped over glass rod. Distilled H_2O (10 ml) was added to each Petri dish before being sealed with parafilm and placed in a growth incubator at 35/25 °C with a 12 hr photoperiod for five days. Once germinated, seeds were removed and placed in a Petri dish containing a disc of filter paper wrapped around a glass rod. Thereafter, the test was repeated in the same manner as for the un-germinated seeds.

3.2.2 Glasshouse dose-response experiment

To confirm the glyphosate response of the Hatfield *E. indica* population a dose-response experiment was conducted in a glasshouse on Hatfield experimental farm under controlled temperature conditions. Seeds were obtained and sterilized as described in the germination experiment. Seeds were planted by placing them on the surface of a sand-coir mixture contained in 12.5-cm-diameter plastic pots. According to Chauhan and Johnson (2008), seedling emergence of *E. indica* is sensitive to seed burial depth. Greatest emergence levels are achieved when seeds are on the soil surface (Chauhan and Johnson 2008). After emergence of seedlings, the growth medium was watered and fertilized with commercial Multifeed Classic fertilizer three times a week at a rate of 1 g/L of tap water, in order to prevent nutrient and water stress from developing. Plants were thinned to four plants per pot at 2 weeks after emergence. At the 4-6 leaf stage, plants were treated with a commercial glyphosate formulation (potassium salt), Roundup Turbo®, at doses of 0 (control), 0.25, 0.5, 1, 2 and 4 times the registered label recommended rate of 900 g a.e. ha^{-1}, or 2 L Roundup Turbo® ha^{-1}.

Control plants were not treated with the herbicide. Glyphosate was applied in a 2% ammonium sulfate (AMS) solution which is a water conditioner which helps facilitate glyphosate effectiveness in "hard" water.

Plants were watered the day before spraying to prevent washing off the herbicide if they were to be watered after spraying. The spraying was conducted in a closed room to avoid contamination of non-target plants. Plants were sprayed with a hand-held Oxford Precision sprayer equipped with RS-MM 110°/04 nozzles, operating at a pressure of 180 kPa, calibrated to deliver a total spray volume equivalent to 200 L per hectare. After spraying, the plants were returned to the glasshouse where watering and fertilization resumed 24 hours after treatment. Plants were visually evaluated for herbicide damage seven, 14 and 21 days after treatment. Plants were harvested 28 days after treatment. They were clipped at the soil surface, and weighed for biomass before oven-drying at 70 °C for 24 hours, followed by weighing to measure dry biomass per plant.

3.2.3 Statistical analysis

The experimental design was a completely randomized design with eight replicates per treatment. Data were subjected to analysis of variance using SAS software version 9.3 and mean separation was done with Tukey's Studentized Range least significant difference (LSD) test at p=0.05.

3.3 Results

3.3.1 Petri dish rapid test

3.3.1.1 Un-germinated seeds

Results obtained from seeds treated with the recommended dose of glyphosate showed a significant reduction in shoot (F=4171.78, p<.0001) and root (F=5567.90, p<.0001) length compared to the untreated control (Figure 3.1). Although germination was not measured, through visual observation, glyphosate had no effect on germination as all seeds germinated. However, the development of the seedlings in glyphosate solution

was significantly affected while normal seedling development occurred at the untreated control (Figure 3.2).
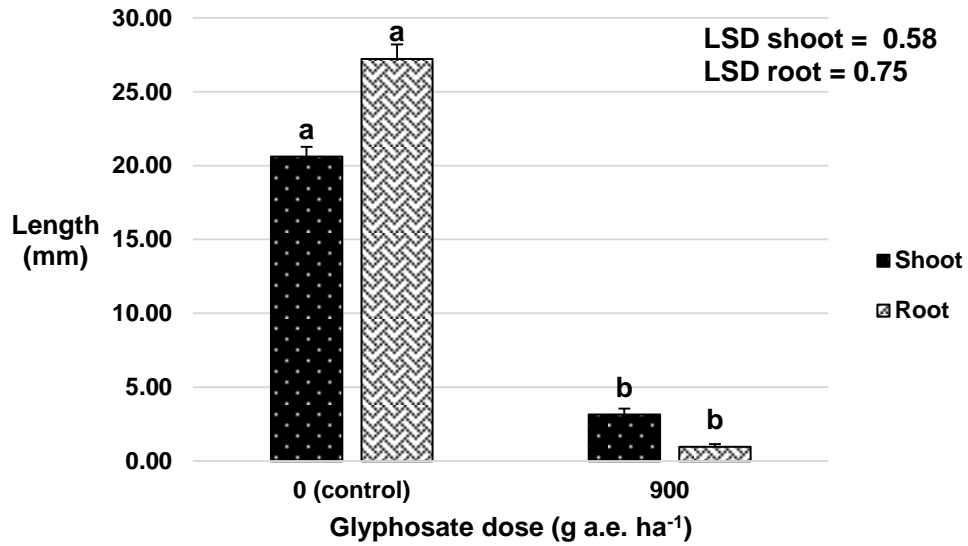
Figure 3.1: Shoot and root length development from un-germinated seeds of *E. indica* as affected by glyphosate at the label recommended rate for Roundup Turbo[®], i.e. 900 g a.e. ha^{-1} (Means with different letters are significantly different from each other at p=0.05; ANOVA presented in Appendix A, Tables A3 and A4)
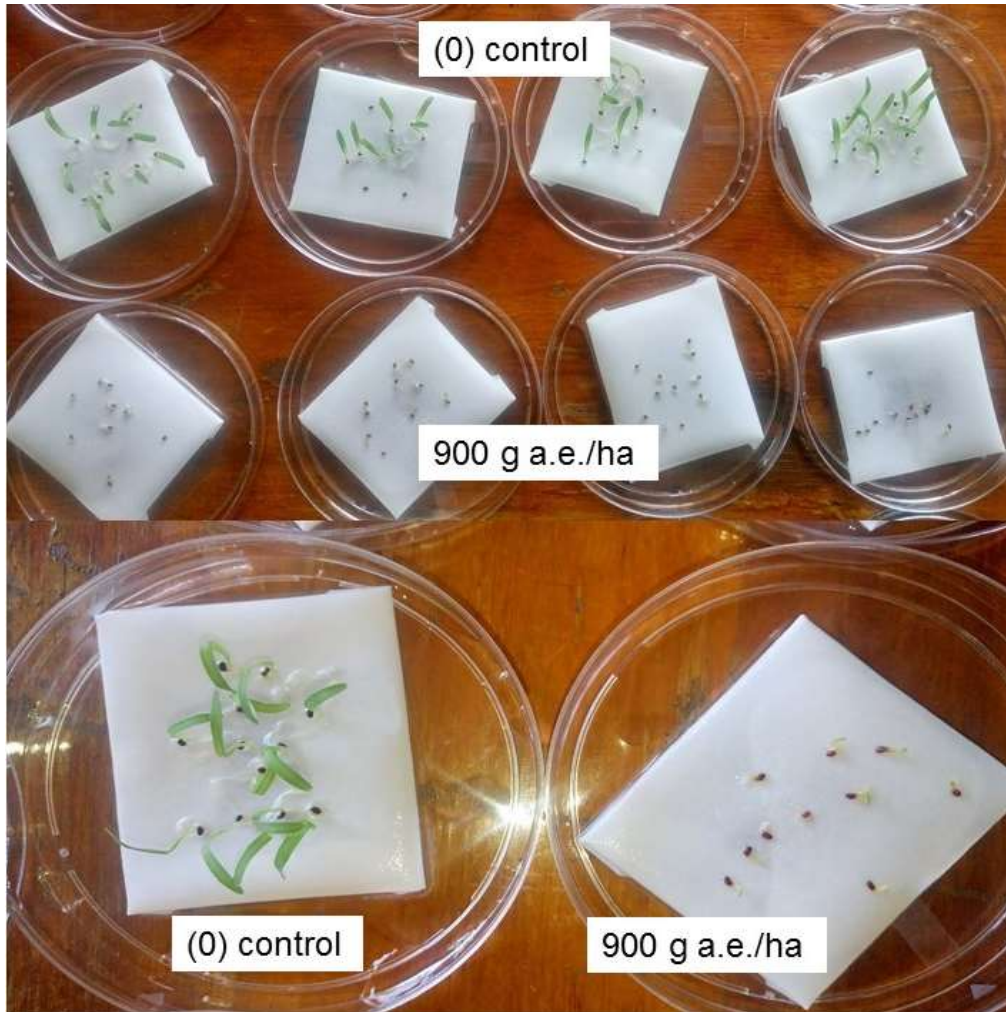
Figure 3.2: Seedlings from *E. indica* seeds germinated in a solution consisting of the label recommended dose of glyphosate, Roundup Turbo®, and the untreated control.

3.3.1.2 Pre-germinated seeds

The seedlings treated with glyphosate showed a significant inhibition in root development compared to the untreated control (F=1076.47, p<.0001). There was also a significant difference between the shoot length of the treated and untreated seedlings (F=12.06, p=0.0037). Further shoot development of the treated seedlings was inhibited by glyphosate treatment (Figure 3.3).
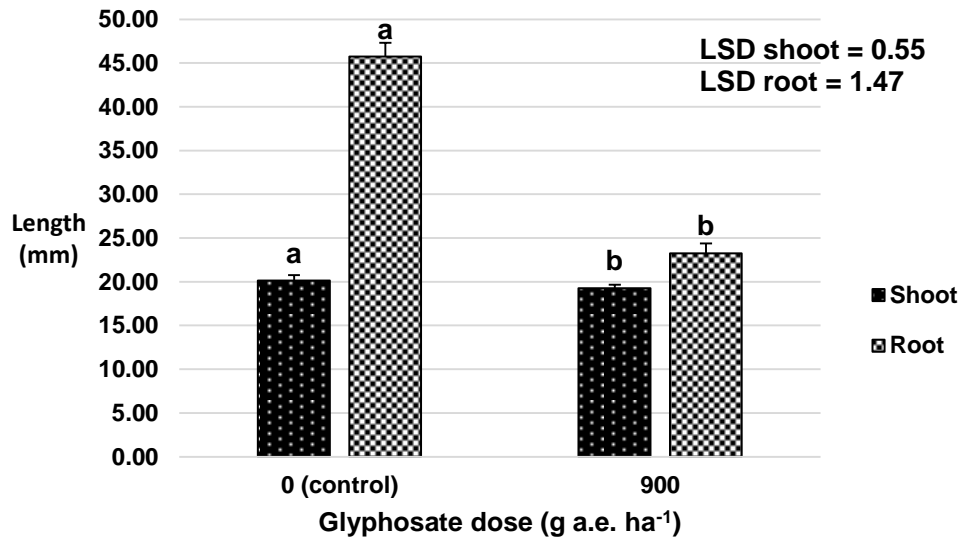
Figure 3.3: Shoot and root length development from pre-germinated seeds of *E. indica* as affected by glyphosate at the label recommended rate for Roundup Turbo[®], i.e. 900 g a.e. ha^{-1} (Means with different letters are significantly different from each other at p=0.05; ANOVA presented in Appendix A, Table A5 and A6)

3.3.2 Glasshouse dose-response experiment

3.3.2.1 Biomass of *E. indica* plants treated with different doses of glyphosate

The Hatfield *E. indica* population suffered significant reduction in biomass with increasing dose of glyphosate (F=116.72, p<.0001) (Figure 3.4). Biomass at 0.25x, 0.5x, 1x (label recommended dosage), 2x and 4x dosages was reduced by 21%, 46%, 79%, 92% and 96%, respectively. The lowest dose (0.25x) already reduced biomass significantly.

Symptoms of glyphosate damage were observed at seven DAT (Figure 3.5). Chlorosis was observed at the growth points of treated plants at all doses, and the intensity of the symptom increased with dose. The yellowing of the plants treated with 4x and 2x rates increased further between seven to 14 and 14 to 21 DAT, followed by wilting and dying of most plants. Plants treated with 1x were significantly different from 4x, 0.5x and 0.25x plants. Although plants treated with the 1x rate showed minor chlorotic lesions

compared to 4x and 2x rates at 21 DAT (Figure 3.7), they were stunted and displayed no potential for further growth and subsequent seed production. Plants treated with 0.5x and 0.25x rates showed minor chlorotic lesions compared to all other rates at 14 and 21 DAT (Figure 3.6 & 3.7).
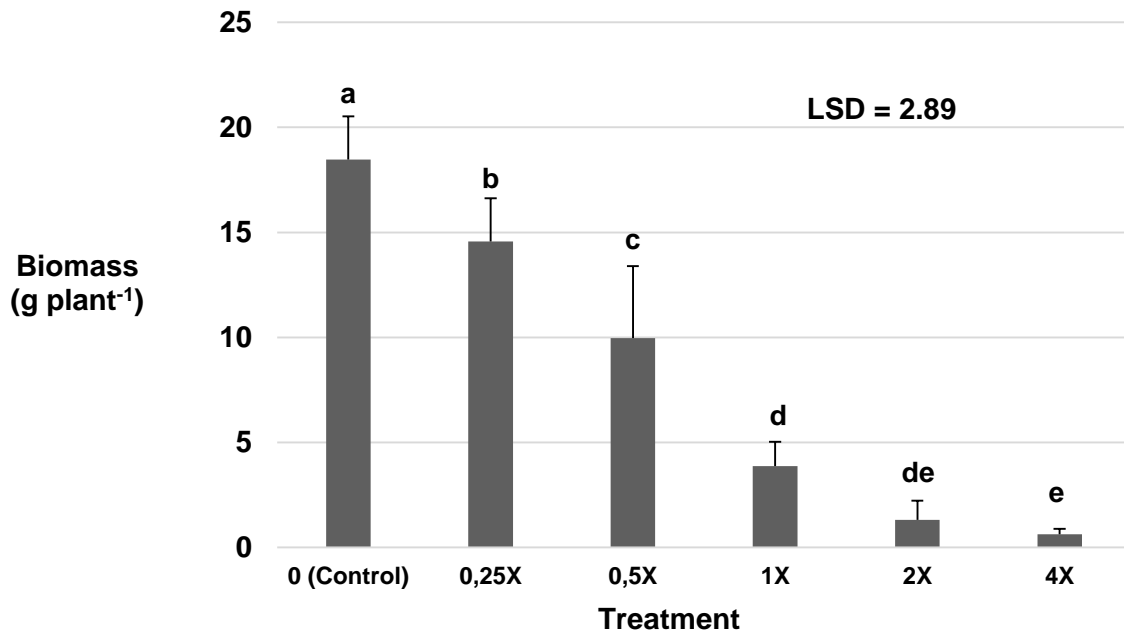
Figure 3.4: Biomass of *E. indica* plants treated with different doses of glyphosate. The "1x" rate for Roundup Turbo$^{®}$ is 900 g a.e. ha^{-1} (Means with different letters are significantly different from each other at p=0.05; ANOVA presented in Appendix A, Table A7).
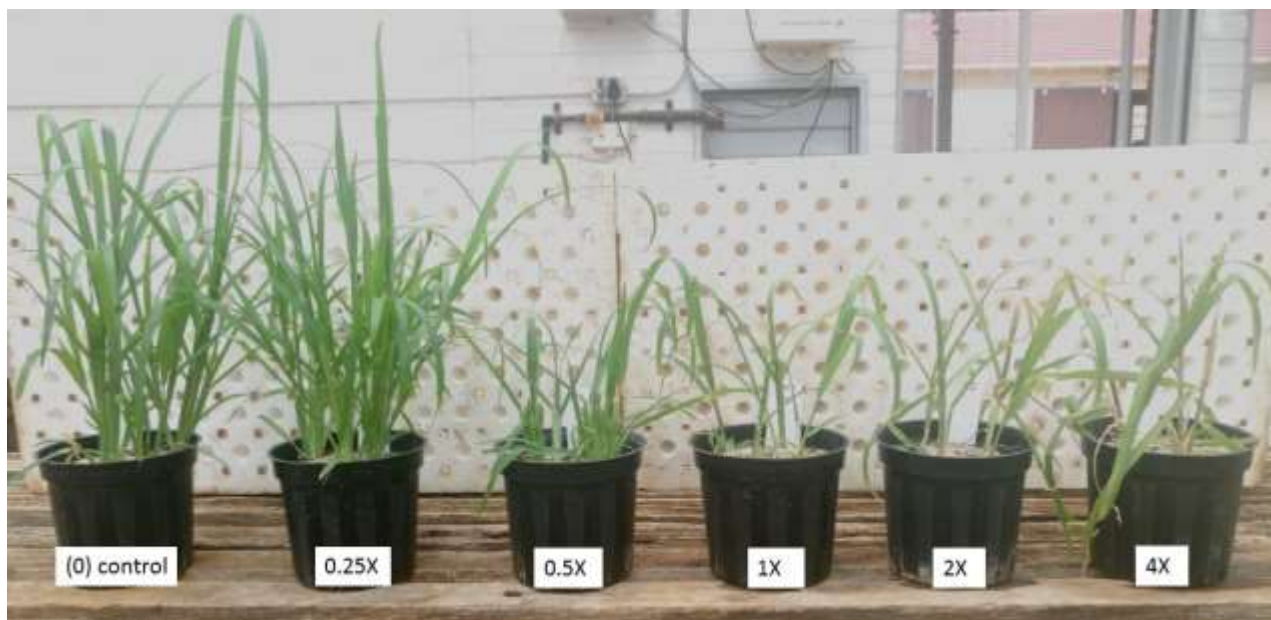
Figure 3.5: *Eleusine indica* plants treated with different doses of glyphosate at seven DAT. The "1x" rate is the label recommended rate for Roundup Turbo®, i.e. 900 g a.e. ha^{-1}.
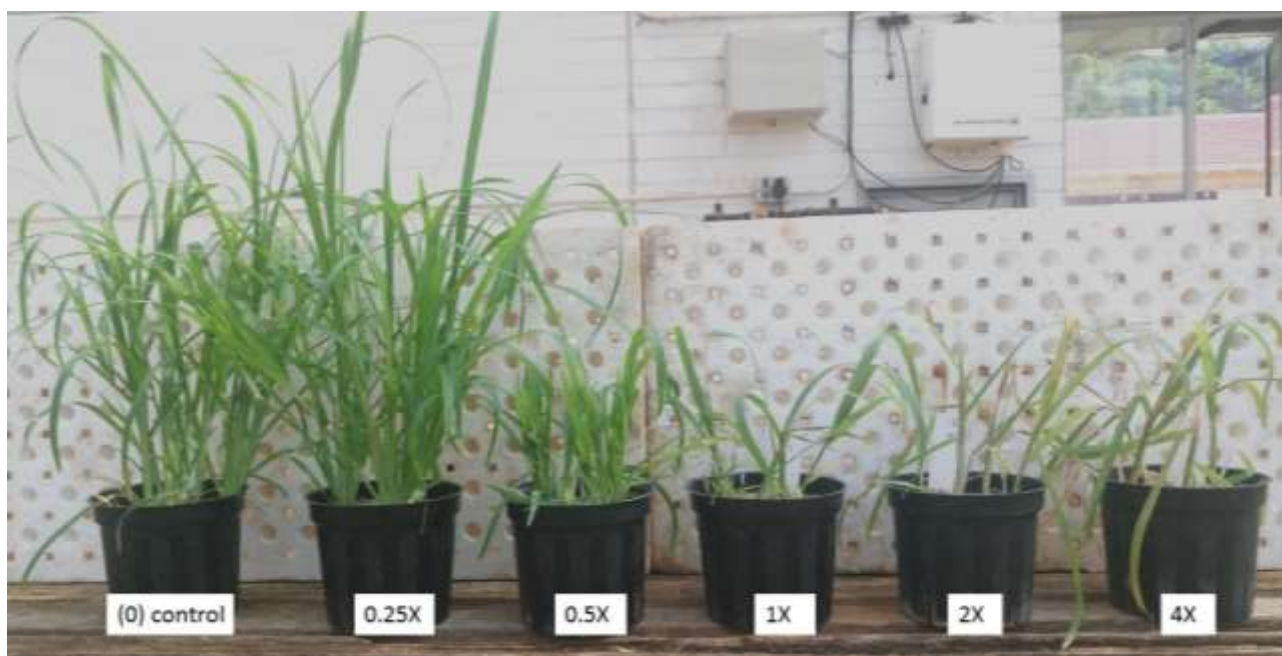
Figure 3.6: *Eleusine indica* plants treated with different doses of glyphosate at 14 DAT. The "1x" rate is the label recommended rate for Roundup Turbo®, i.e. 900 g a.e. ha^{-1}.
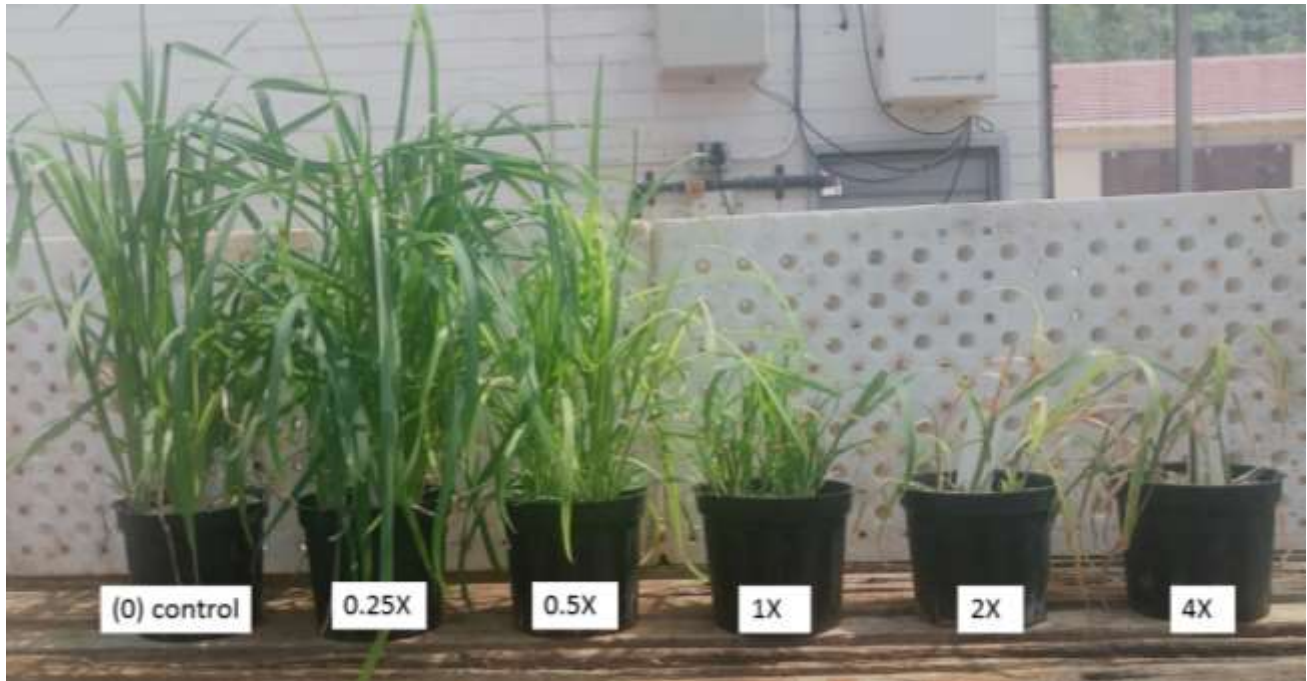
Figure 3.7: *Eleusine indica* plants treated with different doses of Roundup Turbo® (a.i. glyphosate) at 21 DAT. The "1x" rate is the label recommended rate for Roundup Turbo®, i.e. 900 g a.e. ha^{-1}.

3.3.2.1 Dry mass of *E. indica* treated with different doses of glyphosate

The dry mass of *E. indica* from Hatfield experimental farm was significantly reduced (F=106.67, p<.0001) by glyphosate treatment at all dosage rates compared to the untreated control (Figure 3.8). There was a 40% reduction in dry mass at the lowest dose, i.e. 0.25x. The reduction increased with the increase in the dosage rate of glyphosate. At 4x, which was the highest rate of the present study, the dry mass was reduced by 89%.
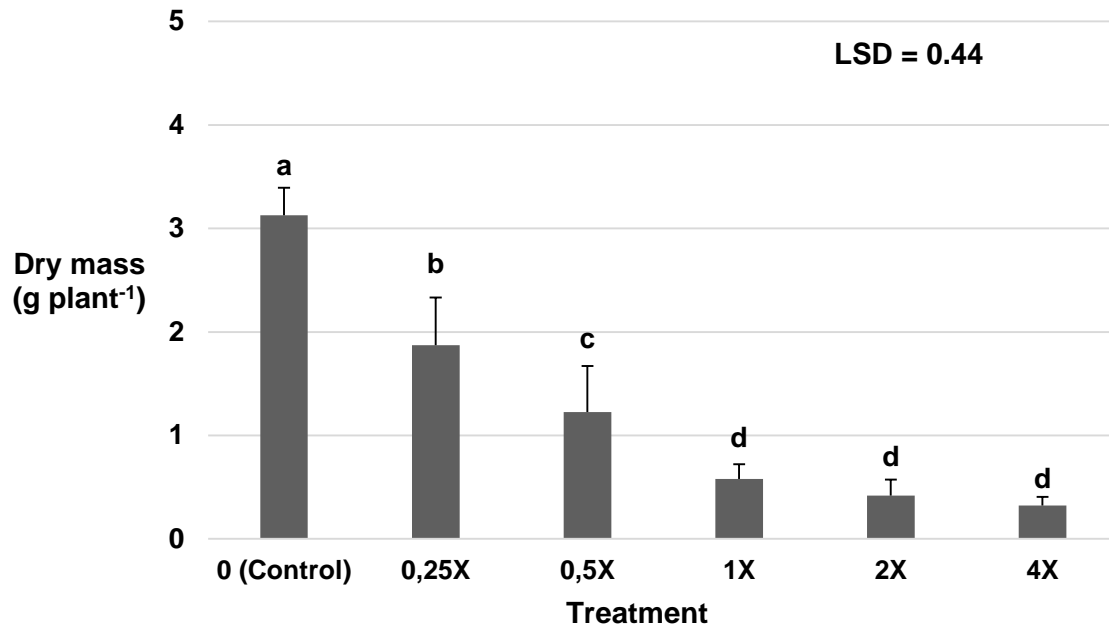
Figure 3.8: Dry mass of *E. indica* treated with different doses of glyphosate. The "1x" rate is the label recommended rate for Roundup Turbo[®], i.e. 900 g a.e. ha^{-1} (Means with different letters are significantly different from each other at p=0.05; ANOVA presented in Appendix A, Table A8)

3.4 Discussion

3.4.1 Petri dish rapid test

The shoot and root lengths of seedlings from both the un-germinated and pre-germinated seeds treated with glyphosate were significantly reduced compared to the untreated control. This is in agreement with previous studies where similar tests were conducted on *Lolium* species. Preston et al. (2015) conducted a study on known susceptible and resistant *L. rigidum* populations and found greater root length inhibition on the glyphosate-susceptible population relative to the glyphosate-resistant (GR) population. Perez and Kogan (2003) reported shoot length reduction after seeds of a putative GR *L. multiflorum* population were germinated in different glyphosate concentrations. Ghanizadeh et al. (2015) tested known susceptible and resistant populations of *L. multiflorum* and *L. perenne* and reported reduction in both shoot and

root length in both populations, i.e. susceptible and resistant populations. However, the glyphosate dose required for reducing the growth of both shoot and root growth was higher for the resistant populations compared to the susceptible population. Furthermore, they found the root and shoot growth responded differently to glyphosate, with root growth being relatively more sensitive. Shoot growth required higher doses for growth reduction that is similar to that observed on roots (Ghanizadeh et al. 2015).

Prior to the present study on the response of *E. indica* to glyphosate, no similar study had been conducted, according to the best of our knowledge. Although the experiment reported here investigated the effect of only the label recommended dose of Roundup Turbo®, the results are in agreement with previous experiments (Perez and Kogan 2003, Ghanizadeh et al. 2015, Preston et al. 2015) where shoot and root growth were significantly affected by glyphosate treatment on seeds of susceptible species. Therefore, the results of the present study reveal that the studied population is susceptible to glyphosate.

3.4.2 Dose-response experiment

The fresh and dry biomass of *E. indica* were significantly reduced by all doses of glyphosate compared to the untreated control. Heap (2005) states that under normal field conditions a population is classified as resistant if it survives the herbicide's recommended dose. In this study, the population was significantly affected by glyphosate at all tested doses. Plants exposed to the 0.5x and 0.25x doses showed less signs of chlorosis at harvest, however, information on their recovery is not available as ability to recover was not investigated in this study. At the recommended dose no signs of survival were displayed by the treated plants as plants were stunted and showed no signs of healthy growth. This confirms susceptibility of the population to glyphosate.

The dose-response method as employed in this study was successful in confirming the results obtained in the *in vitro* study. Both methods demonstrated sensitivity of the population to glyphosate. The Petri dish experiment proved to be a relatively quick and easy method to determine herbicide response in the present study. In practice, based

on problems reported in the field, a quick result may be required for information on the response of a suspected GR weed species, in order to plan alternative control methods where resistance is discovered. In the standard protocol for herbicide resistance evaluation under controlled conditions, which takes about eight weeks or longer to complete, results may come too late for the farmer who has to manage weeds timeously. With the quick Petri dish test, however, results can be obtained within a week after seed collection, thus allowing enough time for taking the necessary action based on guidance received from investigation results. However, as Preston et al. (2015) state, the traditional glasshouse pot experiment method provides a more accurate measurement for weeds' response to herbicides. According to Perez and Kogan (2003), the dose-response experiment better simulates field conditions, because growth stage of the treated plants and the applied herbicide rates are similar to those involved under natural field conditions. This gives emphasis to the use of both tests in tandem.

3.5 Conclusions

Both the Petri dish and glasshouse dose-response experiments were successful in determining the response of the particular *E. indica* population to glyphosate. In the Petri dish trial, the shoots and roots of the seedlings were reduced as a result of exposure to glyphosate. However, the Petri dish experiment carried out in the present Plants in the pot trial conducted in a glasshouse showed high susceptibility, irrespective of glyphosate dosage. Therefore, both methods followed in this study prove that this particular *E. indica* population is sensitive to glyphosate. Findings support those made by other researchers with regard to the suitability of both methods for research of this nature.

CHAPTER 4

Influence of growth stage on the response of an *Eleusine indica* population to glyphosate

4.1 Introduction

Weed growth stage is an important factor in the application timing of herbicides. The growth stage at which a weed species is treated with herbicide plays a critical role in its response to the applied herbicide (Nandula 2010, Qi et al. 2017). The uptake and metabolism of the herbicide is influenced by growth stage which may have an effect on the efficacy of the herbicide (Chauhan and Abugho 2012). According to Khan et al. (2011), the best time for herbicide application is when weeds are at the most susceptible growth stage, coupled with the most tolerant growth stage of the crop.

Generally, weeds become less susceptible to herbicides as they increase in age/size (Faccini and Puricelli 2007). Reports have been made on various weeds and crop species showing increased tolerance to herbicides as they increase in age/size (Khan et al. 2011, Chauhan and Abugho 2012). According to Chauhan and Abugho (2012), herbicide degradation is more rapid in bigger plants which reduces the effectiveness of the herbicide. To achieve the same level of control in some weed species, the herbicide doses applied to bigger plants may have to be increased compared to the rates applied to smaller plants (Chauhan and Abugho 2012). Contact herbicides are more affected by growth stage compared to systemic herbicides (Kudsk 2014).

Information on the efficacy of herbicides on a specific weed species or population at different growth stages is essential for determining growth stages at which the weed is mostly vulnerable to the herbicide (Faccini and Puricelli 2007). According to Takano et al. (2016), effective control of *E. indica* with glyphosate is inversely proportional to its growth stage. Considering that *E. indica* is a problematic weed species worldwide, information on the ideal growth stage for its control is essential, especially in farming

situations. The objective of the present study was to investigate the influence of growth stage on glyphosate response of a South African *E. indica* population.

4.2 Materials and methods

4.2.1 Growth response

To determine the influence of growth stage on a glyphosate-sensitive *E. indica* population an experiment was conducted on the previously screened (refer Chapter 3) Hatfield population. Seeds were planted in two different black plastic pot sizes, 12.5 and 16-cm-diameter. The pots were filled with a potting mix consisting of sand and coir (50:50) and seeds were placed on the surface. After emergence, plants were thinned to two plants per pot and the medium was watered and fertilized with commercial Multifeed Classic fertilizer three times a week at a rate of 1 g/L of tap water, in order to prevent nutrient and water stress from developing. Plants were treated at four different growth stages: 21 DAE (plant height = 160 mm); 35 DAE (plant height = 290 mm); 49 DAE (plant height = 370 mm); and 63 DAE (plant height = 540 mm). Plants treated at 21, 35 and 49 DAE were planted in 12.5-cm-diameter pots, 28, 42 and 56 days before treatment, respectively, and plants treated at 63 DAE were planted in 16-cm-diameter pots 70 days before treatment. The treatment consisted of the commercial glyphosate formulation (potassium salt), Roundup Turbo®, at field recommended dose of 900 g a.e. ha^{-1}, or 2 L Roundup Turbo® ha^{-1}. Glyphosate was applied in a solution of 2% granular ammonium sulphate (VELOCITY-DRYMAX, Villa Crop Protection) and 0.1% isotridecanol (alkylpolyethylene glycol ether) (VILLA 51, Villa Crop Protection) added as surfactants. Ammonium suphate is a water conditioner for improving glyphosate effectiveness in "hard" water, and isotridecanol is a surfactant that increases the wetting and spreading properties of the spray solution. Control treatments, where zero herbicide was applied, were maintained for comparisons for each growth stage.

Treatments were applied using a hand-held Oxford Precision sprayer equipped with RS-MM 110°/04 nozzles, operating at a pressure of 180 kPa, calibrated to deliver a total spray volume equivalent to 200 L per hectare. Plants were watered the day before spraying to prevent washing off the herbicide if watered during the day of spraying. The

standard watering and fertilization procedure resumed 24 hours after treatment. Spraying was conducted in an enclosed room and plants were returned to the glasshouse after spraying. Plants were visually evaluated for herbicide damage 21 days after treatment. At 28 days after treatment all the plants were harvested. They were clipped at the soil surface and weighed for biomass measurement before oven-drying at 70 °C for 24 hours, and afterwards weighed for dry biomass.

4.2.2 Statistical analysis

The experimental design was a completely randomized design with growth stage at four levels and glyphosate dose at two levels, with treatment combinations repeated six times. Data were subjected to analysis of variance using SAS software version 9.3 and mean separation was done with Tukey's Studentized Range least significant difference (LSD) test at $p=0.05$.

4.3 Results

4.3.1 Biomass of *E. indica* treated with glyphosate at different growth stages

Irrespective of the growth stage, the biomass of the plants treated with glyphosate was significantly different (F=5.50, p=0.0029) from their untreated control (Figure 4.1). At all growth stages, glyphosate applied at the recommended dose for Roundup Turbo[®], i.e. 900 g a.e. ha^{-1}, significantly reduced the biomass of *E. indica*. However, growth stage had an effect on the reduction of biomass across growth stages. At 21, 35, 49 and 63 DAE the biomass was reduced by 95, 90, 87 and 56%. Therefore, growth stage had an influence on the response of the population to glyphosate.
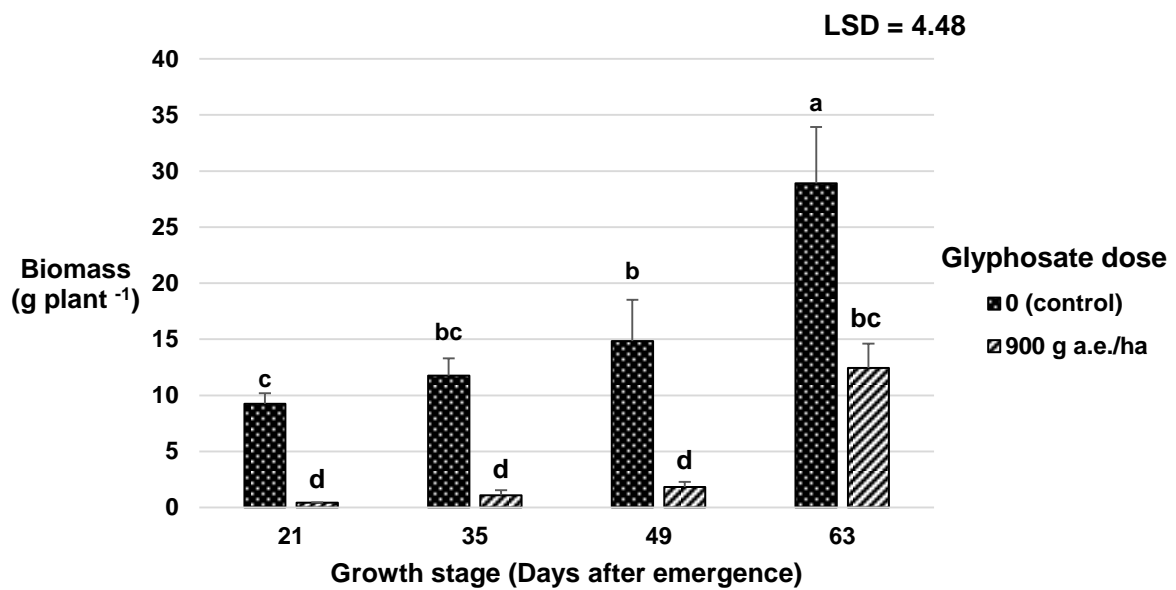
Figure 4.1: Biomass of *E. indica* plants treated with the recommended dose of glyphosate at different growth stages. (Means with different letters are significantly different from each other at p=0.05; ANOVA presented in Appendix A, Table A9).

4.3.2 Dry mass of *E. indica* treated with glyphosate at different growth stages

The dry mass of *E. indica* was significantly reduced (F=5.48, p=0.0030) by glyphosate treatment compared to untreated control plants, irrespective of growth stage (Figure 4.2).
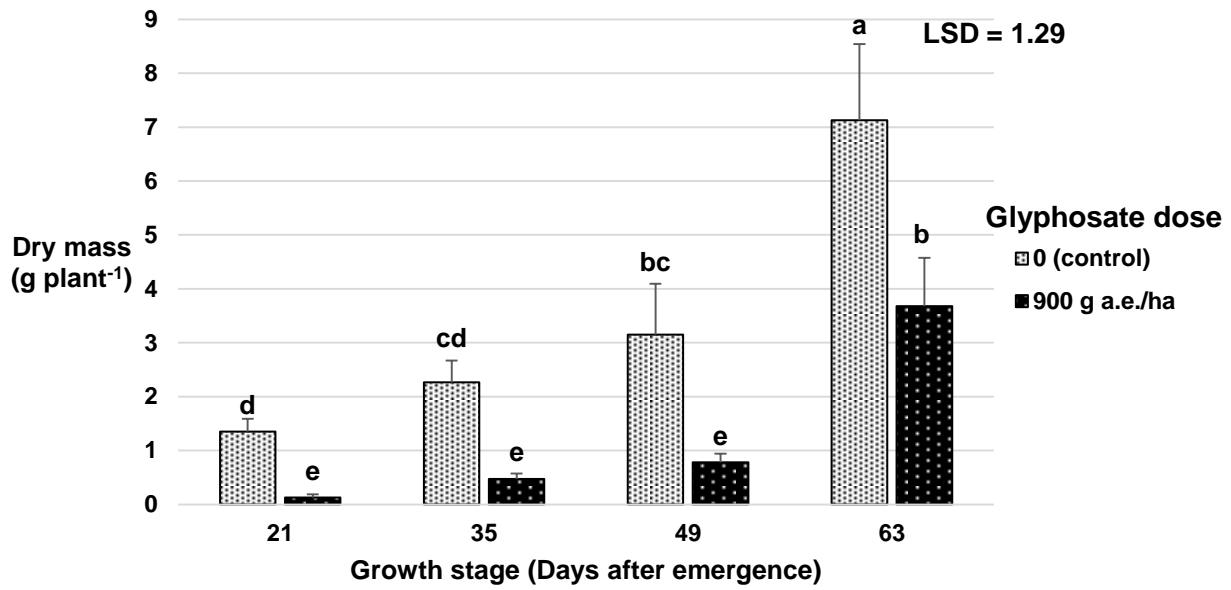
Figure 4.2: Dry mass of *E. indica* plants treated with glyphosate at different growth stages (Means with different letters are significantly different from each other at p=0.05; ANOVA presented in Appendix A, Table A10)

Although there is a significant difference between the treated and the untreated plants at all growth stages, by visual observation, plants treated at 63 DAE appear to be less affected by glyphosate treatment compared to other growth stages (Figure 4.3, 4.4, 4.5 & 4.6). The *E. indica* population appeared to be most susceptible to glyphosate when treated at 21 DAE as only little green material was left on these plants at three weeks after treatment. The oldest plants, which were treated 63 DAE, visibly recovered and continued to grow and eventually reached the tasseling stage and produced seeds (Figure 4.6).

Figure 4.3: Glasshouse experiment showing response of *E. indica* plants 21 days after treatment with glyphosate at 21 DAE

Figure 4.4: Glasshouse experiment showing response of *E. indica* plants 21 days after treatment with glyphosate at 35 DAE

Figure 4.5: Glasshouse experiment showing the response of *E. indica* plants 21 days after treatment with glyphosate at 49 DAE

Figure 4.6: Glasshouse experiment showing the response of *E. indica* plants 21 days after treatment with glyphosate at 63 DAE

4.4 Discussion

As demonstrated by the results of the present study, the *E. indica* population studied was more sensitive to glyphosate treatment at the youngest growth stages compared to treatment of older plants. This is in agreement with various studies that suggest that herbicide efficacy is reduced with increasing weed growth stage. According to other studies conducted to assess the influence of the weed growth stage on glyphosate response, early applications of this herbicide result in improved weed control. Soltani et al. (2016) reported 90% control on 100 to 200 mm tall *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Chenopodium album*, and *Echinochloa crus-galli* biotypes treated with 900 g a.e. ha^{-1} of glyphosate. However, to achieve a similar control rate for bigger plants treated at 300 mm height, a glyphosate dose greater than 900 g a.e. ha^{-1} was required. Similarly, Chauhan and Abugho (2012) reported effective control of *Echinochloa crus-galli, Echinochloa colona,* and *Digitaria sanguinalis* when herbicides were applied during the early growth stage. In the present study, plants treated at 63 DAE were already close to the reproductive growth stage at the time of treatment and these plants displayed relatively poor control by glyphosate as they continued to grow and produce seed. Faccini and Puricelli (2007) suggest that for effective control of weeds at the reproductive stage the herbicide dose might need to be increased to above the normal (registered) recommended dose for that specific herbicide. Cautionary note: In South Africa, according to Act 36, it is unlawful to recommend/use herbicides at dosages that are either lower or higher than the recommended dosage appearing on herbicide product labels. In the present study, only the recommended dose effect was assessed, therefore, no information is available for increased glyphosate doses on older plants of the *E. indica* population under investigation.

In the present study, plants treated at 21, 35 and 49 DAE showed relatively greater susceptibility to the herbicide. At these growth stages plants were ranging from seedling to vegetative growth stages. Glyphosate is a systemic herbicide with movement (translocation) in the plant system that resembles photo-assimilate translocation. According to Futch and Sellers (2016), the movement of systemic herbicides within the plant is rapid. At seedling and vegetative growth stages plant growth is rapid, which aids

the absorption and translocation, and hence, the efficacy of the systemic herbicide applied at these growth stages. On the other hand, plant growth during the reproductive stage is reduced, resulting in slower movement of assimilates and herbicides alike, thus resulting in reduced herbicide efficacy (Futch and Sellers 2016). The growth cycle of *E. indica* is relatively rapid (Takano et al. 2016), and this natural ability might have boosted the effectiveness of glyphosate at the younger growth stages compared to lesser effects observed on the older plants in the present study.

Weed growth stage at time of spraying is influenced by several factors, including environmental factors that determine the time of weed seed germination and emergence, as well as weed growth rate (Hammerton 1967). In rainy situations, growers may not be able to apply post-emergence herbicides at the best time, resulting in weeds having more time to develop and get bigger. By the time of treatment, the weeds might be more tolerant to the herbicide resulting in reduced control with the herbicide. On the other hand, farmers might allow weeds to grow with the intention of giving time for new weeds to emerge, aiming at achieving control of all weeds in the field with just a single application. If such a scenario is allowed to develop in the case of glyphosate, weeds on a particular field will be at different growth stages and thus unlikely to be controlled equally effectively due to variation in growth stage. This kind of practice can be a problem in achieving good control as demonstrated by the results of the present study, which are supported by Hammerton (1967) who found that susceptibility of annual weeds to herbicides decreases with increasing age. Therefore, timing of glyphosate application is essential even where weeds are susceptible to the herbicide, as found in the present study.

Although glyphosate application on older plants may not be effective in achieving the desired result, which is totally killing the target species, several studies suggest that, late growth stage glyphosate application on weeds can be good for reducing future weed infestations (Clay and Griffin 2000, Hill et al. 2016). According to Clay and Griffin (2000), glyphosate applied on *Xanthium strumarium, Sesbania exaltata,* and *Senna obtusifolia* at seed set reduces seed production and subsequent seedling emergence.

Similarly, Shuma et al. (1995) established that treatment of *Avena fatua* with glyphosate at or near flowering inhibits seed development and reduces seed germination. In contrast, Nurse et al. (2015) reported that glyphosate applied to *Eriochloa villosa* post-anthesis has no effect on seed production. However, the viability of the treated seeds was affected due to the effect of the treatment on embryo and endosperm development (Nurse et al. 2015). The observations made in these previous studies suggest that glyphosate applied to weeds during the reproductive growth stage has an effect on both the quantity and quality of the seeds produced by the treated weeds. This implies that late season herbicide application does not only improve harvesting efficiency and crop quality but also future season weed infestations by reducing seeds in the seed bank as well as the quality of the produced seeds. In the present study, plants treated at 63 DAE had produced seeds at the time of harvest, however, no investigation was conducted on the quantity and viability of the produced seeds.

4.5 Conclusions

Weed growth stage was confirmed to be important in the timing of glyphosate application. Younger *E. indica* plants were more susceptible than older plants. In the dose-response experiment conducted in this study (refer Chapter 3), this population showed susceptibility to glyphosate even at the lowest tested doses. This proved that the population is highly susceptible to glyphosate. However, the response of the sensitive population to glyphosate at the recommended dose was affected by growth stage. This suggests that, even in cases where weeds are sensitive to the herbicide, growth stage should always be considered and weed control should be done at the ideal growth stage for attaining effective control.

Chapter 5
General discussion and conclusions

Literature on germination and glyphosate response of *Eleusine indica* in South Africa is lacking. Such information is essential for maximizing the effectiveness of glyphosate as part of control practices employed where the weed is problematic. This study was, therefore, aimed at determining how temperature and light influence seed germination of an *E. indica* population, and to investigate its response to glyphosate herbicide.

Seed germination in grass weeds is an essential process in the establishment of the weed, and is regulated by various factors (Hugo et al. 2014). Knowledge on factors influencing weed seed germination is essential for the improvement of weed control methods for that particular weed species (Lim et al. 2015). Investigations on the germination characteristics of *E. indica* were conducted in a temperature and light controlled growth chamber. Germination of the *E. indica* population investigated in this study shows similarities to several other *E. indica* populations previously examined in various studies (Ismail et al. 2002, Chauhan and Johnson 2008). The results from the germination experiment suggest that *E. indica* can germinate under a wide range of temperature and light regimes. The population examined in the present study displayed good response to alternating temperatures resulting in high germination at both low and high regimes. This is in agreement with several studies conducted on the germination characteristics of various *E. indica* biotypes under both constant and alternating temperature conditions, where higher maximum germination was reported under alternating temperature conditions (Ismail et al. 2002, Chauhan and Johnson 2008). The present study did not investigate germination under constant temperatures, therefore, no information is available on the germination of this population under constant temperatures. However, high germination percentages (>90%) were recorded at all tested alternating temperatures regimes, thus indicating that alternating temperatures are essential for germination of *E. indica*.

Germination in the present study was not negatively affected by the absence of light. The examined *E. indica* population was able to germinate in the absence of light, provided moisture and temperature were adequate. Chauhan and Johnson (2008) made similar observations and concluded that light had no influence on *E. indica* germination. The ability of *E. indica* to germinate under different light conditions combined with its adaptation to varying temperature regimes is an essential characteristic for its survival under various climatic conditions. Considering that *E. indica* produces copious amounts of seeds, 140,000 seeds per plant (Jalaludin et al. 2010), and that it has a rapid development cycle relative to other species (Takano et al. 2016), probabilities for it to establish and reproduce are relatively high. Therefore, information on its seed germination characteristics is critical for a better understanding of the biology of the studied population, and furthermore, for its effective control. Reports on the light and temperature germination responses of *E. indica* varies within the species (Chauhan and Johnson 2008). Therefore, studying various individual populations is essential to obtain information on the true characteristics of the species.

The Petri dish and dose-response experiment conducted in the present study were successful in revealing the response of the tested population to glyphosate. The Petri dish experiment conducted proved a relatively quick, simple and less expensive method compared to the dose-response experiment procedure. The roots and shoots of both the un-germinated and the pre-germinated seeds in the quick test were significantly reduced by glyphosate treatment compared to their untreated controls. The dose-response experiment showed sensitivity of the tested population at all glyphosate treatment rates. This plant response was demonstrated by the significant reduction of biomass of treated plants compared to the untreated plants. Therefore, the outcomes initially provided by the quick Petri dish study were supported by the dose-response experiment. This proves that the Petri dish experiment can be used as a quick alternative to the more resource requiring, time consuming and detailed dose-response experiment. However, Perez-Jones et al. (2007) state that the Petri dish method cannot be used as a replacement for the dose-response (pot experiment) method. A detailed

dose-response experiment should always follow the quick test as it best resembles field conditions (Perez and Kogan 2003, Perez-Jones et al. 2007).

In the present study, the response of the *E. indica* population to glyphosate was affected by growth stage. This is in agreement with several other studies that report the importance of application timing for effective herbicide control. Those studies suggest early herbicide application as the ideal time, whereas delayed application results in reduced control (Chauhan and Abugho 2012, Soltani et al. 2016). In the present study, best control was achieved in the younger plants while older plants showed recovery and grew to attain the reproductive stage. This suggests that even for glyphosate-sensitive species, application should be timed for the ideal growth stage in order to achieve best results. In practice, such an aim will be thwarted by various growth stages of a particular weed occurring on the same field. Such scenarios often characterize zero-tillage and reduced tillage cropping systems because mechanical weed control, which is an excellent tool for "equalizing" growth stage differences, is not an option.

According to Coble and Schroeder (2016), there is a general decrease in weed susceptibility to herbicides that is promoted by reliance on herbicides as a single solution to weed problems. Continued reliance on a particular herbicide despite decreasing efficacy can only exacerbate the situation, and in fact, result in the evolvement of herbicide resistance (Pieterse 2010). In the present study, the tested *E. indica* population was found to be highly sensitive to glyphosate. This is confirmed by the *in vitro* test, dose-response and growth stage response experiments. However, the discovery that the weed is sensitive to the herbicide does not mean there should be less caution on the use of the herbicide. Duke (2018) predicts that, in the near future, the use of glyphosate might be limited by the evolution of glyphosate-resistant weeds. Therefore, precautionary measures for avoiding the occurrence of this problem are essential.

In the present study, only one population of *E. indica* was examined in the experiments. Even though a single population was used, the study was successful in developing a

protocol and showing the way forward for future studies on *E. indica* in South Africa. Most studies on glyphosate response use the traditional dose-response method. The *in vitro* test used in the present study can be replicated in future studies for quick results on glyphosate response. However, one must take into consideration and carefully select the rates of glyphosate used in the Petri dish experiment. Considering the fact that under normal field conditions, due to certain conditions including environmental conditions, not all active ingredient present in the spray solution reaches the target.

According to Khan et al. (2011), the susceptibility of weeds to herbicides vary even within weed species. Therefore, further research on glyphosate response of various *E. indica* populations from different geographic locations and cropping situations across South Africa would provide better insight on glyphosate response outcomes. Future studies on the effect of glyphosate on the viability of seeds produced by *E. indica* plants treated at, or close to, the reproductive stage can also be valuable by providing alternative methods for controlling this serious grass weed. Discovering that late glyphosate treatment results in unviable seed production may provide new approaches for weed control should this approach prove to be practical.

SUMMARY

Eleusine indica is regarded as one of the world's worst weeds. It is a major competitor in a wide variety of ecosystems worldwide. In agro-ecosystems it infests a wide range of crops including fruit orchards. *Eleusine indica* causes a major reduction in both crop quality and quantity in agro-ecosystems. Furthermore, *E. indica* is a secondary host to diseases, nematodes, and viruses that affect various crops including maize. Glyphosate, described as a "once in a century" herbicide, is a systemic, non-selective herbicide that is used for controlling annual and perennial weed species as well as volunteer crops under various situations in both crop and non-crop lands. It has become the most historically successful herbicide throughout the world. In South Africa, glyphosate is the most popular and widely used herbicide. It is among the leading products used to control weeds and invading alien plant species in the country. In 1996, rigid ryegrass (*Lolium rigidum*) was the first weed species for which a case of a glyphosate resistance was reported. This was as a result of intensive use of glyphosate for weed control. Since then, several cases of resistance to the herbicide have been reported around the world.

The first case of glyphosate resistance in *E. indica* was reported in Malaysia in 1997, making it the second weed found to be GR worldwide. Since then, glyphosate resistance in *E. indica* has been reported in several other countries under various cropping situations. As a result of this, there has been increasing interest in the response of various *E. indica* populations occurring in fields where glyphosate has been the most used herbicide in various cropping systems. In South Africa there is a lack of information on the response to glyphosate in *E. indica* species.

In the present study, initial investigations were aimed at the germination of *E. indica* as affected by temperature and light. The experiments were conducted in Petri dishes in a growth chamber for 14 days at three alternating day/night temperature regimes of 20/10, 30/20 and 35/25 °C (day/night). Germinated seeds were counted and removed from the Petri dishes at seven and 14 days. Data were subjected to analysis of variance

(ANOVA) using SAS software version 9.3. A completely randomized design was used in all experiments. Mean separation was done with Tukey's Studentized Range least significant difference (LSD) test. The investigated *E. indica* population was able to reach high germination rates under all tested temperature and light conditions. The results of the germination study suggest that the *E. indica* population examined in the present study is able to germinate under a wide range of temperature and light conditions.

To determine the response of the *E. indica* population to glyphosate, growth chamber and glasshouse experiments were conducted. Firstly, in the growth chamber seeds of *E. indica* were germinated in a glyphosate stock solution (Roundup Turbo[®], label recommended rate 900 g a.e. ha^{-1} or 2 L ha^{-1}) in Petri dishes. Control seeds were treated with distilled water. Root and shoot length development was determined after five days. This was followed by glasshouse dose-response experiments. Seeds were planted in plastic pots and plants were treated with glyphosate at the 4-6 leaf stage with Roundup Turbo[®] at doses of 0 (control), 0.25, 0.5, 1, 2 and 4 times the registered label recommended rate of 900 g a.e. ha^{-1}, or 2 L ha^{-1}. Secondly, a growth response experiment was conducted to determine the effect of growth stage on the response of the *E. indica* population to glyphosate. Plants were treated with glyphosate, (Roundup Turbo[®]) at field recommended dose, at four different growth stages. Control plants were maintained for each growth stage. Biomass and dry mass were measured 28 days after treatment in both experiments. Data were subjected to analysis of variance (ANOVA) using SAS software version 9.3. A completely randomized design was used in all experiments. Mean separation was done with Tukey's Studentized Range least significant difference (LSD) test.

The results of the above mentioned studies reveal that the *E. indica* population is sensitive to glyphosate. Glyphosate reduced shoot and root development of the treated seeds and the biomass of the treated plants was also significantly reduced by the herbicide. However, despite the sensitivity of the population to glyphosate, growth stage had an effect on the response of the population to the herbicide. Older plants treated with glyphosate continued to grow and reach reproductive stage. This suggests that

even where weeds are sensitive to glyphosate, growth stage should be considered for effective weed control. The results reported in the present study are based on a single population. Further research is required for populations from different location across South Africa for better insight on the weed's biology and sensitivity to glyphosate in the country.

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APPENDIX A: STATISTICAL ANALYSIS

Chapter 2: Germination of *Eleusine indica* as affected by temperature and light

Table A1: ANOVA table for effect of temperature and light on germination of E. indica seeds for 7-day incubation period (Figure 2.1)

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Temp	2	578.375000	289.187500	18.54	<.0001
Light	1	954.083333	954.083333	61.17	<.0001
Treatment	1	1200.000000	1200.000000	76.94	<.0001
Temp*Light	2	282.291667	141.145833	9.05	0.0007
Temp*Treatment	2	333.375000	166.687500	10.69	0.0002
Light*Treatment	1	208.333333	208.333333	13.36	0.0008
Temp*Light*Treatment	2	219.291667	109.645833	7.03	0.0026
Error	36	561.500000	15.597222		
Corrected Total	47	4337.250000			

Table A2: ANOVA table for effect of temperature and light on germination of *E. indica* for 14-day incubation period (Figure 2.2, 2.3 and 2.4)

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Temp	2	209.625000	104.812500	7.25	0.0023
Light	1	623.520833	623.520833	43.15	<.0001
Treatment	1	1092.520833	1092.520833	75.60	<.0001
Temp*Light	2	107.041667	53.520833	3.70	0.0345
Temp*Treatment	2	95.791667	47.895833	3.31	0.0477
Light*Treatment	1	196.020833	196.020833	13.56	0.0008
Temp*Light*Treatment	2	24.541667	12.270833	0.85	0.4362
Error	36	520.250000	14.451389		
Corrected Total	47	2869.312500			

Chapter 3: Response of an *Eleusine indica* population to glyphosate herbicide

Table A3: ANOVA table for shoot length development of un-germinated *E. indica* seeds (Figure 3.1)

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Treatment	1	1218.010000	1218.010000	4171.78	<.0001
Error	14	4.087500	0.291964		
Corrected total	15	1222.097500			

Table A4: ANOVA table for root length development of un-germinated *E. indica* seeds (Figure 3.1)

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Treatment	1	2753.625625	2753.625625	5567.90	<.0001
Error	14	6.923750	0.494554		
Corrected Total	15	2760.549375			

Table A5: ANOVA table for shoot length development of pre-germinated *E. indica* seeds (Figure 3.3)

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Treatment	1	3.15062500	3.15062500	12.06	0.0037
Error	14	3.65875000	0.26133929		
Corrected total	15	6.80937500			

Table A6: ANOVA table for root length development of pre-germinated *E. indica* seeds (Figure 3.3)

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Treatment	1	2020.502500	2020.502500	1076.47	<.0001
Error	14	26.277500	1.876964		
Corrected total	15	2046.780000			

Table A7: ANOVA table for biomass of *E. indica* plants treated with different rates of glyphosate (Figure 3.4)

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Treatment	5	2181.231814	436.246363	116.72	<.0001
Error	42	156.980323	3.737627		
Corrected total	47	2338.212136			

Table A8: ANOVA table for dry mass of *E. indica* plants treated with different rates of glyphosate (Figure 3.8)

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Treatment	5	47.26353346	9.45270669	106.67	<.0001
Error	42	3.72191953	0.08861713		
Corrected Total	47	50.98545299			

Chapter 4: Influence of growth stage on the response of an *Eleusine indica* population to glyphosate

Table A9: ANOVA table for biomass of *E. indica* plants treated with glyphosate at different growth stages (Figure 4.1)

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Treatment	1	1801.546338	1801.546338	305.58	<.0001
DAE	3	1873.098493	624.366164	105.91	<.0001
Treatment*DAE	3	97.214093	32.404698	5.50	0.0029
Error	40	235.820012	5.895500		
Corrected Total	47	4007.678937			

Table A10: ANOVA table for dry mass of *E. indica* plants treated with glyphosate at different growth stages (Figure 4.2)

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Treatment	1	58.6644630	58.6644630	119.04	<.0001
DAE	3	156.3978807	52.1326269	105.78	<.0001
Treatment*DAE	3	8.0980474	2.6993491	5.48	0.0030
Error	40	19.7129208	0.4928230		
Corrected Total	47	242.8733120			