

**SOIL FACTORS AFFECTING GLYPHOSATE EFFICACY IN
*LOLIUM SPP.***

by
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Declaration

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To my family, their endless love and support was my strength.

ABSTRACT

Glyphosate remains an important herbicide in weed control. This is due to several positive attributes it has including systemicity, wide spectrum of weed control and environmental friendliness. Its efficacy and lack of residual activity are therefore important to ensure adequate weed control without imposing hazards to the environment. Despite these favourable attributes for weed control glyphosate has its shortcomings.

Evolution of resistance to glyphosate has been a major concern from 1996. However, there are other factors that reduce the maximum potential of glyphosate. Any factors that reduce glyphosate efficacy may result in the target plant being subjected to non-lethal concentrations of glyphosate. This in turn may predispose the plants to developing herbicide resistance. Some factors that may influence efficacy of glyphosate, and therefore be possibly selecting for resistance were investigated in this study.

Although glyphosate is a postemergence herbicide, its efficacy is not exempt from the effect of soil and nutrients in which the weeds occur. The possibility of this occurring was investigated in a greenhouse study on ryegrass (*Lolium* spp.) In this study ryegrass was grown in three soils: pure sand (SS), soil from pasture paddock (PS) and soil from crop field (CS). The soils varied in nutrient composition and, although all were classified as sand, they had varying proportions of sand, loam and clay. This investigation consisted of four experiments. The first experiment was investigating the effect of growing a susceptible commercial ryegrass cultivar on PS, CS and SS soils on the efficacy of glyphosate (360 g a.i. L⁻¹ formulation) applied at five glyphosate application rates (GAR). The GARs were 0 (0x), 67.5 (1/8x), 135 (1/4x), 270 (1/2x) and 540 (1x) g a.i. ha⁻¹. The second experiment investigated the effect of growing a susceptible commercial ryegrass cultivar and a glyphosate resistant ryegrass biotype on PS and CS soils on the efficacy of glyphosate. The application rates were 0 (0x), 270 (1/2x), 540 (1x), 1080 (2x) and 2160 (4x) g a.i. ha⁻¹. The third and the fourth experiments were similar to the first experiment except: The latter investigated the role of nutrient content of irrigation water (pure water or balanced nutrient solution) and; the former investigated the effect of soil activity (by covering the soil surface with cotton at the time of spraying) of glyphosate with regard to the role it plays on efficacy of glyphosate. Our findings showed that: i) soil affects the efficacy of glyphosate with more control (19% survivors) found

in the PS soil compared to 50% and 62% survivors in CS and SS soils respectively, this effect may be dependent upon the species resistance as; ii) the effect in the resistant ryegrass biotype was reversed with about 95% of survivors in the PS soil compared to about 78% in CS soil; iii) efficacy of glyphosate is influenced by the soil nutrient status and the nutrient content of the irrigation water. This was shown by decrease in the control of ryegrass (100% survivors) grown in SS soil when fed with pure water compared to 45% when nutrient fed. In PS soil there was no significant effect. This was probably due to inherently higher nutrient content of the PS soil; and iv) glyphosate efficacy is influenced by the amount of glyphosate reaching the soil (absorbed through the roots). This was shown in PS soil where 1/8x GAR resulted in 93.3% survivors in covered soil compared to 60% in uncovered soil. A similar trend was also observed at 1/4x GAR. An opposite effect was shown in SS soil with 0% and 40% survival at 1/4x GAR in the covered and uncovered soil respectively.

Glyphosate has been hailed as an environmentally friendly herbicide as it rapidly degrades in soil and it sorbs on metals embedded in soil matrix. However, reports in the literature have showed reduction in crop yield due to soil glyphosate residues. In these studies, glyphosate phytotoxicity was found to be dependent on certain soil characteristics and nutrient content. Following this, a greenhouse study was conducted to assess the phytotoxic activity of glyphosate on a susceptible commercial ryegrass cultivar grown in PS, CS and SS soils. Glyphosate was applied at 0 (G1), 540 (G2) and 3240 (G3) g a.i. ha⁻¹. Ryegrass seedlings of comparable size were transplanted into the soil at intervals of two hours, three weeks and four weeks after glyphosate application referred to as TAS1, TAS2 and TAS3 respectively. Evidence of soil glyphosate activity was shown by the decrease in percentage survival with the application of glyphosate. This was significant in the SS soil where about 60% and 48% survival in G1 and G2 GAR respectively was observed compared to about 100% in the untreated control when transplanted three weeks after glyphosate application. The decrease in percentage survival was time mediated with significant effect of G2 GAR shown at TAS 1 whereas at G3 GAR the effect was significant at TAS1 and TAS 2. At TAS 3 there was no effect at all GARs. Similar trends were observed with dry mass and shoot length.

Trace metals required for normal plant growth have been implicated in the reduction of glyphosate efficacy. This follows glyphosate's original development as a metal chelator. Glyphosate-trace metal antagonism has recently sparked interest following co-application in glyphosate resistant soybeans. Molybdenum (Mo), an anion, may play a role at the physiological level on the antagonism of glyphosate. A greenhouse assay was carried out

where seedlings grown from seeds (of susceptible commercial ryegrass cultivar (S biotype) and glyphosate resistant biotype (R biotype)) were grown with nutrient solutions containing 0x, 1x and 2x molybdenum (Mo) concentrations where 1x is 0.05 mg L⁻¹ Mo. Glyphosate was applied at 0 (0x), 135 (1/4x), 270 (1/2x), 540 (1x) and 1040 (2x) g a.i. ha⁻¹ rates. In the R biotype applying 2x Mo resulted in 0% survival in the R biotype at 1x GAR compared to 50% and 90% survival at the same GAR with 0x and 1x Mo. In terms of dry mass and shoot length the results did not show any conclusive trends.

UITTREKSEL

Glifosaat is 'n baie belangrike onkruidodder wat in verskeie onkruidbeheerstelsels gebruik word. Dit is as gevolg van verskeie positiewe eienskappe waaroor dit besit onder andere sistemiese werking, wye spektrum van onkruidbeheer en omgewingsvriendelike werking. Die onkruidodder se effektiwiteit en gebrek aan residuele aktiwiteit is dus belangrik om bevredigende onkruidbeheer te bewerkstellig sonder om skade aan die omgewing te veroorsaak. Ten spyte van hierdie voordelige eienskappe het glifosaat ook tekortkominge.

Vanaf 1996 is ontwikkeling van weerstand teen glifosaat in onkruid 'n groot bron van kommer. Daar is egter ook ander faktore wat die maksimum potensiaal van glifosaat strem. Enige faktore wat die effektiwiteit van glifosaat strem mag veroorsaak dat die teikenplant aan subletale dosisse van glifosaat blootgestel word. Dit mag weer daartoe lei dat sulke plante blootgestel word aan die ontwikkeling van weerstand. Sommige faktore wat die effektiwiteit van glifosaat mag strem en dus moontlik kan lei tot seleksie vir weerstand is in hierdie studie ondersoek.

Alhoewel glifosaat 'n na-opkoms middel is kan die effektiwiteit moontlik beïnvloed word deur grondfaktore en nutriënte. Hierdie moontlikheid is ondersoek in 'n glashuisstudie waarin raaigras (*Lolium* spp.) gebruik is. In hierdie studie is raaigras geplant in drie verskillende grondsoorte nl. suiwer sand (SS), grond vanaf 'n weidingskamp (PS) en grond vanaf 'n gewasland (CS). Die gronde het gevarieer in terme van nutriëntinhoud en alhoewel al drie gronde as sand geklassifiseer is, was daar tog verskille in fisiese eienskappe. Hierdie afdeling het uit vier eksperimente bestaan. In die eerste eksperiment is die invloed van verskillende gronde (PS, CS en SS) waarin die kommersiële raaigras kultivar geplant was op die effektiwiteit van glifosaat (360 g a.b. L⁻¹ formulasie) teen vyf verskillende dosisse (GAR) ondersoek. Die dosisse was 0 (0x), 67.5 (1/8x), 135 (1/4x), 270 (1/2x) and 540 (1x) g a.b. ha⁻¹. Die tweede eksperiment het die invloed van twee grondsoorte (PS en CS) waarin 'n vatbare kommersiële raaigras kultivar en 'n glifosaat weerstandbiedende raaigras biotipe geplant is, se invloed op die effektiwiteit van glifosaat ondersoek. Die dosisse was 0 (0x), 270 (1/2x), 540 (1x), 1080 (2x) and 2160 (4x) g a.b. ha⁻¹. Die derde en vierde eksperimente was soortgelyk aan die eerste eksperiment behalwe dat die derde eksperiment die invloed van voeding (suiwer gedistilleerde water teenoor 'n gebalanseerde voedingsoplossing) saam met besproeiing

ondersoek het. In die vierde eksperiment is die grondwerking van glifosaat wat as blaarbespuiting toegedien is ondersoek deurdat sommige potte wat gespuit is se oppervlakte met 'n laag watte bedek is tydens die spuitproses en dadelik na spuit verwyder is teenoor die ander behandeling waar die grondoppervlakte nie bedek is nie. Die resultate het getoon dat i) grondtipe die effektiwiteit van glifosaat beïnvloed met beter beheer (19% oorlewing) in die PS grond vergeleke met 50% en 62% oorlewing in die CS en SS grondtipes respektiewelik. Hierdie effek kan moontlik beïnvloed word deur die weerstandsvlak van spesies omdat ii) die effek in die weerstandbiedende biotipe omgekeer is met ongeveer 95% oorlewing in the PS grondtipe vergeleke met 78% in die CS grondtipe; iii) effektiwiteit van glifosaat is beïnvloed deur die voedingstatus van die grond en die besproeiingswater. Dit word aangedui deur die afname in beheer van raaigras (100% oorlewing) wat in SS grond gegroei het en met suiwer gedistilleerde water besproei is vergeleke met 45% oorlewing in dieselfde grond wanneer met 'n gebalanseerde voedingsoplossing besproei is. Plante wat in PS grond gegroei het het geen betekenisvolle verskille tussen die besproeiingsbehandelings getoon in hulle reaksie op glifosaattoediening nie, waarskynlik as gevolg van die inherente hoër nutriëntinhoud van die grond en iv) glifosaat effektiwiteit word beïnvloed deur die hoeveelheid glifosaat wat die grond bereik en deur die wortels opgeneem word. Dit is bewys in plante wat in PS grond gegroei het waar 93.3% plante oorleef het waar die grondoppervlakte bedek was teenoor 60% oorlewendes waar die grondoppervlakte nie bedek was nie indien glifosaat teen 1/8x toegedien is. 'n Soortgelyke tendens is by die 1/4x dosis waargeneem. 'n Teenoorgestelde effek is in SS grond waargeneem waar die oorlewingspersentasie in bedekte en onbedekte grond by 1/4x glifosaatdosis 0% en 40% onderskeidelik was.

Glifosaat is aanvanklik aangeprys as 'n omgewingsvriendelike onkruidodder omdat dit vinnig in grond afgebreek word en omdat dit geadsorbeer word aan metale in die grondmatriks. In teenstelling hiermee is daar egter verslae in die literatuur wat dui daarop dat glifosaatresidue in die grond gewasopbrengste kan verlaag. In die gemelde studies is gevind dat fitotoksisiteit van glifosaat residue afhang van grondeienskappe en grondvrugbaarheid. Na aanleiding hiervan is 'n glashuisstudie uitgevoer waarin die fitotoksisiteit van residuele glifosaat op 'n kommersiële raaigraskultivar wat in PS, CS en SS gronde groei, ondersoek is. Glifosaat is op die grond in potte toegedien teen 0 (G1), 540 (G2) and 3240 (G3) g a.b. ha⁻¹. Raaigras saailinge is daarna in die potte ingeplant twee ure, drie weke en vier weke nadat die glifosaat toegedien is. Bewys van grondaktiwiteit van glifosaat is gelever deur die vermindering in persentasie oorlewing van die saailinge met toediening van glifosaat. Die

vermindering in oorlewing was betekenisvol in die SS grond waar ongeveer 60% en 48% oorlewing van saailinge was by G2 en G3 dosisse onderskeidelik teenoor 100% oorlewing in die onbehandelde kontrole. Die afname in persentasie oorlewing is deur tyd beïnvloed deurdat die G2 dosis slegs by die twee ure behandeling betekenisvolle verlaging in oorlewing veroorsaak het terwyl die G3 dosis by die twee ure sowel as die drie weke behandeling betekenisvolle verlaging veroorsaak het. Vier weke na toediening was daar geen effek van glifosaat op die saailinge in enige van die gronde gewees nie. Soortgelyke tendense is waargeneem by die droëmassa en lengte data.

Spoorelemente wat noodsaaklik is vir normale plantgroeiprosesse is al geïmpliseer in verlaging van glifosaat effektiwiteit. Dit is waarskynlik omdat glifosaat oorspronklik ontwikkel is as 'n metaal cheleerder. Glifosaat-spoorelement antagonisme was onlangs in die nuus nadat glifosaat saam met sulke elemente toegedien is op glifosaat weerstandbiedende sojabone. Molibdeen (Mo), 'n anioon, mag 'n rol op fisiologiese vlak speel in die effektiwiteit van glifosaat. 'n Glashuisstudie is uitgevoer waarin saailinge van 'n glifosaat vatbare kommersiële raaigras kultivar en 'n glifosaat weerstandbiedende raaigras biotipe besproei is met voedingsmengsels wat 0x, 1x en 2x Mo bevat waar 1x 0.05 mg L⁻¹ Mo is. Glifosaat is op die plante toegedien teen 0 (0x), 135 (1/4x), 270 (1/2x), 540 (1x) and 1040 (2x) g a.b. ha⁻¹ dosisse. Die 2x Mo toediening het gelei tot 0% oorlewing in die R biotipe by 1x GAR vergeleke met 50% en 90% oorlewing by dieselfde GAR met 0x en 1x Mo. In terme van droëmateriaal en lengtegroei was daar geen konkrete tendense nie.

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CHAPTER 1

INTRODUCTION

1.1 General

Weeds represent a major threat to crop production hence herbicide use is important to overcome crop-weed competition and reduce yield losses. Among the herbicides employed in weed control, glyphosate remains one of the best. It is a 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) inhibitor. Its activity prevents the synthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) in the shikimic acid pathway (Jasieniuk *et al.*, 2008; Powles & Yu, 2010). This post-emergence applied herbicide has been referred to as a “once in a hundred years” herbicide due to its many favourable attributes including broad spectrum weed control, low mammalian toxicity and no soil activity (Duke & Powles, 2008; Jasieniuk *et al.*, 2008). However, its use has been restricted to weed control in non agricultural areas or to preplant weed control in crops due to non selectivity. The advent of glyphosate resistant crops has since extended its scope of use.

1.2 Effect of soil on glyphosate activity

As in any herbicide, glyphosate efficacy is important to bring about adequate weed control. Primarily, efficacy can be affected by spray components such as spray carrier and effective calibration of the sprayer. In addition, efficacy can be reduced due to: resistance evolution or the inherent ability of the weed species to withstand the herbicide; growth conditions, and the environment in which plants are growing (weather, soil type and pH). Glyphosate, although post-emergence applied, can indirectly be affected by the soil (Waltz *et al.*, 2004; Zhou *et al.*, 2006).

The attribute of “no soil activity” has made glyphosate a key weed management tool in modern weed control (Jasieniuk *et al.*, 2008). This is due to glyphosate being known to rapidly degrade chemically and microbially to produce environmentally friendly products (ammonia and carbon dioxide) (Borggaard & Gimsig, 2008). However, there are reports on: glyphosate found in environmental sites; and the reduction of crop growth after planting where preplant glyphosate weed control was employed (Struger *et al.*, 2008; Ludvigsen *et al.*, 2003). In light

of these reports, residual glyphosate persistence could be a problem (leading to weed resistance) to subsequent crops after preplant glyphosate weed control (Ruepel *et al.*, 1977; Kjaer *et al.*, 2005). The activity of glyphosate in soil has been found to be soil dependent due to their differential effects on glyphosate absorption (Sprankle *et al.*, 1975). In addition, Mithila *et al.* (2008) reported that soil nutrients may influence phytotoxicity of pre- and post-emergence herbicides.

Glyphosate-tracemetal chelation is another factor that might lead, indirectly, to glyphosate antagonism. Glyphosate was initially developed as a metal chelator. This is why co-application with trace metals results in reduced efficacy of glyphosate and deficiency of the trace metal it is applied with. Co-application of glyphosate with trace metals is a common practice in glyphosate-resistant soybean (Bernards *et al.*, 2005; Zobiolo *et al.*, 2010). This is due to glyphosate application time coinciding with the time for plant requirement of these nutrients (Bernards *et al.*, 2005). The reaction is termed antagonism and may not be restricted to glyphosate-trace metals co-application but also trace metals within the plant leaf or its surface (Hall *et al.*, 2000; Eker *et al.*, 2006).

Objectives of the Study

Research on factors affecting glyphosate efficacy and establishing facts on its soil activity is important for the sustainable use of this invaluable tool in weed control. In this study species of *Lolium* (ryegrass) were used. The aim of this study was to: i) investigate the effect of soil on the efficacy of foliar applied glyphosate; ii) to determine phytotoxic activity of glyphosate applied on different soils; and iii) the effect of varying molybdenum (Mo) ion applications on the efficacy of foliar-applied glyphosate.

Thesis outline

This thesis is written according to the South African Journal of Plant and Soil article format. Thus each chapter is written with its own materials and methods. It is embodied into six chapters. Chapter 1 is the general introduction to the thesis. Chapter 2 is the review of literature on this topic. Chapter 3 is the first experimental chapter where the effect of different sandy soils on the efficacy of glyphosate was investigated. Chapter 4 was aimed at investigating the soil activity of glyphosate on ryegrass species grown in three different sandy soils. Chapter 5 was addressing the question of the effect of Mo on the efficacy of glyphosate. The general conclusions form the body of Chapter 6 where all chapters are collectively dealt with in summary with recommendations for further research.

References

- BERNARDS, M.L., THELEN, K.D. & PENNER, D., 2005. Glyphosate efficacy is antagonized by manganese. *Weed Technol.* 19, 27-34.
- BORGGGAARD, O.K. & GIMSIG, A.L., 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Manag. Sci.* 64, 441-456.
- DUKE, S.O. & POWLES, S.B., 2008. Glyphosate: a once-in -a-century herbicide. *Pest. Manag. Sci.* 64, 319-325.
- EKER, S., OZTURK, L., YAZICI, A., ERENOGLU, B., ROMHELD, V. & CAKMAK, I., 2006. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. *J. Agric. Food Chem.* 54, 10019-10025.
- HALL, G.J., HART, C.A. & JONES, C.A., 2000. Plants as sources of cations antagonistic to glyphosate activity. *Pest. Manag. Sci.* 56, 351-358.
- JASIENIUK, M., AHMAD, R., SHERWOOD, A.M., FIRESTONE, J.L., PEREZ-JONES, A., LANINI, W.T., MALLORY-SMITH, C. & STEDNIK, Z., 2008. Glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in California: Distribution, response to glyphosate, and molecular evidence for an altered enzyme. *Weed Sci.* 56, 496-502.
- KJAER, J., OLSEN, P., ULLUM, M. & GRANT, R., 2005. Vadose zone processes and chemical transport. *J. Environ. Qual.* 34, 608-620.
- LUDVIGSEN, G.H., LODE, O. & SKJVDAL, R., 2003. Retrieval of glyphosate and AMPA in Norwegian streams, including studies on leaching due to heavy rainfall. Proceedings of the XII Symposium Pesticides Chemistry. Pesticides in air, plant soil and water system. on velvet varies with application time of the day. 4-6 June 2003, Piacenza, Italy.
- MITHILA, J., SWANTON, J.C., BLACKSHAW, R.E., CATHCART, R.J. & HALL, C., 2008. Physiological basis for reduced glyphosate efficacy on weeds grown under low soil nitrogen. *Weed Sci.* 56, 12-17.
- POWLES, S.B. & YU, Q., 2010. Evolution in action: Plants resistance to herbicides. *Annu. Rev. Plant Biol.* 61, 317-47.
- RUEPPEL, M.L., BRIGHTWELL, B.B., SCHAEFER, J. & MARVEL, J.T., 1977. Metabolism and degradation of glyphosate in soil and water. *J. Agric. Food Chem.* 25, 517-528.
- SPRANKLE, P., MEGGIT, W. F. & PENNER, D., 1975. Rapid inactivation of glyphosate in the soil. *Weed Sci.* 23, 224-228.

- STRUGER, J., THOMPSON, D., STAZNIK, B., MARTIN, P., MCDANIEL, T. & MARVIN, C., 2008. Occurrence of glyphosate in surface waters of Southern Ontario. *B. Environ. Contam. Tox.* 80, 378-384.
- WALTZ, A.L., MARTIN, A.R., ROETH, F.W. & LINDQUIST, J.L., 2004. Glyphosate efficacy. *Weed Sci.* 54, 1132-1136.
- ZHOU, J., TAO, B. & MESSERSMITH, C.G., 2006. Soil dust reduces glyphosate efficacy. *Weed Sci.* 54, 1132-1136.
- ZOBIOLE, L.H.S., de OLIVEIRA JR, R.S., HUBER, D.M., CONSTANTIN, J., DECASTRO, C., DEOLIVEIRA, F.A. & OLIVEIRA JR, A., 2010. Glyphosate reduces shoot concentration of mineral nutrients in glyphosate resistant soybean. *Plant Soil.* 328, 57-69.

CHAPTER 2

LITERATURE REVIEW

2.1 Glyphosate

Glyphosate (Figure 2.1) is the most widely used herbicide in the history of weed control which is why Duke and Powles (2008) deemed it a “once in a century herbicide”. Initially it was patented as a metal chelator and was later used for weed control. Its use, as a herbicide, has been limited, mainly, to non-crop use as exemplified by weed control in industrialized areas, recreational areas, road sides and in no-till weed control systems. This is due to its mechanism of action that inhibits an essential pathway in plants which results to its broad spectrum activity and thus non selectivity (Wolfenbarger & Phifer, 2000; Duke & Powles, 2008; Zobiolo *et al.*, 2010).

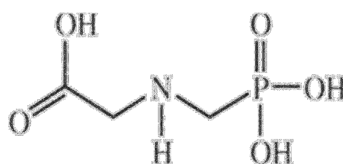


Figure 2.1 The structure of glyphosate (After: Coutinho & Mazo, 2005).

2.1.1 Glyphosate mechanism of action

Glyphosate kills susceptible plants by inhibiting the action of 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) enzyme of the shikimate pathway (Figure 2.2). This enzyme is involved in the synthesis of aromatic amino-acids and secondary compounds. *EPSPS* is a nuclear encoded enzyme, chloroplast located and catalyses the reaction of shikimate-3-phosphate (S-3-P) and phosphoenolpyruvate (PEP) to form EPSP and inorganic phosphate (Stoltenberg & Jeschke, 2003; Pedersen *et al.*, 2007; Chua *et al.*, 2008; Preston & Wakelin, 2008). Susceptible plants, and almost all green plants, lack the ability to withstand the herbicide hence are damaged with glyphosate application (Powles & Preston, 2006). In these plants glyphosate application hinders the production of chorismate (Figure 2.2) which is a precursor of aromatic amino acids (phenylalanine, tryptophan and tyrosine). This results in the arrest of protein synthesis, vitamins (K&D) and other plant secondary compounds, such as phytoalexin and lignin. The carbon flow into the pathway becomes massive and results in the

accumulation of shikimate (Holt & LeBaron, 1990; Bradshaw *et al.*, 1997; Cerdeira & Duke, 2006; Jasieniuk *et al.*, 2008; Shaner, 2009).

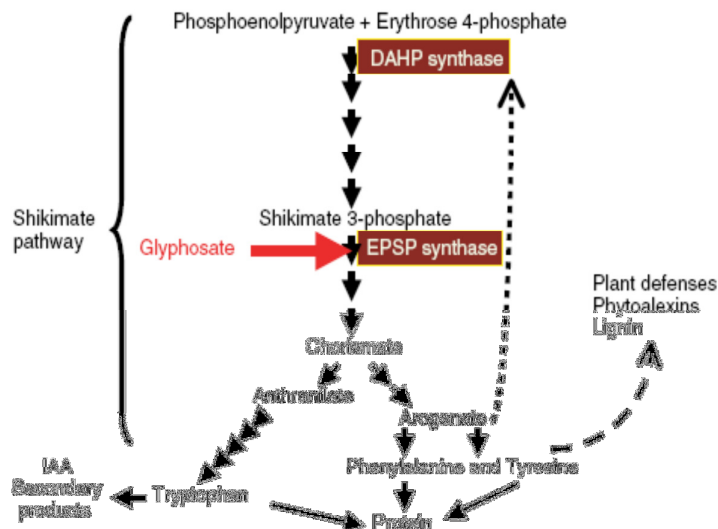


Figure 2.2 The shikimate pathway and the site of its inhibition by glyphosate (After Duke & Powles, 2008).

Plants respond to glyphosate treatment by continuing to attempt pushing away carbon through the shikimate pathway. This subsequently leads to diversion of carbon in the form of PEP and D-erythrose-4-phosphate to accumulate shikimate and other alicyclic hydroxyl acid intermediates from the shikimic acid pathway in susceptible plants, this also result in reduction in energy in the form of ATP (Kaundun *et al.*, 2008; Mueller *et al.*, 2008). Accumulation of shikimate is an indication that glyphosate reaches the target enzyme and such accumulation is a good biomarker of glyphosate activity in plants (Powles & Preston, 2006; Mueller *et al.*, 2008). However, the actual phytotoxic effect of glyphosate is not known, except that there is reduced protein production due to glyphosate's mechanism of action (Duke & Powles, 2008).

Visual symptoms due to glyphosate phytotoxicity in plants include chlorosis, pigmentation, stunting and reduction in apical dominance. Other effects include the epinastic response which is similar to the effects of ethylene. It is this mechanism that makes glyphosate a unique herbicide with several favourable attributes. These favourable attributes include effectiveness over a wide range of species with no less than 180 annual, perennial and biennial species of grasses, sedges, broad leaf weed species, woody brush and tree species, and

volunteer crops. Low activity in the soil and low mammalian toxicity has made it a major tool of weed control (Jasieniuk *et al.*, 2008). The herbicide derives its environmental friendliness from its low volatility, short half-life and minimal movement to groundwater, and rapid degradation (Wehtje *et al.*, 2008; Webster & Sosnokie, 2010). It is used ideally for pre-plant weed control in crop fields, or post-emergence weed control in herbicide resistant crop fields, inter-crop row weed control and weed control in fallow fields (Bradshaw *et al.*, 1997; Powles *et al.*, 1998; Powles & Preston, 2006; Kaundun *et al.*, 2008; Tranel & Horvath, 2009; Zobiolo *et al.*, 2010). In most instances glyphosate application to weeds that are up to 10 cm high provides adequate control (Webster & Sosnokie, 2010).

Other major non-agricultural glyphosate weed control applications include those along road sides, irrigation channels, in recreational areas, and for woody weed control (Powles & Preston, 2006). Glyphosate is also useful in the technique known as pasture topping. This involves the use of glyphosate in the pasture phase late in the growing season to reduce weed seed production (Neve *et al.*, 2003). Low frequency of resistant genes adds to the advantage of this herbicide because of reduced chances of resistance evolution (Perez & Kogan, 2003; Jasieniuk *et al.*, 2008). However, glyphosate overuse increases the prevalence of resistant biotypes which dominate and leads to the spread of resistant biotypes (Nandula, 2010).

2.1.2 Glyphosate - an environmental threat?

In spite of glyphosate regarded as an “environmental friendly herbicide”, there are reports of glyphosate build up in ground water resources. Because glyphosate is said to degrade rapidly to AMPA and due to its tendency to tightly bind into the soil colloids, it was reported that glyphosate could not reach ground water resources (Zhao *et al.*, 2009). However, this was challenged by the findings that the glyphosate-soil bonds can quickly be broken. Soil texture and pH are determinants of the extent and duration of glyphosate degradation and adsorption in soil colloids (Cox, 1995; Hanke *et al.*, 2008).

2.1.3 Effect of soil physical and chemical characteristics on efficacy of glyphosate

Effects of soil characteristics on herbicides have been well researched in terms of pre-emergence herbicides. Glyphosate is among the herbicides which have been poorly researched when it concerns soil physical and chemical properties. This is due to glyphosate being a post-emergence herbicide and has been reported to have limited or no soil activity (Salazar &

Appleby, 1982; VanGessel, 2001). Investigations by Zhou *et al.* (2006) addressed soil and pH effect on weed control. Their findings showed that efficacy was slightly influenced by soil dust with the trend toward increased efficacy as pH increased. Soil texture and pH play an important role in soil nutrient availability for plant use. This in turn is a determinant of leaf nutrient availability in plants. The indirect involvement of soil and pH on glyphosate efficacy makes it a less well-understood subject. Mithila *et al.* (2008) found that low nutrient levels contributed to poor glyphosate efficacy.

2.2 Antagonism, additivity and synergism

Antagonism is defined by Penner (1989) as an interaction of two or more chemicals such that the effect when combined is less than the predicted effect based on the activity of each chemical applied separately. The antagonistic interaction in herbicide mixtures is not a new phenomenon, reports date it from as early as the 1960s (Tammes, 1964). These reactions result from the interaction of the herbicide with the chemicals applied simultaneously or with residual amounts already present at the treatment time. The antagonistic reactions are classified into four types: biochemical, competitive, physiological and chemical antagonism (Green, 1989).

Antagonism is not the only reaction between herbicides and other chemicals. There is also additivity and synergism (Green, 1989). While antagonism results in less control of the weed than the predicted control; additivity is when the weed control is equal to the predicted control; and synergism is when the weed control is greater than the predicted control (Hydrick & Shaw, 1994; Hoagland, 1996; Chua *et al.*, 2008). An example of additivity could be that played by adjuvants in herbicides. They play a significant role in herbicide efficacy, especially the post-emergence applied herbicides. This is due to the fact that spray retention and herbicide absorption (especially post-emergence herbicides) by the target plant are essential for efficacy (Nalewaja, 2002).

2.2.1 Types of antagonism

Antagonism may result in adverse effects such as alteration of absorption, translocation and/or biotransformation characteristics (Hoagland, 1989; Hoagland, 1996). Herbicides interact with ions that they are in solution with. These range from polyvalent cations found in water to nutrient ions found in fertiliser. In addition herbicides may interact with each other in non-registered mixtures made in an attempt to increase weed control spectrum. Tank mixtures of

glyphosate with other herbicides often results in antagonism (Hydrick & Shaw, 1994; Scott *et al.*, 1998; Bradley *et al.*, 2000). These are exemplified by the reaction of glyphosate and paraquat in mixtures and diquat-glyphosate mixtures although it was earlier thought that diquat makes glyphosate work faster (Wehtje *et al.*, 2008). In fact, diquat antagonised the activity of glyphosate. A mixture of atrazine and glyphosate resulted in less control of shattercane compared to application of glyphosate alone and a mixture of glyphosate or glufosinate with atrazine had an antagonistic effect for the control of rye and horseweed (Hoagland, 1989). The interaction can occur before entry or after entry to the plant leaf or foliage.

2.2.1.1 Biochemical

In biochemical antagonism, one chemical antagonist reacts with the herbicide to decrease the amount of herbicide available at the site of action. This occurs in one of the following ways, *viz*: reduced herbicide absorption or penetration; reduced herbicide transport or altered herbicide transport; enhanced metabolic inactivation or an increased rate of herbicide biotransformation within the leaf cellular system and sequestration. An example of an antagonist causing reduced herbicide absorption is that of Na-bentazon on sethoxydim absorption (Green, 1989; Penner, 1989).

2.2.1.2 Competitive

In competitive antagonism, the antagonist acts at the same site of action as the herbicide intended for killing the weed. Competitive antagonism is a function of the concentration of the antagonist and the herbicide as well as the affinity of each for the site of action. Where an antagonist has the highest affinity for the site of action compared to the herbicide, the efficacy of the herbicide is reduced due to the antagonist occupying the active site. Antagonism of 2,4-D by 2,4,6-T is an example of competitive antagonism; the antagonism of paraquat activity by the organic polyamine putrescine in the susceptible biotype of *Conyza bonariensis* is another example of competitive antagonism, through competitive inhibition of paraquat uptake by the plasmalemma (Penner, 1989; Norman & Fuerst, 1997).

2.2.1.3 Physiological

The attributes of physiological antagonism can be seen when the herbicide and the antagonist act at different sites, as opposed to the biological effects, and have the same mechanism of

action, but counteracting each other by producing opposite effects on the physiological process. An example of physiological antagonism is that of 2, 4-D on the growth inhibition caused by EPTC and another example is when wild oat herbicides diclofop and difenzoquat are mixed with sulfonyl ureas or phenoxy herbicides that control broadleaf weeds (Green, 1989; Penner, 1989). Other antagonistic reactions may occur due to changes in physiological function of the plant (Selleck & Baird, 1981). The effect of antagonism is often reduced with increase in dose of herbicide or increasing with increase in antagonist concentration e.g. the efficacy of glyphosate was reduced with increase in the amount of Mn applied (Cakmak *et al.* 2009). Glyphosate antagonism in velvetleaf (*Abutilon theophrasti*) was decreased by increasing the time interval between glyphosate and Mn application (Bernards *et al.*, 2005).

2.2.1.4 Chemical

In chemical antagonism, the antagonist and the herbicide react chemically resulting in the formation of an inactive complex leaving less active herbicide available at the site of action. The interaction is a function of the available concentration of herbicide and antagonist. An example of chemical antagonism is the formation of a complex between FeCl_3 and amitrole (Glass, 1984; Penner, 1989).

2.3 Glyphosate antagonism

Glyphosate antagonism was observed when in mixture with iron (Fe), calcium (Ca), magnesium (Mg), sodium (Na), manganese (Mn) and potassium (K) (Nalewaja & Metysiak, 1991). This emanates from glyphosate having multiple functional groups (amine, carboxylate and phosphonate) which can form strong coordination with metal cations to give bidentate and tridentate complexes (Wang *et al.*, 2009). Cases of glyphosate-Mn complex formation (Figure 2.3) have been noted in glyphosate resistant soybean.

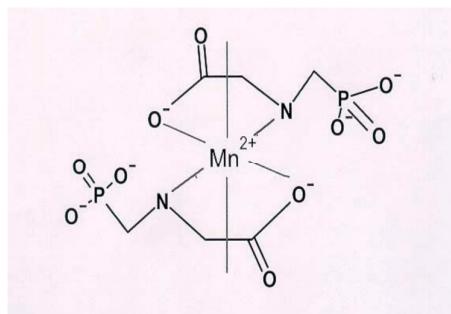


Figure 2.3 Glyphosate antagonism by manganese (After: Gednalske, Undated).

Manganese is a tetravalent transition metal close to Fe and Zn that forms insoluble salt complexes with glyphosate and similar herbicides (Bailey *et al.*, 2002). This essential trace metal acts as an enzyme cofactor. The metal ion activates about 35 different enzymes, leading to the biosynthesis of aromatic amino acids such as tyrosine and secondary products such as lignin and flavanoids. Manganese deficiency results in interveinal chlorosis (also known as “yellow flashing”) in newly emerged tissue. The ion also imparts plant processes in higher plants, photosynthesis in general and photosynthetic oxygen evolution in photosystem II (Bernards *et al.*, 2005; Gordon, 2007; Zobiolo *et al.*, 2010).

The explanation of the yellow flashing symptom is derived from the knowledge that glyphosate was initially developed as a chelating agent, meaning its molecule wraps around the molecules of other elements making them unavailable and also closing the immobilization of divalent cations (Cakmak *et al.*, 2009; Halbeisen, 2011). In maize, Mn deficiency is marked by poor tasseling and delayed anther development. It also can result in fewer and smaller pollen grains with reduced cytoplasmic contents due to the significance of *in vitro* pollen grains germination (Sharma *et al.*, 1991). Mn deficiency in crops is a great concern because it results in decreased yield (Diedrick *et al.*, 2008). Because Mn acts as a cofactor of enzymes that leads to the biosynthesis of aromatic amino acids, flavanoids and lignin, a lack of it results in decrease in disease resistance (Gordon, 2007). Plants differ in their susceptibility to Mn deficiency i.e. those of the *Poaceae* family are less affected compared to *Brassicaceae* which seems to be affected more. It is common among the phosphonic acids to act as chelating agents and form stable complexes with divalent and trivalent metal cations. Mn is one of the divalent cations that complexes with glyphosate. In such cases, absorption into or translocation within the treated tissue becomes impeded (Sayyari-Zahan *et al.*, 2009).

Glyphosate resistant soybeans were introduced in 1996 (Bailey *et al.*, 2002). Application of Mn with glyphosate is a common practice in glyphosate resistant crops (GRC). This according to Bernards *et al.* (2005) is due to the coincidence of glyphosate application with the time where Mn application is required. Soybean Mn deficiency is common among the soybeans grown in mildly acidic soils, alkaline sands, alluvial soil derived from calcareous materials and due to low Mn availability and high soil pH (as soil pH increases plant available manganese decreases) or both (Bernards *et al.*, 2005; Gordon, 2007).

Herbicide antagonism in weed biotypes could lead to propagation of resistant weeds due to low doses of glyphosate that reaches the active site. This can be due to weed biotypes being exposed to sub-lethal doses which according to Busi and Powles (2009) results in the selection of minor resistance genes. In addition, glyphosate activity in soil may predispose subsequent weed populations to sub-lethal doses which in the same manner could trigger the development of resistance. This is similar to glyphosate resistance developing as a result of subjecting weeds to label rates below manufacturer's recommendations (Zhang *et al.*, 2000)

2.4 Development of glyphosate resistance

It took more than twenty years before glyphosate resistance was observed in weeds. Weeds have a low propensity to develop glyphosate resistance due to: rare resistance traits endowing resistance to glyphosate; plants not readily metabolising glyphosate; poor soil activity; existence of few plants with inherent resistance to glyphosate (Bradshaw *et al.*, 1997; Pratley *et al.*, 1999; Powles & Preston, 2006; Yu *et al.*, 2007).

Resistance is referred to as an evolutionary process that occurs through natural selection. The otherwise susceptible biotype, due to exposure to selection pressure (herbicide), withstands a normal lethal dose. In this case a rare resistant trait carrying biotype survives and reproduces after application of the herbicide normally lethal to the vast majority of individuals of that species (Stoltenberg & Jeschke, 2003). Generally, the time it takes for the evolution of resistance to occur depends upon the initial frequency of the rare resistant individual and the extent of the selection pressure of the herbicide (i.e. how often the herbicide is applied) (Jasieniuk *et al.*, 1996). This could explain the longer time it took weeds to evolve glyphosate resistance (low initial frequency for the rare resistant traits) and the sudden increase in the glyphosate resistant biotypes following the initial discovery (increase in glyphosate use in GRC and the decrease in the price after the expiry of the patent).

The first confirmed case of evolution of glyphosate resistance was observed in 1996. This was in ryegrass (*Lolium rigidum* L.) of Australia (Heap, 2003; Neve *et al.*, 2004; Perez-Jones *et al.*, 2005). Following this, glyphosate was reported in goosegrass (*Eleusine indica* (L.) Gaertn.) in Malaysia; horseweed (*Conyza canadensis* (L.) Conq.) in United States; and *Italian ryegrass* in Chile, rigid ryegrass in California and South Africa (Perez-Jones *et al.*,

2005). To date there are 18 known glyphosate resistant weed species (Owen & Powles, 2010). Lack of judicious practices in weed control systems using glyphosate commonly in orchards, GRC systems and no till farm practises have been blamed for the evolution of resistance. GRCs and perennial crops share common features such as high levels of glyphosate usage, repeated usage during a growing season, monoculture and lack of herbicide rotation (Dinelli *et al.*, 2008).

As mentioned previously, exposure to sub-lethal doses as a result of glyphosate being in an antagonistic reaction, predisposition through glyphosate residual soil activity; or by deliberate reduction of application rates are tools by which rare resistance traits could be selected. Busi and Powles (2009) reported that glyphosate resistance (endowed by single major genes) can emanate from minor genes due to exposure to sub-lethal doses. Known mechanisms in glyphosate resistance are target site mutation and limited or reduced glyphosate translocation. These mechanisms fall into two categories: target and non target site alteration. Target site mechanisms involve the alteration of EPSPS or the over-expression of the gene and non target site modification are all other mechanisms (differential uptake and or translocation, sequestration or increased metabolic detoxification) (Nandula, 2010).

Due to possible involvement of residual soil activity and antagonism in evolution of herbicide resistance, it is important to investigate antagonism and residual soil activity of glyphosate, as they are indirectly involved in selection of resistance traits.

References

- BAILEY, W.A., POSTON, D.H., WILSON, H.P. & HINES, T., 2002. Glyphosate interaction with manganese. *Weed Technol.* 16, 792-799.
- BERNARDS, M.L., THELEN, K.D. & PENNER, D., 2005. Glyphosate efficacy is antagonized by manganese. *Weed Technol.* 19, 27-34.
- BRADLEY, P.R., JOHNSON, W.G. & SMEDA, R.J., 2000. Response of sorghum (*Sorghum bicolor*) to atrazine, ammonium sulphate, and glyphosate. *Weed Technol.* 14, 15-18.
- BRADSHAW, L.D., PADGETTE, S.R., KIMBALL, S.L. & WELLS, B.H., 1997. Perspectives on glyphosate resistance. *Weed Technol.* 11, 189-198.
- BUSI, R. & POWLES, S.B., 2009. Evolution of glyphosate resistance in a *Lolium rigidum* biotype by glyphosate selection at sublethal doses. *Heredity* 103, 318-325.

- CAKMAK, I., YAZICI, A., TUTUS, Y. & OZTURK, L., 2009. Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. *Europ. J. Agronomy* 31, 126-132.
- CERDEIRA, A.L. & DUKE, S.O., 2006. The current status and environmental impacts of glyphosate –resistant crops: a review. *J. Environ. Qual.* 35, 1633-1658.
- CHUA, T.S., NOR ASMAH, B.J., CHA, T.S., HASAN, S.M.Z. & SAHID, I.B., 2008. The use of reduced rates of herbicide combination in tank mixes for goose grass (*Eluesine indica* (L.) Gaetrn.) control. *World Appl. Sci. J.* 5, 358-362.
- COUTINHO, C.F.B. & MAZO, L.H., 2005. Metallic complexes with glyphosate: A review. *Quim. Nova* 28, 1038-1045.
- COX, C., 1995. Glyphosate part 2: human exposure and ecological effects. *J. Pest. Reform.* 15, 14-20.
- DIEDRICK, K.A., MULLEN, R.W. & DYGERT, C.E., 2008. Evaluation of manganese and glyphosate formulation and timing on soybean yield. *The Joint Annual Meeting of the GSA, SSSA, ASA, CSSA, GCAGS and HGS* Houston, Texas, 5-9 October 2008.
- DINELLI, G., MAROTTI, I., CATIZONE, P., URBANO, J.M. & BARNES, J., 2008. Physiological and molecular bases of glyphosate resistance in *Conyza bonariensis* biotype from Spain. *Weed Res.* 48, 257-265.
- DUKE, S.O. & POWLES, S.B., 2008. Glyphosate: a once-in -a-century herbicide. *Pest. Manag. Sci.* 64, 319-325.
- GEDNALSKE, J.V., Undated. Herbicide antagonism and herbicide –fertiliser interactions. Online, Internet. www.agronomy.cfans.umn.edu (Accessed 13/11/ 2009).
- GLASS, R.L., 1984. Metal complex formation by glyphosate. *J. Agric. Food Chem.* 32, 1249-1253.
- GORDON, W.B., 2007. Manganese nutrition of glyphosate-resistant and conventional soybeans. *Better Crop* 91, 12–13.
- GREEN, J.M., 1989. Herbicide antagonism at the whole plant level. *Weed Technol.* 3, 217-226.
- HALBEISEN, J., 2011. Glyphosate and micronutrients. Growers mineral solutions. www.growersmineral.com (Accessed 05/01/2011).
- HANKE, I., SINGER, H. & HOLLENDER, J., 2008. Ultratrace-level determination of glyphosate, aminomethyl phosphonic acid and glufosinate in natural environments by solid phase extraction followed by liquid chromatography-tandem mass spectrometry:

- performance tuning of derivatization, enrichment and detection. *Anal. Bioanal. Chem.* 398, 2265-2276.
- HEAP, I., 2003. International survey of herbicide resistant weeds. Herbicide Resistance Action Committee, North American Herbicide Resistance Action Committee, and *Weed Sci. Soc. Am.* Online, Internet. www.weedscience.org (accessed 08/11/03).
- HOAGLAND, R.E., 1989. Biochemical interactions of atrazine and glyphosate in soybean (*Glycine max*) seedlings. *Weed Sci.* 37, 491-497.
- HOAGLAND, R.E., 1996. Chemical interaction with bioherbicides to improve efficacy. *Weed Technol.* 10, 651-674.
- HOLT, J.S. & LEBARON, H.M., 1990. Significance and distribution of herbicide resistance. *Weed Technol.* 4, 141-149.
- HYDRICK, D.E. & SHAW, D.R., 1994. Effect of tank-mix combination of non-selective foliar and selective soil-applied herbicides on three weed species. *Weed Technol.* 8, 129-133.
- JASIENIUK, M., BRÛLÉ-BABEL A.L. & MORRISON, I. N., 1996. The evolution and genetics of herbicide resistance in weeds. *Weed Sci.* 44, 176-193.
- JASIENIUK, M., AHMAD, R., SHERWOOD, A.M., FIRESTONE, J.L., PEREZ-JONES, A., LANINI, W.T., MALLORY-SMITH, C. & STEDNIK, Z., 2008. Glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in California: Distribution, response to glyphosate, and molecular evidence for an altered enzyme. *Weed Sci.* 56, 496-502.
- KAUNDUN, S.S., ZELAYA, I.A., DALE, R.P., LYCETT, J.A., CARTER, P., SHARPLES, K.R. & MCINDOE, E., 2008. Importance of the P106S target-site mutation in conferring resistance to glyphosate in a goosegrass (*Eleusine indica*) biotypes from the Phillipines. *Weed Sci.* 56, 637-647.
- MITHILA, J., SWANTON, J.C., BLACKSHAW, R.E., CATHCART, R.J. & HALL, C., 2008. Physiological basis for reduced glyphosate efficacy on weeds grown under low soil nitrogen. *Weed Sci.* 56, 12-17.
- MUELLER, T.C., ELLIS, A.T., BEELER, J.E., SHARMA, S.D. & SINGH, M., 2008. Shikimate accumulation in nine weedy species following glyphosate application. *Weed Res.* 48, 455-560.
- NALEWAJA, J.D., 2002. Oils as with herbicide. In: G.A.C. Beattie (ed.). Spray Oil Beyond 2000. University of Western Sydney, New South Wales, Australia.
- NALEWAJA, J.D. & MATYSIAK, R., 1991. Salt antagonism of glyphosate. *Weed Sci.* 39, 622-628.

- NANDULA, V.K., 2010. Herbicide resistance: definitions and concepts. In: V.K. Nandula (ed.) *Glyphosate Resistance in Crops and Weeds: History, Development and Management*. John Wiley & Sons. Hoboken.
- NEVE, P., SADLER, J. & POWLES, S.B., 2004. Multiple herbicide resistance in a glyphosate-resistant rigid ryegrass (*Lolium rigidum*) population. *Weed Sci.* 52, 920-928.
- NEVE, P., DIGGLE, A.J., SMITH, F.P. & POWLES, S.B., 2003. Simulating the evolution of glyphosate resistance *Lolium rigidum* II: past, present and the future glyphosate use in Australian cropping. *Weed Res.* 43, 418-427.
- NORMAN, M.A. & FUERST, P.E., 1997. Interaction of cations with paraquat in leaf section of resistant and sensitive biotypes of *Conyza bonariensis*. *Pestic. Biochem. Phys.* 57, 181-191.
- OWEN, M.J. & POWLES, S.B., 2010. Glyphosate resistant rigid ryegrass (*Lolium rigidum*) populations in the Western Australian Grain Belt. *Weed Technol.* 24, 44-49.
- PEDERSEN, B.P., NEVE, P., ANDREASEN, C. & POWLES, S.B., 2007. Ecological fitness of a glyphosate – resistant *Lolium rigidum* biotype: Growth and seed production along a competition gradient. *Basic Appl. Ecol.* 8, 258-268.
- PENNER, D., 1989. The impact of adjuvants on herbicide antagonism. *Weed Technol.* 3, 227-231.
- PEREZ, A. & KOGAN, M., 2003. Glyphosate resistant *Lolium multiflorum* in Chilean orchards. *Weed Res.* 43, 12-19.
- PEREZ-JONES, A., PARK, K.W., COLQUHOUN, J., MALLORY-SMITH, C. & SHANER, D., 2005. Identification of glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in Oregon. *Weed Sci.* 53, 775-779.
- POWLES, S.B. & PRESTON, C., 2006. Evolved glyphosate resistance in plants: Biochemical and genetic basis of resistance. *Weed Technol.* 20, 282-289.
- POWLES, S.B., LORRAINE-COLWILL, D.F., DELLOW, J.J. & PRESTON, C., 1998. Evolved resistance to glyphosate in ryegrass (*Lolium rigidum*) in Australia. *Weed Sci.* 46, 604-607.
- PRATLEY, J., URWIN, N. & STANTON, R., 1999. Resistance to glyphosate in *Lolium rigidum*. I. Bioevaluation. *Weed Sci.* 47, 405-411.
- PRESTON, C. & WAKELIN, A.M., 2008. Resistance to glyphosate from altered herbicides translocation patterns. *Pest. Manag. Sci.* 64, 372-376.

- SALAZAR, L.C. & APPLEBY, A.P., 1982. Herbicidal activity of glyphosate in soil. *Weed Sci.* 30, 463-466.
- SAYYARI-ZAHAN, M.H., SADANA, U.S., STEINGROBE, B. & CLASSEN, N., 2009. Manganese efficiency and manganese uptake kinetics of raya (*Brassica juncea*), wheat (*Triticum aestivum*), and oat (*Avena sativa*) grown in nutrient solution and soil. *J. Plant Nutr. Soil Sci.* 172, 425-434.
- SCOTT, R., SHAW, D.R. & BARRENTINE, W.L., 1998. Glyphosate tank mixtures with SAN 582 for burndown or post-emergence application in glyphosate-tolerant soybean (*Glycine max*). *Weed Technol.* 12, 23-26.
- SELLECK, G.W. & BAIRD, D.D., 1981. Antagonism with glyphosate and residual herbicide combinations. *Weed Sci.* 29, 185-190.
- SHANER, D.L., 2009. Role of translocation as a mechanism of resistance to glyphosate. *Weed Sci.* 57, 118-123.
- SHARMA, C.P., SHARMA, P.N., CHATTERJEE, C. & AGARWALA., 1991. Manganese deficiency in maize affects pollen viability. *Plant Soil.* 139-142.
- STOLTENBERG, D.E. & JESCHKE, M.R., 2003. Occurrence and mechanism of weed resistance in glyphosate. <http://www.soils.wisc.edu> (Accessed 12/08/ 2009).
- TAMMES, P.M.L., 1964. Isoboles a graphic representation of synergism in pesticides. *Neth. J. Pathol.* 70, 73-80.
- TRANEL, P.J. & HORVATH, D.P., 2009. Molecular biology and genomics: New tools for weed science. *Bioscience* 59, 207-215.
- VAN GESSEL, M.J., 2001. Glyphosate resistant horseweed from Delaware. *Weed Sci.* 49, 703-705.
- WANG, Y., CUI, Y., ZHOU, D., WANG, S., XIAO, A., WANG, R. & ZHANG, H., 2009. Adsorption kinetics of glyphosate and copper (II) alone and together on two types of soils. *Soil Sci. Soc. Am. J.* 73, 1995-2001.
- WEBSTER, T.M. & SOSNOKIE, L.M., 2010. Loss of glyphosate efficacy: a changing weed spectrum in Georgia cotton. *Weed Sci.* 53, 73-79.
- WEHTJE, G., ATLAND, J.E. & GILLION, C.H., 2008. Interaction of glyphosate and diquat in ready-to-use weed control practices. *Weed Technol.* 22, 472-476.
- WOLFENBARGER, L.L. & PHIFER, P.R., 2000. The ecological risk and benefits of genetically engineered plants. *Science* 290, 2088-2093.
- YU, Q., CAIRNS, A. & POWLES, S.B., 2007. Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. *Weed Sci.* 225, 499-513.

- ZHANG, J., WEAVER, S.E. & HAMILL, A.S., 2000. Risk and reliability of using herbicides at below label rates. *Weed Technol.* 14, 106-115.
- ZHAO, B., ZHANG, J., GONG, J., ZHANG, H. & ZHANG, C., 2009. Glyphosate mobility in soils by phosphate application: Laboratory column experiments. *Geoderma.* 149, 290-297.
- ZHOU, J., TAO, B. & MESSERSMITH, C.G., 2006. Soil dust reduces glyphosate efficacy. *Weed Sci.* 54, 1132-1136.
- ZOBIOLE, L.H.S., DE OLIVEIRA JR, R.S., HUBER, D.M., CONSTANTIN, J., DE CASTRO, C., DE OLIVEIRA, F.A. & OLIVEIRA JR, A., 2010. Glyphosate reduces shoot concentration of mineral nutrients in glyphosate resistant soybean. *Plant Soil* 328, 57-69.

CHAPTER 3

THE EFFECT OF THREE DIFFERENT SANDY SOILS AND NUTRIENTS ON THE EFFICACY OF GLYPHOSATE ON RYEGRASS (*LOLIUM SPP.*)

Abstract

Glyphosate is one of the most widely applied herbicides in the world. The advent of glyphosate resistant crops boosted the usage of glyphosate even more. With increased usage of glyphosate weeds resistant to glyphosate started to emerge. Non-target site resistance may be caused by sub-lethal doses of herbicide. In this study the effect of certain soil characteristics were investigated to determine their effect on glyphosate efficacy. In the first experiment the effect of three different sandy soils and five different glyphosate application rates on glyphosate efficacy on a susceptible commercial ryegrass cultivar were investigated. In the second experiment the same investigations were applied to two ryegrass biotypes viz. the commercial susceptible cultivar and a glyphosate resistant biotype. The third experiment investigated the effect of nutrient content of irrigation water on glyphosate efficacy on commercial ryegrass grown in the three soils and in the fourth experiment the effect of soil activity of glyphosate in the three soils were evaluated with regard to the contribution that soil activity play in foliar applied glyphosate. Results from the four experiments can be summarised as follows: High nutrient levels, irrespective of whether they were applied with irrigation water or whether they were part of the inherent higher fertility of a specific soil type, improved glyphosate efficacy in susceptible ryegrass plants. The opposite was true in resistant plants where higher nutrient levels appeared to decrease glyphosate efficacy. Soil activity of glyphosate appeared to contribute substantially to glyphosate efficacy in two of the sandy soils but in the third soil which had a small clay fraction, the soil activity appeared to be negated. It was concluded that soil factors such as fertility and soil texture might improve or reduce the efficacy of glyphosate to such an extent that recommended doses could be sub-lethal under certain conditions and thus could contribute to the development of non-target site resistance.

Keywords: efficacy, glyphosate, nutrient, ryegrass, sandy soils

3.1 Introduction

Herbicides are invaluable tools in weed control. They provide an easy and less tedious method of weed control compared to the earlier methods such as hand and hoe weeding. However,

their value as weed control tools depends on the ability to exert adequate phytotoxic effect (efficacy) on the weed species within their spectrum of weed control. There are several factors that are implicated in impeding the efficacy of herbicides (Nandula, 2010). These include environmental factors such as light duration and intensity, temperature, relative humidity, storm, and drought (Feng *et al.*, 2003; Waltz *et al.*, 2004; Zhou *et al.*, 2006; Mohr *et al.*, 2007) plant morphology and the degree of sensitivity to the herbicide; application time of the herbicide and the growth stage of the weeds (Singh & Singh, 2004; Feng *et al.*, 2004; Waltz *et al.*, 2004). This is especially so for glyphosate where its activity on specific weeds results from a complex interaction between the herbicide and the environment (Zhou *et al.*, 2007).

The impact of soil characteristics on the efficacy of herbicides has, in a number of cases, been in the case of pre-emergence soil-applied herbicides. This is due to the fact that the soil applied herbicides are directly affected by soil pH, clay content, mineralogy and organic matter and a high correlation of some soil-applied herbicide activities with these soil properties were reported (Blumhorst *et al.*, 1990; Stouggard *et al.*, 1990). However, Steward *et al.* (2010) reported that environmental and soil conditions affect efficacy of both pre- and post-emergence herbicides. Post-emergence applied herbicides may, indirectly, be affected by pH, soil texture and nutrient content - particularly nitrogen (N) is implicated in affecting glyphosate efficacy. Mithila *et al.* (2008) reported that soil nutrients may influence phytotoxicity of pre- and post-emergence herbicides. In green foxtail (*Setaria viridis* (L.) Beauv.), low nitrogen negatively affected the efficacy of glyphosate, glufosinate, nitrosulfuron and mesotrione (Mithila *et al.*, 2008).

Soil characteristics may affect the physiology and nutrient contents of the plant which in turn may affect the activity of the herbicide in the plant. When growth conditions do not favour optimum growth of weeds they exert abiotic stress which may result in decreased efficacy of herbicides. This is because growth conditions of the weed and the environment is important for the action of the herbicide, as was reported for glyphosate by Zhou *et al.* (2007) and is true for other post-emergence herbicides as well. Dependency of glyphosate efficacy on the pH was noted in a soil dust pH study by Zhou *et al.* (2006) where an increase in pH resulted in increased glyphosate efficacy.

The aim of this study was to investigate the: (i) Effect of soil type on the efficacy of glyphosate on a susceptible ryegrass (*Lolium multiflorum*) cultivar, (ii) Effect of soil type and nutrient level on the efficacy of glyphosate on a susceptible ryegrass cultivar and a resistant

ryegrass (*Lolium* spp.) biotype, and (iii) Effect of covering the soil surface during foliar glyphosate application on the efficacy thereof.

3.2 Materials and Methods

This experiment was carried out as a way of establishing whether soil has an effect on glyphosate efficacy and if this differs between a susceptible commercial ryegrass cultivar (*L. multiflorum*) and a resistant ryegrass biotype (*Lolium* spp.) from Groenkloof farm in the Tulbagh district (33°20'S, 19°10'E).

3.2.1 Experiment 1. Effect of different sandy soils on the efficacy of glyphosate on a commercial ryegrass cultivar

This experiment was carried out to investigate the effect of soil type on the efficacy of glyphosate for the control of ryegrass. In this study, a pure sand (SS), soils from a pasture paddock (PS) and crop field (CS) were used. The SS soil was collected from a sand mine located near Malmesbury (33°30'S, 18°40'E). Both CS and PS soil were collected from Welgevallen Experimental Farm in Stellenbosch (33°56'S, 18°52'E). These soils were analysed for nutrient content and texture (Table 3.1 and 3.2 respectively).

Seeds of ryegrass (*L. multiflorum* cv. Agri Hilton - putative susceptible) were sown into three plastic trays (21 cm long, 15 cm wide and 8 cm deep) containing one of PS, CS and SS soils. The pots were adequately moistened with balanced nutrient solution adjusted to pH 6 (Appendix A1: Table A1.1). Two weeks after germination, seedlings (cut to a uniform size) from the plastic trays were transferred to wetted 8 cm x 8 cm pots with corresponding soil type respectively. Four seedlings per pot were transplanted. The pots were adequately irrigated with a balanced nutrient solution. The experiment was a complete randomised design replicated four times. The treatments were: soil type (PS, CS and SS); and glyphosate (360 g a.i. L⁻¹ formulation) application rate (GAR) (0 (0x), 67.5 (1/8x), 135 (1/4x), 270 (1/2x) and 540 (1x) g a.i. ha⁻¹) arranged in a 3 x 5 factorial design. The recommended glyphosate dose for seedlings in pre-sow situation is 540g a.i. ha⁻¹. Glyphosate was applied with a pneumatically driven pot spraying apparatus fitted with a flat fan 8001 nozzle. The sprayer was operated at a constant pressure of 2 bars and glyphosate was applied at 100 litres of water ha⁻¹. Care was taken that the plants were not irrigated within 24 hours of the herbicide being applied. The other three experiments were carried out in a similar way, and the parts where they differ are explained below.

3.2.2 Experiment 2. Effect of different sandy soils on the efficacy of glyphosate applied to resistant and susceptible biotypes of ryegrass

Crop soil (CS) and soil from a pasture paddock (PS), as described above, were used in the experiment. The experiment was a completely randomised design with 2 x 2 x 5 factorial arrangement with factors: soil type (PS and CS); Ryegrass biotypes (resistant (R) and susceptible (S)) and glyphosate application rate (0 (0x), 270 (1/2x), 540 (1x), 1080 (2x) and 2160 (4x) g a.i. ha⁻¹).

3.2.3 Experiment 3. Effect of nutrient content (pure distilled water and a fully balanced nutrient solution) on the efficacy of glyphosate on a commercial ryegrass cultivar grown in different sandy soils

This experiment was a 3 x 2 x 5 factorial design. The first factor was soil type (paddock soil (PS), cropping soil (CS) and sandy soil (SS)), the second factor was feeding solution (balanced nutrient solution (Appendix A1: Table A1.1) and pure distilled water) and the third factor was glyphosate application rate (GAR) (0 (0x), 67.5 (1/8x), 135 (1/4x), 270 (1/2x) and 540 (1x) g a.i. ha⁻¹).

3.2.4 Experiment 4. Effect of soil glyphosate activity in three sandy soils on the efficacy of glyphosate

A fourth experiment was conducted where the objective was to establish the effect of soil activity when applying glyphosate to foliage. To test this aspect the soil surface was either covered with cotton wool during the time of spraying or not. The covering of the soil surface would result in the effect of glyphosate on the plant being purely because of foliar absorption. In this study (See Chapter 4) it was demonstrated that the different soils exhibited varying levels of glyphosate soil activity. The experimental layout was a 3 x 5 x 2 factorial in a completely randomised design, replicated three times. The factors were: 3 soils PS, CS and SS; 5 GAR's, 0 (0x), 67.5 (1/8x), 135 (1/4x), 270 (1/2x) and 540 (1x) g a.i. ha⁻¹ and; 2 soil covering treatments where the soils were either covered with cotton wool or not covered prior to glyphosate application. Pots were covered with cotton wool to prevent glyphosate reaching the soil. To make removal of the cotton wool easy and to minimize contact between the damp cotton wool and the stems of the plants, the plants were arrayed diagonally in the pots (Plate 3.1). Immediately after spraying the cotton wool was removed carefully from the pots to prevent contact with the plants.

3.2.5 Determination of test parameters

In all experiments determination of the test parameters was similar. One week after glyphosate application shoot length was measured by measuring the height from the soil surface to the tip of the tallest leaf making use of a ruler. Percentage survival of the plants was recorded 28 days after application of glyphosate. The number of actively growing seedlings was recorded and the percentage survival was calculated. After survival rating of the plants, the above ground material were excised, oven-dried for 48 hours at 80°C and the dry mass was determined.

3.2.6 Statistical analysis

The experimental data was subjected to analysis of variance (ANOVA) using STATISTICA, software version 9 programme (Statsoft, 2009). This was performed using factorial ANOVA analyses in the PROC GLM command. Where the experiment showed significant interaction, the means were separated using Fisher's protected LSD test at $p = 0.05$.



Plate 3.1 Covering of the soil surface with cotton wool (pot on the left) before applying glyphosate to prevent soil activity of glyphosate.

Table 3.1 Soil nutrient analyses of paddock soil (PS), crop soil (CS) and sand soil (SS) before the experiment commenced and after being irrigated with a balanced nutrient solution at pH 6 for 28 days (PSPH6, CSPH6 and SSPH6)

Sample	Lab. No.	Soil	pH	Resistance (KCl) (Ohm)	Stone (Vol %)	P Bray II mg/kg	K mg/kg	Exchangeable cations (cmol(+)/kg)				Cu	Zn	Mn	B	Fe	Ca %
								Na	K	Ca	Mg						
Paddock soil (PS)	15943	Sand	7.0	550	1	294	479	0.19	1.23	13.24	1.93	4.60	26.8	31.1	0.32	149.82	2.48
PS pH6	15945	Sand	7.1	520	2	312	435	0.20	1.11	13.41	1.98	5.34	34.6	23.7	0.36	136.96	2.27
Crop soil (CS)	15947	Sand	6.4	1380	1	81	113	0.09	0.29	3.56	0.87	2.54	4.2	15.9	0.14	74.79	0.55
CS pH6	15949	Sand	6.9	1630	1	78	143	0.12	0.37	5.28	0.85	2.33	4.4	15.4	0.07	85.64	0.63
Sand soil (SS)	15951	Sand	8.6	4380	5	33	14	0.06	0.04	12.90	0.26	0.06	0.4	0.7	0.05	10.50	0.10
SS pH6	15953	Sand	8.6	3410	4	32	9	0.08	0.02	12.16	0.25	0.01	1.3	0.5	0.06	11.84	0.13

Table 3.2 Physical properties of three sandy soils (paddock soil (PS), crop soil (CS) and sand soil (SS)) used in the study

Sample	Lab. No.	Clay %	Silt %	Sand %	Fine Sand %	Medium Sand %	Rough Sand %	Classification
Crop soil	1847	1.2	11.0	87.8	64.6	18.60	4.60	Sand
CS								
Paddock soil	1848	0.0	5.2	94.8	59.8	22.20	12.80	Sand
PS								
Sand soil	1849	0.2	0.0	99.8	56.0	24.20	19.70	Sand
SS								

Table 3.3 Analysis of nutrient content on the leaves of susceptible plants grown in paddock soil (PS), crop soil (CS) and sand soil (SS) at pH 4, 6 and 8

pH	Soil	N	P	K	Ca	Mg	S	Na	Mn	Fe	Cu	Zn	B	Mo
		%							mg/kg				μg/kg	
4	PS	4.01	0.51	5.65	0.96	0.33	0.28	1962	21	303	9	109	32	2325
4	CS	2.64	0.53	9.01	1.02	0.54	0.30	1664	45	282	10	69	37	2239
4	SS	3.49	0.38	6.67	1.03	0.47	0.28	2122	53	207	8	87	50	4277
6	PS	2.46	0.55	5.66	0.73	0.31	0.33	1703	26	206	9	51	22	2311
6	CS	3.63	0.46	7.95	0.96	0.46	0.28	1285	37	133	8	50	32	2179
6	SS	3.44	0.42	10.36	1.09	0.65	0.30	2049	60	171	7	42	58	4041
8	PS	2.81	0.48	7.72	0.91	0.45	0.33	4168	26	221	7	64	23	2795
8	CS	3.92	0.49	8.94	0.87	0.54	0.32	3929	49	229	8	56	33	2284
8	SS	2.64	0.32	8.52	1.13	0.54	0.33	4431	47	756	8	53	46	4329

3.3 Results and Discussion

The dry mass data will not be discussed in this section because the effect of only one plant surviving per pot complicates the effect of glyphosate on the total dry mass per pot. This effect is exacerbated by the differential growth rates of the plants in the different soils and the resulting data detracts from the relatively clear trends indicated by the survival data. The same applies to the shoot length data. Since the main objective of applying herbicide is to control weeds by killing them the results presented and the discussion thereof in this section will focus exclusively on the survival data.

3.3.1 Experiment 1. Effect of different sandy soils on the efficacy of glyphosate on a commercial ryegrass cultivar

There was a rate-mediated increase in the efficacy of glyphosate as expressed by survival rate of the plants. This was shown by a significant interaction between glyphosate application rate and soil type ($p < 0.05$). In all soils there was a trend of decrease in survival with increase in glyphosate application rate (GAR). However, ryegrass treated with 1/2x GAR showed a significantly lower survival rate in PS soil compared to CS and SS soil with 19, 50 and 62 % survival respectively (Figure 3.1). The increase of glyphosate in the tissue content is the probable reason for improved efficacy with increase in dose because glyphosate treated seedlings will die when all tissue have been killed (Feng *et al.*, 2004). Any surviving tissue provides the opportunity to regrow under favourable conditions (Feng *et al.*, 2003). This is due to the totipotency in higher plant cells (Vasil & Vasil, 1972). Survival at low concentrations is a result of low tissue concentrations of glyphosate. Thus Feng *et al.* (2004) indicated that the fate of individual tissues is dependent on whether sufficient glyphosate concentrations were attained to cause phytotoxic effects (or inhibition of activity of EPSPS).

The increased efficacy of glyphosate in the PS soil is probably due to the fact that the seedlings were growing more vigorously in the PS soil as indicated by the shoot length and dry mass data (results not shown). This can be attributed to the higher nutrient content of the PS soils, even after all three soils were irrigated with a balanced nutrient solution for the duration of the experiment (Table 3.1). Mithila *et al.* (2008) showed that low nutrient levels that put plants under stress reduced the efficacy of glyphosate.

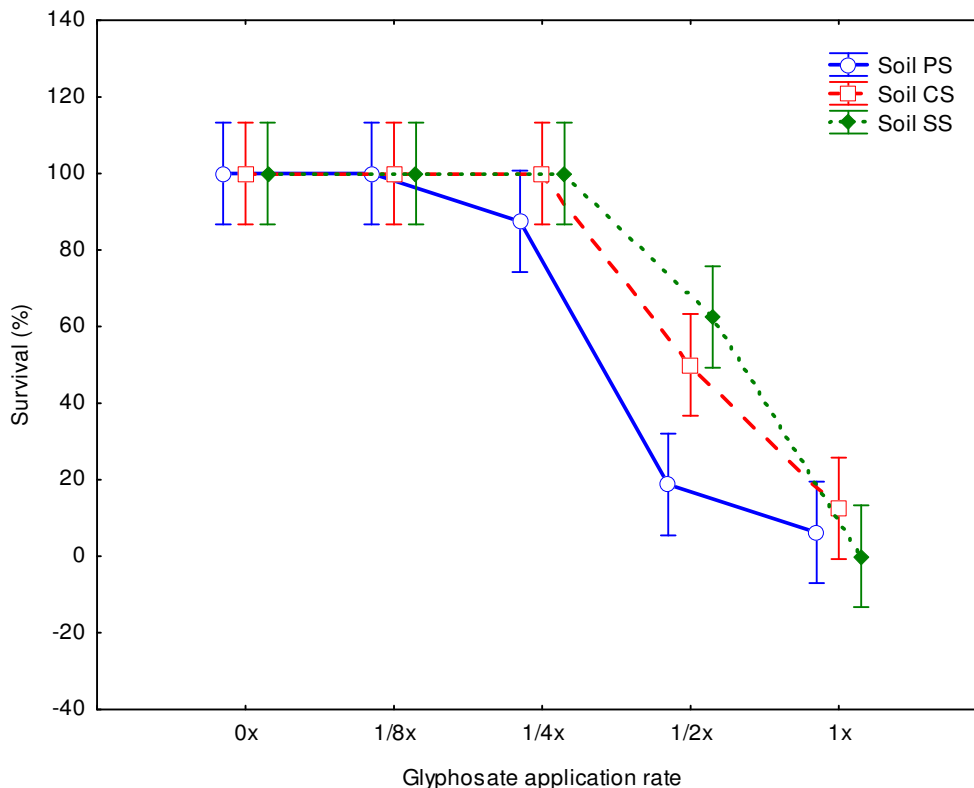


Figure 3.1 Reduction in percentage survival of ryegrass seedlings grown in different soil types (PS = paddock soil, CS = crop soil and SS = pure sand) with increase in glyphosate application rate (0 (0x), 67.5 (1/8x), 135 (1/4x), 270 (1/2x) and 540 (1x) g a.i. ha⁻¹). The vertical bars represent the standard error (1±SE) of the mean of each treatment.

Glyphosate efficacy is determined by its efficient inhibition of the EPSPS (Feng *et al.*, 2003). In this study effects of glyphosate activity were observed in terms of plant mortality. However, the response of ryegrass on different soils showed variation. This is a confirmation that weed growth conditions play a role in glyphosate efficacy. Steward *et al.* (2010) reported that environment and soil condition play a role in efficacy of pre- and post-emergence herbicides.

In the current study the soils were not significantly different in texture (they all were classified as sands) (Table 3.2). Visual observation, however, showed that SS soil was pure sand (coarse and more porous) and the applied nutrient solution infiltrated easily into the soil particles. The PS and CS soils felt and appeared more closely related to clay soil than sand soils. Upon wetting PS soil retained water better than the CS soil. Thus, according to visual

observation, the latter soils would not fit the definition of sandy soil– very porous soil with large spaces between the particles (Davis & Wilson, 2007).

Although all three soils were classified as sand soils, the percentage of sand type (coarse, fine and medium sand) content varied. The results of the physical analysis showed that the SS soil had a high content of coarse sand (19.70%), followed by PS soil (12.80 %) and CS soil (4.60 %). Fine sand percentage in the SS (56%) soil was lower, followed by 59.8% in PS soil and CS had 64.6 %. This could explain the reason of finer texture (according to the feel) in the latter two soils. The total sand content of the SS soil was higher (99.8%) followed by PS soil (94.8 %) and SS soil (87.8%). In addition, differences in nutrient content between soils may have contributed in the variation observed. The PS soil had higher nutrient content than the CS and SS soils, and seedling grown in this soil had increase shoot length and dry weight compared to the other soils (results not shown).

3.3.2 Experiment 2. Effect of different sandy soils on the efficacy of glyphosate applied to resistant and susceptible biotypes of ryegrass

Analysis revealed a significant ($p < 0.05$) two-way interaction between soil type and population in terms of percentage survival (Figure 3.2). The S biotype exhibited high mortality compared to the R biotype, this was regardless of the soil type (not significant at $p = 0.05$). This, however, in the R population was not the case as the PS soil showed a significantly ($p < 0.05$) higher percentage survival compared to the CS soil. The increased survival rate of the plants in the PS soil can possibly be ascribed to the increased content of Mn, Fe and Cu in this medium.

The survival advantage in the R biotype over the S biotype was contributed by the resistance mechanism that exists in the R population. Webster and Sosnokie (2010) referred to weed tolerance and resistance as one of the ways in which weeds escape herbicide control. Resistance of weeds to glyphosate appeared due to selection of rare resistance traits. The selection pressure arises from overreliance and high glyphosate application which are common in glyphosate resistant crops and perennial crops (Tranel, 2005; Hidayat *et al.*, 2006). Travlos and Chachalis (2010) reported that overreliance on glyphosate can increase the risk of reduced efficacy on weeds and weed resistance.

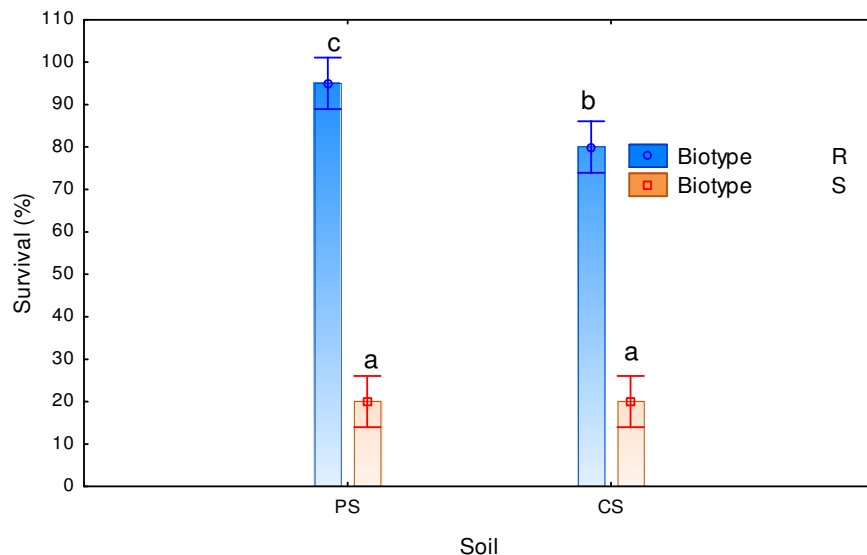


Figure 3.2 Percentage survival of resistant (R) and susceptible (S) ryegrass biotypes grown in PS (paddock soil) and CS (cropping soil). The vertical bars represent the standard error ($1\pm SE$) of the mean of each treatment. Bars denoted with the same letter do not differ significantly at $p = 0.05$.

The significantly higher survival rate of the resistant biotype grown in PS soil is difficult to explain. In the previous experiment glyphosate had a higher efficacy on plants growing in PS soils (Fig 3.1). In this case however, glyphosate had a higher efficacy on plants growing in CS soils. It is possible that resistant plants may react differently to the higher nutrient content in PS soil than susceptible plants. The PS soil is nutrient rich with high cation exchange capacity (CEC) and very high micronutrient contents compared to the other two soils (Table 3.1). Depending on the mechanism of resistance in these plants, high nutrient content could possibly lead to an enhanced metabolism rate of glyphosate in the plants. In the case of polygenic non-target site resistance mechanisms enhanced growth rates could increase the rate of detoxification or sequestration of glyphosate.

There was also a two-way interaction of GAR and biotype (Figure 3.3). Increase in GAR significantly affected the S population. At 1/2x the recommended dose, 100% control was achieved. The R population was poorly controlled. Significant differences between survival rates of the untreated control (0x) and the treated plants was observed at 2x and 4x GAR with 84% and 76% survival respectively. This is an indication of the high level of resistance existing in this population where even four times the recommended application rate could not control the plants satisfactorily.

The effect of soil on the efficacy of glyphosate is somewhat obscure. This is due to indirect relationships between the working of post-emergence herbicide and soil. With regards to soil: Mithila *et al.* (2008) studied the effect of nitrogen on the efficacy of glyphosate; and Adkins *et al.* (1998) studied the effect of soil moisture content, irradiance, temperature and relative humidity on glyphosate efficacy. Several studies have reported that high moisture deficit reduced glyphosate efficacy (Dickson *et al.*, 1990). Efficacy of glyphosate increased with increase in soil moisture in johnson grass (*Sorghum halepense* (L.)) (Sivesand *et al.*, 2011). However, none of the studies have looked on the effect of soil texture on glyphosate efficacy for weed control.

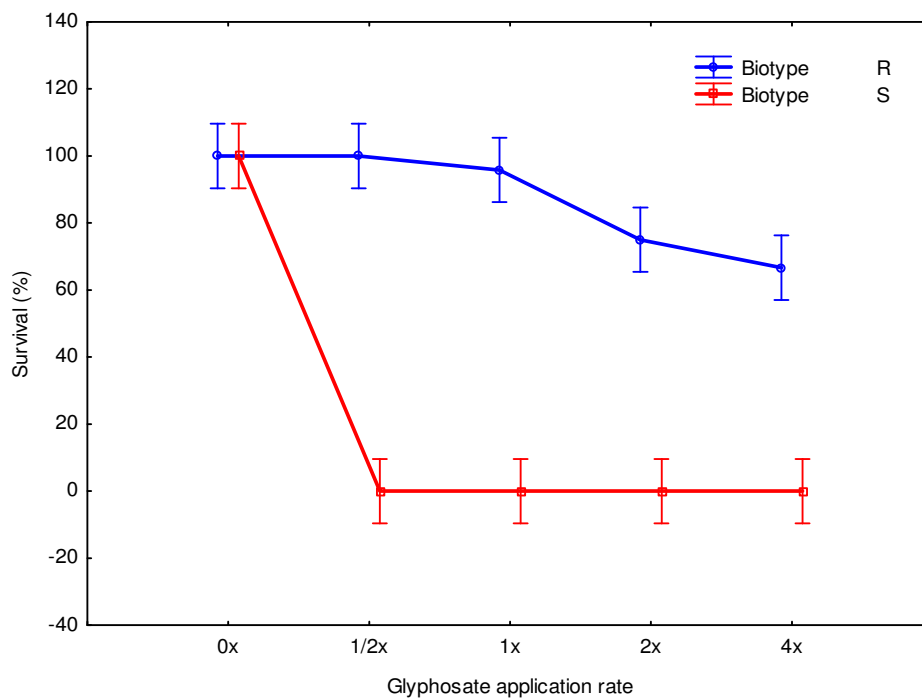


Figure 3.3 Effect of glyphosate application rate (0 (0x), 270 (1/2x), 540 (1x), 1080 (2x) and 2160 (4x) g a.i. ha⁻¹) on the efficacy of glyphosate as measured by percentage survival of the resistant (R) and susceptible (S) ryegrass biotypes. The vertical bars represent the standard error (1±SE) of the mean of each treatment.

3.3.3 Experiment 3. Effect of nutrient content (pure distilled water and a fully balanced nutrient solution) on the efficacy of glyphosate on a commercial ryegrass cultivar grown in different sandy soils

There was a difference in the percentage survival of the seedlings irrigated with a balanced nutrient solution and those irrigated with pure distilled water. This was revealed in a

significant ($p < 0.05$) three-way interaction between soil type, glyphosate application rate (GAR) and nutrient level (water or balanced nutrient solution) (Figure 3.4). There was a decrease of about 60 % in percentage survival of the CS and SS soil grown (nutrient fed) seedlings at 1/8x GAR compared to the untreated control; whereas in the PS soil, survival was decreased by almost 100% at the same GAR. Further increase in GAR showed no significant difference in the percentage survival of seedling in all soils of the nutrient solution treatment. The response of PS grown seedlings to GAR was the same with or without the nutrient solution. It showed high efficacy of glyphosate in this soil despite the nutrient content of the feeding solution. The high nutrient content in the PS soil probably contributed to the lack of significant differences in glyphosate efficacy between pure water and nutrient solution irrigated seedlings (i.e. pure water irrigated seedlings may have been exposed to less stress due to nutrient availability in soil). The percentage survival of seedling grown in the CS and SS soil (water treatment) were significantly different from the PS soil at the 1/8x GAR with about 70% and 100% percentage survival respectively. Further increase of GAR in the CS soil (pure water irrigated) grown seedlings did not result in significant different from nutrient solution irrigated seedlings. An increase at 1/4x GAR by 40% in pure water irrigated seedlings compared with 1/4x GAR in nutrient solution irrigated seedlings was observed in the SS soil treatment. At 1/2x GAR, although not significant different, there was 26% survival in pure water irrigated seedlings compared to 0% in the nutrient solution irrigated seedlings in the SS soil. Cathcart *et al.* (2004) reported that weeds growing in nutrient poor soils require higher herbicide doses compared to those growing in nutrient rich areas.

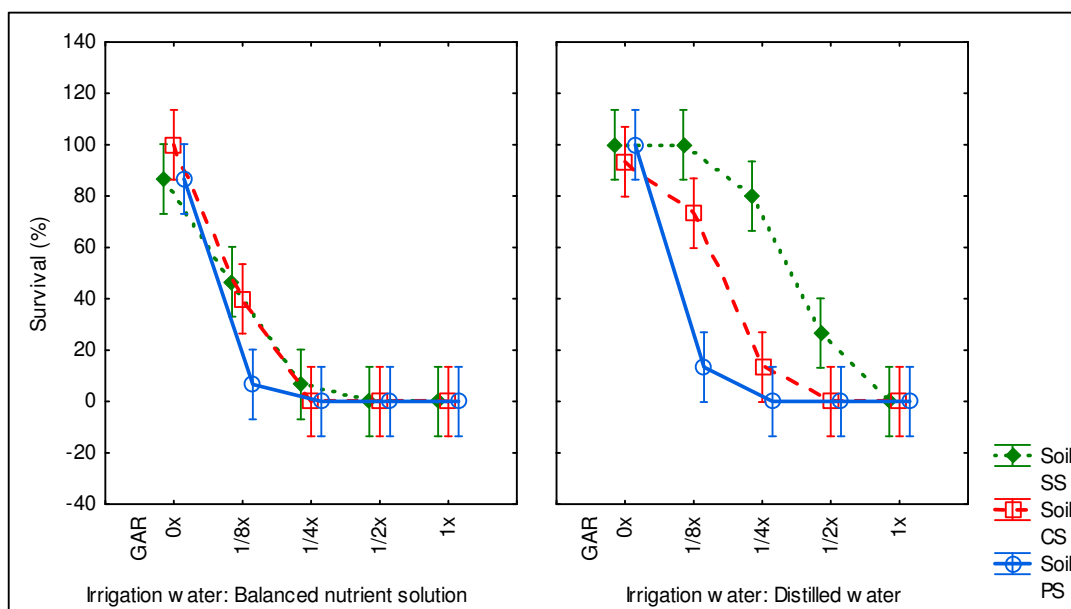


Figure 3.4 Effect of nutrient application with irrigation water (Pure distilled water and balanced nutrient solution) on the percentage survival of ryegrass seedlings in sandy soil (SS), crop soil (CS) and paddock soil at different glyphosate application rates (GAR) (0 (0x), 67.5 (1/8x), 135 (1/4x), 270 (1/2x) and 540 (1x) g a.i. ha⁻¹). The vertical bars represent the standard error (1±SE) of the mean of each treatment.

The difference observed in response to glyphosate of seedlings grown in these soils in the pure water treatment, may have emanated from differences in nutrient content (Table 3.1). This might have posed as a stress factor and resulted in poor efficacy of glyphosate. Post- and pre-emergence herbicides according to Mithila *et al.* (2008) are influenced by nutrient status especially nitrogen (N). It also is reported that weeds in agronomic soil benefit more from nutrient application compared to the crops themselves (Mashingaidze, 2004). This is due to the low nutritive value of weeds (Abaye *et al.*, 2009). Some of the nutrients are important components of chlorophyll such as N (major component of chlorophyll), copper (Cu), manganese (Mn), Zinc (Zn), magnesium (Mg) and potassium (K) (Uchida, 2000; Renuka & Chimmad, 2006). The lack of these nutrients may result in decreased photosynthesis and hence decreased photosynthate (sucrose) to be translocated through the phloem and thus impeding translocation of glyphosate too.

These results are an indication that response of the seedlings to glyphosate application is not only dependent on environmental growth conditions (Krausz *et al.*, 1996; Adkins *et al.*, 1998) but also on nutrient status and the soil texture (Cathcart *et al.*, 2004). Adkins *et al.* (1998) reported that the efficacy of glyphosate was reduced by adverse environmental effects. From the report of Mithila *et al.* (2008) low N results in decreased efficacy of glyphosate on common lambsquatter (*Chenopodium album* L. CHEAL) and velvetleaf (*Abutilon theophrasti* Medic. ABUTH). Low N was found to reduce photoassimilate synthesis and its translocation thus resulting in the concomitant decrease in glyphosate translocation. Glyphosate in many plants follows the source and sink translocation pattern similar to photoassimilates (Martin & Edgington, 1981). Also the soil itself has played a role in the response of seedlings to GARs. Krausz *et al.* (1996) reported that glyphosate efficacy improved under favourable growth conditions. Soil texture was also reported as playing a role in glyphosate efficacy (Cathcart *et al.*, 2004).

The tested soils being of similar texture, in this study, their ability to withhold water was different, probably due to more gravel sand in SS soil compared to the lesser proportion

in the CS and PS soil (Table 3.2). This was additionally being impeded by the different nutrient content that is available in the PS soil which was high compared to CS and SS soils. Nutrients that are involved in chlorophyll synthesis (Cu, Mn, Zn, Mg and K) (Uchida, 2000) were available in abundance in PS soil. Hence irrigating with distilled water may not have resulted in photosynthate decrease leading to decreased glyphosate translocation. Nutrient deficient plants seem to have a survival advantage as observed in plants growing in SS soil compared to plants growing in the CS soil and even more to plants growing in the PS soil.

3.3.4 Experiment 4. Effect of soil glyphosate activity in three sandy soils on the efficacy of glyphosate

Percentage survival showed a trend of decrease with increased GAR. The decrease in percentage survival varied with soil type and soil surface covering treatments. This was shown by a significant ($p = 0.01$) three-way interaction between soil surface covering, GAR and soil type. No significant difference was observed when the CS soil surface was covered or uncovered in the 1/8x and 1/4x GAR. In the SS soil a significant difference in the percentage survival of seedlings was observed at 1/4x GAR between covered and uncovered soil. In the PS soil, survival at the 1/8x and 1/4x GAR's were significantly lower in the uncovered treatments than in the covered treatments.

Soils covered with cotton wool should yield reduced glyphosate efficacy compared to the uncovered soil. This is due to the fact that in the uncovered soil, the amount of glyphosate in the plant will be sum of the foliar retained glyphosate and the amount absorbed by the roots from soil. Cornish and Burgin (2005) reported that glyphosate is absorbed through the roots. In the CS soil there was no significant difference between the percentage survival of seedlings grown in cotton wool covered and uncovered soil (Figure 3.5). This could be due to the CS soil being the only soil with a (albeit low) clay content. It is possible that the small amount of clay in the CS soil could have adsorbed enough of the glyphosate entering the soil to negate the soil action of the glyphosate. The PS and SS soils showed differences in the percentage survival of seedlings growing in the covered and uncovered soil. A difference in control was shown in the PS soil at 1/8x GAR with percentage survival of 93.3% when the soil was covered compared to the 60 % survival percentage in the uncovered soil. In the same soil, at 1/4x GAR there was 53.3% survival in covered soil compared to 13.3 % survival in the uncovered soil. In SS soil an opposite effect was shown at 1/4 x GAR with 0% and 40% survival in the covered and uncovered soil respectively. These in the PS soil results indicate that root absorption of glyphosate played a role in these treatments. The fact that the

seedlings were watered with a balanced nutrient solution probably prevented significant differences between the PS and SS treatments at 1/8x and 1/4x GAR's in the uncovered treatment. The significantly better control in the SS treatment in the covered treatment is inexplicable. The fact that the percentage survival at the 0% GAR was about 60% may be an indication of an unknown factor that influenced the survival of the plants in the SS soil. This aspect should be investigated further.

3.4 Conclusions

Efficacy of glyphosate is influenced by several factors such as weed growth stage, environmental and growth conditions. Covering the soil to prevent any glyphosate reaching the soil seems to have an impact on glyphosate efficacy. This effect is an indication that glyphosate, upon reaching the soil does not become completely inactive. A report on effect of soil-applied glyphosate on wheat and soybean validates this (Devlin *et al.*, 1986).

This study showed that soil type or components (nutrient status) relating to soil affect post-emergence applied glyphosate indirectly. They do so by putting the plant under stress or survival advantage (such as growth in the PS soil) and thus affecting glyphosate efficacy negatively. In this study, findings showed that seedlings response to glyphosate varied between soils. The variation could have been brought about by the soil nutrient content. This clearly indicates that the nutrient content plays a significant role in glyphosate efficacy. In light of this, glyphosate application rate would need to be altered so as to suit the weed population growing in varied soils of different nutrient content. This study also shows the importance of fertilization on the efficacy of glyphosate in experiment 3. This shows that glyphosate application in nutrient deficient soils would need to be higher than the normal recommended rate.

The recommended lethal dose of glyphosate in normal fertile soil could therefore be a sub-lethal dose in nutrient poor soils. This could lead to favourable conditions for the development of polygenic non-target site resistance (Moss, 2002).

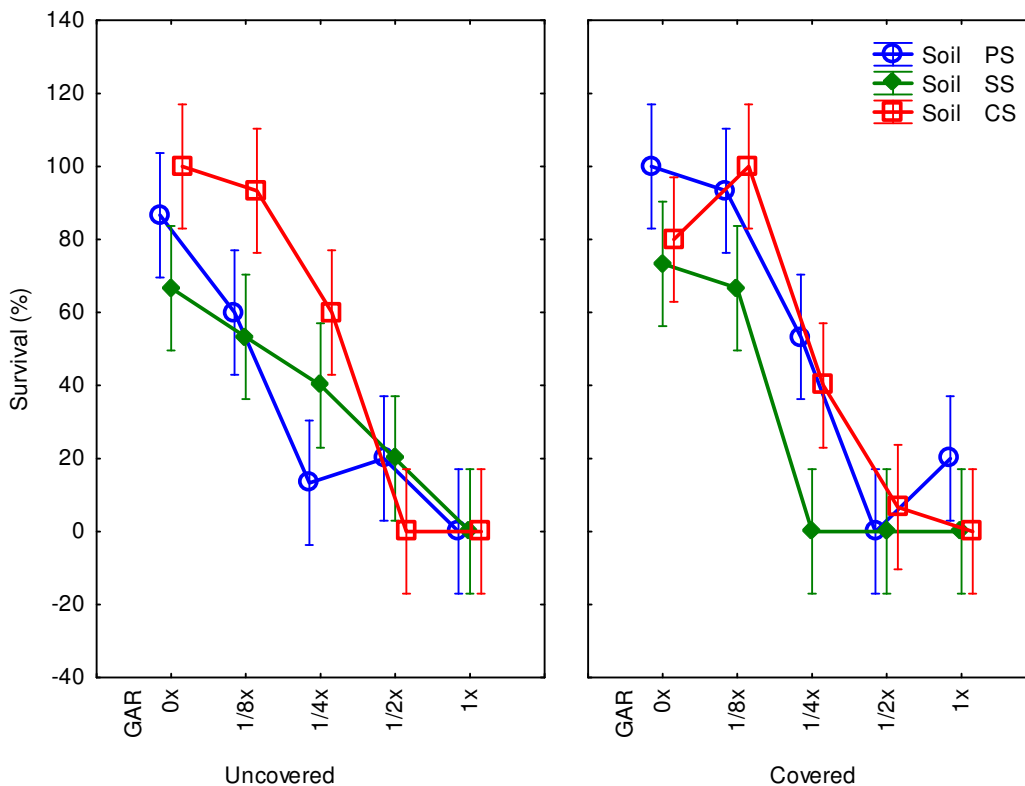


Figure 3.5 Percentage survival of seedling grown in paddock soil (PS), cropping soil (CS) and sand soil (SS). The soil were either covered (with cotton wool) or not covered when glyphosate was applied at glyphosate application rates (GAR) of 0 (0x); 67.5 (1/8x); 135 (1/4x); 270 (1/2x); and 540 (1x) g a.i. ha⁻¹. The vertical bars represent the standard error (1±SE) of the mean of each treatment.

References

- ABAYE, O.A., SCAGLIA, G., TEUTSCH, C. & RAINES, P., 2009. The nutritive value of common pasture weeds and their relation to live stock nutrient requirement. <http://pubs.ext.vt.edu/418/418-150/418-150.pdf>.
- ADKINS, S.W., TANPIPAT, S., SWARBRICK, J.T. & BOERSMA, M., 1998. Influence of environmental factors on glyphosate efficacy when applied to *Avena fatua* or *Urochloa panicoides*. *Weed Res.* 38, 129-138.
- BLUMHORST, M.R., WEBER, J.B. & SWAIN, L.R., 1990. Efficacy of selected herbicides as influenced by soil properties. *Weed Technol.* 4, 279-283.

- CATHCART, R.J., CHANDLER, K. & SWANTON, C.J., 2004. Fertilizer nitrogen rate and the response of weeds to herbicides. *Weed Sci.* 52, 291-296.
- CORNISH, P.S. & BURGIN, S., 2005. Residual effects of glyphosate herbicide in ecological restoration. *Restor. Ecol.* 13, 695-702.
- DAVIS, J.G. & WILSON, C.R., 2007. Choosing a soil amendment. <http://www.ext.colostate.edu/pubs/garden/07235.html>.
- DEVLIN, R.M., KARCZMARCZYK, S.J., ZBIEC, I.I. & KOSZANSKI, Z.K., 1986. Initial and residual activity of glyphosate and SC-0224 in sandy soil. *Crop Prot.* 8, 293-296.
- DICKSON, R.L., ANDREW, M., FIELD, R.J. & DICKSON, E.L., 1990. Effects of water stress, nitrogen and gibberellic acid on fluazifop and glyphosate activity on oats (*Avena sativa*). *Weed Sci.* 38, 54-61.
- FENG, P.C.C., CHIU, T. & SAMMONS, R.D., 2003. Glyphosate efficacy is contributed by its tissue concentration and sensitivity in velvetleaf (*Abutilon theophrasti*). *Pest Biochem. Physiol.* 77, 83-91.
- FENG, P.C.C., TRAN, M., CHIU, T., SAMMONS, R.D., HECK, G.R. & CAJACOB, C.A., 2004. Investigations into glyphosate-resistant horseweed (*Conyza canadensis*): retention, uptake, translocation, and metabolism. *Weed Sci.* 52, 498-505.
- HIDAYAT, I., BAKER, J. & PRESTON, C., 2006. Pollen mediated gene flow between paraquat-resistant susceptible hare barley (*Hordeum leporinum*). *Weed Sci.* 54, 685-689.
- KRAUSZ, R.F., KAPUSTA, G. & MATTHEWS, J.L., 1996. Control of annual weeds with glyphosate. *Weed Technol.* 10, 957-962.
- MARTIN, R.A. & EDGINTON, L.V., 1981. Comparative systemic translocation of several xenobiotics and sucrose. *Pest. Biochem. Physiol.* 16, 87-97.
- MASHINGAIDZE, A.B., 2004. Improving weed management and crop productivity in maize systems in Zimbabwe. Ph.D. Thesis, Wageningen University.
- MITHILA, J., SWANTON, J.C., BLACKSHAW, R.E., CATHCART, R.J. & HALL, C., 2008. Physiological basis for reduced glyphosate efficacy on weeds grown under low soil nitrogen. *Weed Sci.* 56, 12-17.
- MOHR, K., SELLERS, B.A. & SMEDA, R.J., 2007. Application time of day influences glyphosate efficacy. *Weed Tech.* 21, 7-13.
- MOSS, S.R., 2002. Herbicide-resistant weeds. In: R.E.L. Nayler (Ed.) *Weed management handbook*. John Wiley & Sons, U.K.

- NANDULA, V.K., 2010. Herbicide resistance: definitions and concepts. *In*: V.K. Nandula (ed.). Glyphosate resistance in crops and weeds: History, development and management. John Wiley & Sons. Hoboken.
- RENUKA, K. & CHIMMAD, V.P., 2006. Effect of irrigation at different post anthesis stages on stay green trait in Rabi sorghum genotypes in relation to chlorophyll, nitrogen and sugar. *Karnal Journal of Agricultural Science* 19, 523-528.
- SINGH, S. & SINGH, M., 2004. Effect of growth stage on trifloxysulfuron and glyphosate efficacy in twelve weed species of citrus groves. *Weed Technol.* 18, 1031-1036.
- SIVESAND, E.C., GASKA, J.M., JESCHKE, M.R., BOERBOOM, C.M. & STOLTENBERG, D.E., 2011. Common lambsquarters response to glyphosate across environments. *Weed Technol.* 25, 44-50.
- STATSOFT, 2009. STATISTICA (data analyses software systems) version 9, StatSoft. Inc., Tulsa, Oklahoma, USA.
- STEWARD, C.L., NURSE, R.E., HAMILL, A.S. & SIKKEMA, P.H., 2010. Environment and soil conditions influence pre- and post-emergence herbicide in soybean. *Weed Technol.* 24, 234-243.
- STOUGGARD, R.N., SHEA, P.J. & MARTIN, A.R., 1990. Effect of soil type and pH on adsorption, mobility, and efficacy of imazaquin and imazethapyr. *Weed Sci.* 38, 67-73.
- TRANEL, P.J., 2005. Glyphosate resistant weeds in Illinois: Down the road or right around the corner? *Illinois Crop Protection Technology Conference*. University of Illinois Extension College of Agricultural and Environmental Sciences.
- TRAVLOS, I.S. & CHACHALIS, D., 2010. Glyphosate-resistant hairy fleabane (*Conyza boraniensis*) is reported in Greece. *Weed Technol.* 24, 569-573.
- UCHIDA, R., 2000. Essential nutrients for plant growth: Nutrient functions and deficiency symptoms. *In*: J.A. Silva & R. Uchida (eds.). Plants nutrient management in Hawaii soils, approaches for tropical agriculture and human resources. University of Hawaii, Manoa, USA.
- VASIL, I.K. & VASIL, V., 1972. Totipotency and embryogenesis in plant cell and tissue cultures. *In Vitro Cell. Dev. Biol.* 3, 117 - 125.
- WALTZ, A.L., MARTIN, A.R., ROETH, F.W. & LINDQUIST, J.L., 2004. Glyphosate efficacy on velvetleaf varies with application time of the day. *Weed Technol.* 18, 931-939.

- WEBSTER, T.M. & SOSNOKIE, L.M., 2010. Loss of glyphosate efficacy: A changing weed spectrum in Georgia cotton. *Weed Sci.* 58, 73-79.
- ZHOU, J., TAO, B. & MESSERSMITH, C.G., 2006. Soil dust reduces glyphosate efficacy. *Weed Sci.* 54, 1132-1136.
- ZHOU, J., TAO, B., MESSERSMITH, C.G. & NALEWAJA, J.D., 2007. Glyphosate efficacy on velvetleaf (*Abutilon theophrasti*) is affected by stress. *Weed Sci.* 55, 240-244.

CHAPTER 4

PHYTOTOXICITY OF SOIL APPLIED GLYPHOSATE ON RYEGRASS (*LOLIUM MULTIFLORUM*) IN THREE SANDY SOILS

Abstract

Despite glyphosate being well known for ‘no soil activity’, there is a number of reports that negate this. Reduction in growth, germination and dry mass had been recorded as a result of glyphosate soil phytotoxic activity. To investigate this, seeds of a commercial ryegrass cultivar (*Lolium multiflorum* cv Agri-Hilton) were germinated in a greenhouse in three different sandy soils (pasture paddock soil (PS), cropping soil (CS) and pure sand (SS)), and was irrigated with a balanced nutrient solution. Glyphosate (360 g L⁻¹ a.i. formulation) were applied to pots containing the same sandy soils mentioned above at three different application rates (G1 = 0 g a.i. ha⁻¹, G2 = 540 g a.i. ha⁻¹ and G3 = 3240 g a.i. ha⁻¹). Ryegrass seedlings were transplanted into the treated soil at three times after spraying (TAS) viz. 2 hours, 3 weeks and 4 weeks after glyphosate application. Percentage survival, length and dry mass of seedlings transferred to glyphosate treated soil were investigated 28 days after being transplanted into the glyphosate treated soil. Percentage survival decreased with increase in glyphosate application rate (GAR). The effect of increase in GAR on ryegrass seedlings was more significant in the SS soil compared to CS and PS soils. Seedlings exhibited an increase in percentage survival with increase in time after GAR. The trend varied with soil type with the effect of high GAR still prominent in SS soil at four weeks TAS. Dry mass accumulation increased with increase in time after application (TAS) proportional to the GAR. Shoot length reacted similarly in response to treatment. Soil type (slight variation in texture and large variation in chemical characteristics) therefore appeared to influence phytotoxicity of glyphosate on transplanted seedlings with the most sandy soil (SS) that was also the least fertile, having the most detrimental effects on seedling growth.

Keywords: Glyphosate, phytotoxicity, ryegrass, sandy soils, time after spraying

4.1 Introduction

For a long time glyphosate has been dubbed an ‘environmentally friendly’ herbicide. This follows reports that it is readily degradable and sometimes strongly adsorbed to the soil matrix when it is sprayed onto soil or makes it to the soil through decomposing plant material (Campbell, 1974; Egley & Williams, 1978; Segura *et al.*, 1978; Devlin *et al.*, 1986; Cornish,

1992; Borggaard & Gimsig, 2008; Tesfamariam *et al.*, 2009) thus, making glyphosate a practically immobile herbicide and leaching almost improbable (Laitinen *et al.*, 2007). It is due to this lack of residual activity that glyphosate has been commonly used as a post-emergence herbicide. Despite reports that glyphosate is environmentally friendly, Klingman and Murray (1976) and Baylis (2000) reported that there were several cases of glyphosate activity found in soil.

Cases of glyphosate soil activity were speculated as a result of one of the following (i) an acidic form of glyphosate that does not easily degrade in soils; (ii) poor sorption capacity of some soils or (iii) leaching of undegraded glyphosate into natural water bodies. Reports from Canada, Germany and Netherlands have shown that glyphosate and its metabolite, aminomethyl-phosphonic acid (AMPA) were found in water (Ludvigsen *et al.*, 2003; Struger *et al.*, 2008). Despite all these cases Cerdeira and Duke (2006) reported that information on leaching of glyphosate at the field scale is still limited.

Where significant amounts of glyphosate are used, its persistence in soil could be a problem and pre-plant application threatened (weed control prior to crop planting) or an interval before spraying and surface sowing should be allowed after herbicide application (Campbell, 1974). Crop failure was reported as result of glyphosate availability in soil (Ruepel *et al.*, 1977; Ludvigsen *et al.*, 2003; Kjaer *et al.*, 2005; Vereecken, 2005; Cerdeira & Duke, 2006; Chen *et al.*, 2007; Laitinen *et al.*, 2007; Struger *et al.*, 2008). In these cases there was a characteristic decrease in growth, for example a reduction in soybean growth following glyphosate application in soil and a reduction of wheat growth due to glyphosate application in sandy soil (Devlin *et al.*, 1986). Cornish and Burgin (2005) reported that glyphosate is absorbed through the roots.

In soils, activity varies according to the soil type based on composition and properties and its nutrient contents. In a study on soybeans, glyphosate degradation was more rapid in loamy soil compared to sandy clay loam soils (Sprankle *et al.*, 1975). Clay plays an important role in glyphosate adsorption (Strange-Hansen *et al.*, 2004). More nutrients are found in clay soils than in sandy soils. Soil nutrients important to glyphosate adsorption are copper, phosphate and amorphous iron content (Mamy & Barriuso, 2005). Along railway lines glyphosate was found strongly adsorbed to iron (an abundant metal in material of railway embankments) (Torstensson *et al.*, 2005).

Phosphate also plays a major role in glyphosate adsorption. The higher the phosphate content the lesser is the adsorption capacity of glyphosate due to competition for adsorption sites. Glyphosate adsorption into soil colloids is mainly through its phosphonic moiety and the soil available phosphate could exclude glyphosate from sorption sites (Mamy & Barriuso, 2005). In addition, persistence of herbicides in soils is affected by the acidity and alkalinity of the herbicide itself and the soil. This in turn could affect the effectiveness and duration of weed control (Aledesanwa & Akinbobola, 2008).

Persistence of glyphosate in soils also depends on glyphosate half-life. Half-life is defined as the time it takes for a decaying substance to decrease by half (Nilsson, 2009). The half-life of glyphosate varies greatly, and this is attributed to variation (microbial or adsorption properties of the soils) in the sites where the herbicide is found. The ranges of half-life of glyphosate varied from: 3-22.8 days, 1-174 days and 11-17 days in different studies. Determination of the half-life has been through radioactive tracking of the radiolabelled ^{14}C in glyphosate compounds (Grunewald *et al.*, 2001; Anon, 2005; Kolpin *et al.*, 2006).

Apart from glyphosate being sequestered from availability due to sorption the amount of glyphosate can also decrease through microbial and chemical degradations. Degradation of glyphosate is a two-way path (Figure 4.1) that yields environmentally friendly products. Microbial degradation of glyphosate played a bigger role than chemical degradation in different studies. The products of degradation result in the release of naturally occurring substances (hence environmentally friendly) (Borggaard & Gimsig, 2008). The rate of carbon dioxide evolution is used as a measure of herbicide breakdown (Rueppel *et al.*, 1977; Smith & Aubin, 1993; Simonsen *et al.*, 2008). Aminomethyl-phosphonic acid (AMPA) and sarcosine (Figure 4.1) are the major metabolites of glyphosate. It is further degraded in the soils by soil microorganisms into water, carbon dioxide and phosphate. Slow degradation of glyphosate to its component metabolites is blamed to glyphosate binding to the soil colloids making it difficult for chemical and microbial degradation to occur (Balthazor & Hallas, 1986; Smith & Aubin, 1993; Veiga *et al.*, 2001). Some of the microorganisms involved in the degradation of glyphosate include bacteria of *Pseudomonas* species, *Flavobacteria* species, *Arthrobacteria atrocyaneus*, *Achromobacter* and *Rhizobia* species while chemical

degradation of glyphosate is by photolysis (Balthazor & Hallas, 1986; Liu *et al.*, 1991; Forlani *et al.*, 1999; Veiga *et al.* 2001; Chen *et al.*, 2007).

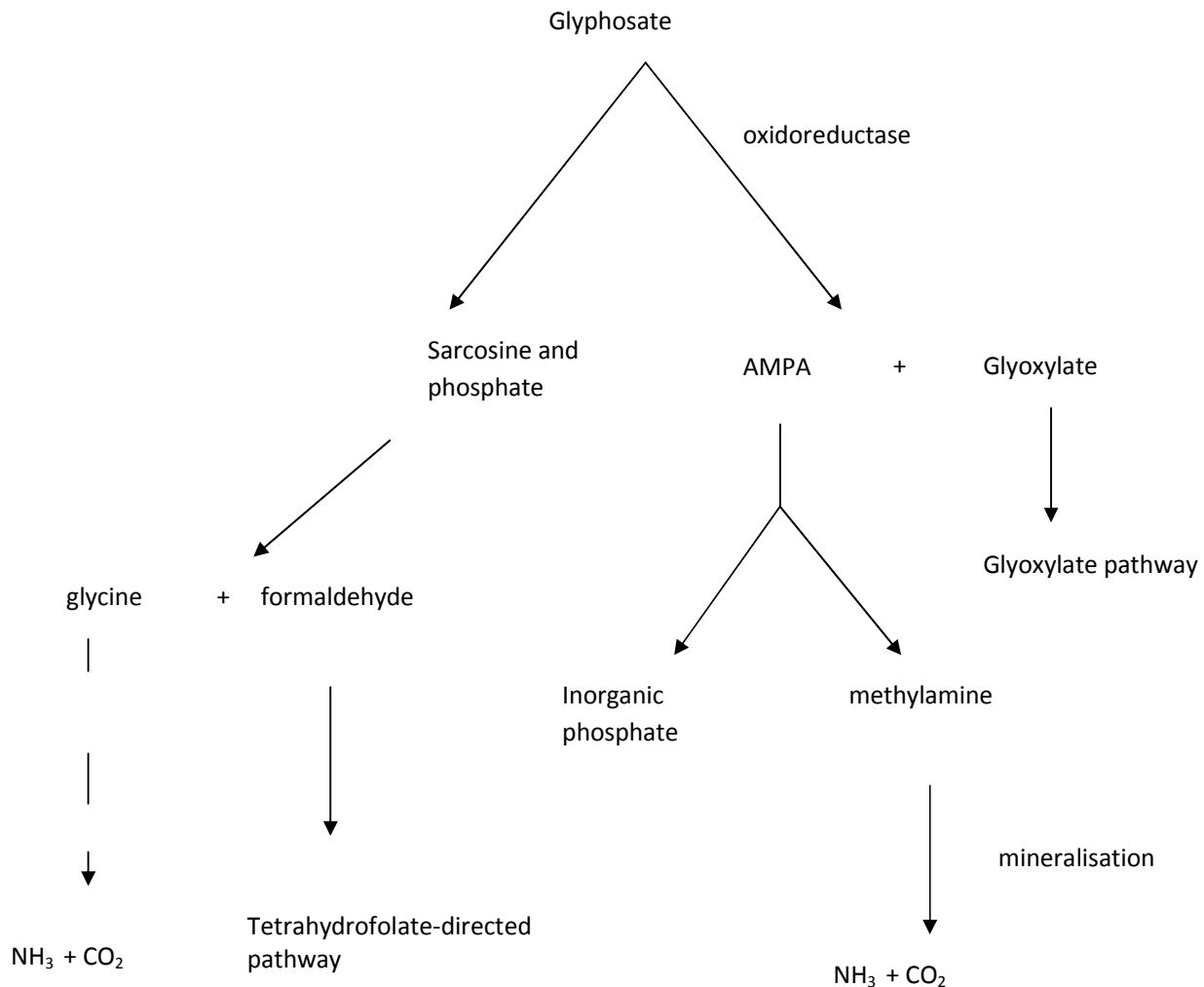


Figure 4.1 Two-way path of glyphosate degradation (Borggaard & Gimsig, 2008).

Glyphosate in soils is derived from glyphosate applied to weeds before crop planting (direct contact). It may also be derived from foliar-applied glyphosate that translocates to the roots and exudes into the soil or wash off from leaves. This may affect subsequent crops due to the phytotoxic effect that glyphosate exerts on the plant (Eker *et al.*, 2006; Aledesanwa & Akinbobola, 2008). Glyphosate residue accumulation in soil is said to be in the first 15 cm of the soil in fields. Thus, if seedlings are to be transferred to soil treated with glyphosate, a thin layer of soil is advisable to be used as a barrier between plant shoot and the surface of the soil (Egley & Williams, 1978; Eker *et al.*, 2006) to prevent direct contact with the herbicide.

This study was aimed at investigating the effect of three different sandy soils on the phytotoxic effect of soil applied glyphosate by making use of bio-assays. This was done by: transplanting ryegrass (*Lolium multiflorum* cv Agri Hilton) seedlings into glyphosate-treated soils at different times after spraying (TAS). A commercial ryegrass cultivar was chosen as source of seeds because it was assumed that the seeds would (i) have a good viability and with little or no dormancy and (ii) not have genes conveying herbicide resistance.

4.2 Materials and Methods

4.2.1 Seedling transfer to treated soil

Ryegrass seeds (cv Agri Hilton - putative susceptible) were sown into nine plastic trays (21 cm long, 15 cm wide and 8 cm deep) of which three each were filled with soil from a pasture paddock (PS), soil from a crop field (CS) and soil from a sand mine (SS), respectively. The pasture paddock and crop field are both located on the Welgevallen Experimental Farm in Stellenbosch (33°56'S, 18°52'E) and the sand mine is located near the town of Malmesbury (33°30'S, 18°40'E). The trays of each soil were irrigated with a balanced nutrient solution (Appendix A1: Table A1.1). The trays were kept moist by adding nutrient solutions when needed to enable the seeds to germinate. The procedure was repeated after three weeks, and again after four weeks.

Small square (8 cm x 8 cm) plastic pots were also filled at the same time with the same soils mentioned above, levelled and firmed. These were pre-treated with balanced nutrient solution described above for about two weeks until the seedlings in the plastic trays were ready to be transplanted. At this stage the pots with soil were sprayed with glyphosate at rates of 0, 540 and 3240 g a.i. ha⁻¹ using the 360 g a.i. L⁻¹ glyphosate formulation. The herbicide was applied by means of an automated cabinet sprayer equipped with a moving boom fitted with a flat fan nozzle. Spraying pressure was kept at a constant pressure of 2 bar and water delivery was 100 L. ha⁻¹. The spray carrier was deionised water.

The emerged seedlings in the plastic trays were transplanted into glyphosate treated soil in 8 cm x 8 cm pots of corresponding soil after two hours. Seedlings were transplanted at the two- to three-leaf stage. Before transplanting seedlings were cut to a uniform length of 2 cm. During transplanting care was taken to prevent direct soil contact with seedling leaf

blades. Six seedlings were transplanted into each pot. The plastic pots were placed into round plastic trays to which the nutrient solution was added so that the pots could be watered from the bottom by capillary force to prevent glyphosate from leaching out if watered from the top. One set of pots were not sown to enable soil analyses to be made without interference from plant nutrient uptake. All pots were kept moist including those without seedlings to retain the activity of the herbicide. The experiment was carried out in a glasshouse with night/day temperatures of 20/30 °C.

In the same manner as above, seedlings at two to three leaf stage were transplanted into pots three and four weeks after glyphosate application. This resulted in a time factor of two hours, three weeks and four weeks after spraying. The experiment was therefore a completely randomized 3 x 3 x 3 factorial design. Treatments (factors) were soil (PS, CS and SS); glyphosate application rates (0 (G1), 540 (G2) and 3240 (G3) g a.i ha⁻¹) and time after spraying (TAS) (two hours (TAS 1), three weeks (TAS 2) and four weeks (TAS 3)). The treatments were replicated twice.

4.2.2 Plant height, dry shoot weight and percentage survival recording

Plant height was recorded one week after transplanting by measuring the distance from the soil surface to the tip of the tallest leaf by means of a ruler and on the fourth week the number of surviving plants was recorded per pot. These were converted to percentage survival. The above ground parts of surviving plants were excised and oven-dried at 80°C for 48 hours and subsequently weighed to determine total dry mass produced per pot. These dry mass figures were then divided by the number of seedlings per pot to calculate the mean dry mass per seedling. Similarly the same procedure was carried out for plants transplanted three and four weeks after spraying.

4.2.3 Statistical analysis

The percentage survival, shoot length and dry mass data was analysed using the STATISTICA, software version 9 programme (Statsoft, 2009). This was performed using factorial ANOVA analyses in the PROC GLM command. Where the experiment showed significant interaction, the means were separated using Fisher's protected LSD test at $p = 0.05$.

Table 4.1 Soil nutrient analyses of paddock soil (PS), crop soil (CS) and sand soil (SS) before the experiment commenced and after being irrigated with a balanced nutrient solution at pH 6 for 28 days (PS pH6, CS pH6 and SS pH6)

Sample	Lab. No.	Soil	pH	Resistanc e (KCl) (Ohm)	Stone (Vol %)	P Bray II	K	Exchangeable cations (cmol(+)/kg)				Cu	Zn	Mn	B	Fe	Ca
						mg/kg	mg/kg	Na	K	Ca	Mg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Paddock soil (PS)	15943	Sand	7.0	550	1	294	479	0.19	1.23	13.24	1.93	4.60	26.8	31.1	0.32	149.82	2.48
PS pH6	15945	Sand	7.1	520	2	312	435	0.20	1.11	13.41	1.98	5.34	34.6	23.7	0.36	136.96	2.27
Crop soil (CS)	15947	Sand	6.4	1380	1	81	113	0.09	0.29	3.56	0.87	2.54	4.2	15.9	0.14	74.79	0.55
CS pH6	15949	Sand	6.9	1630	1	78	143	0.12	0.37	5.28	0.85	2.33	4.4	15.4	0.07	85.64	0.63
Sand soil (SS)	15951	Sand	8.6	4380	5	33	14	0.06	0.04	12.90	0.26	0.06	0.4	0.7	0.05	10.50	0.10
SS pH6	15953	Sand	8.6	3410	4	32	9	0.08	0.02	12.16	0.25	0.01	1.3	0.5	0.06	11.84	0.13

Table 4.2 Physical properties of three sandy soils (paddock soil (PS), crop soil (CS) and sand soil (SS)) used in the study

Sample	Lab. No.	Clay %	Silt %	Sand %	Fine Sand %	Medium Sand %	Rough Sand %	Classification
Crop soil	1847	1.2	11.0	87.8	64.6	18.60	4.60	Sand
CS								
Paddock soil	1848	0.0	5.2	94.8	59.8	22.20	12.80	Sand
PS								
Sand soil	1849	0.2	0.0	99.8	56.0	24.20	19.70	Sand
SS								

4.3 Results and Discussion

As reported by Campbell (1974) glyphosate had a deleterious effect on seedling establishment. In this study seedlings transferred to glyphosate treated soils were impacted by the factors examined. The response of the parameters (percentage survival, length and weight) measured varied with the treatments (time after spraying (TAS), glyphosate application rate and soil type).

4.3.1 Percentage survival

Statistical analysis revealed a two-way interaction between glyphosate application rate (GAR) and soil type for percentage survival (Figure 4.2). The effect of glyphosate on seedlings grown in CS and PS soil was similar. GAR did not have significant effects on the percentage survival of the seedlings in the CS soil, whereas G3 glyphosate application showed a decrease in the percentage survival of the PS soil grown seedlings. Seedlings in the SS soil were significantly affected by application of glyphosate with the untreated control significantly different from both glyphosate application treatments (G2 and G3).

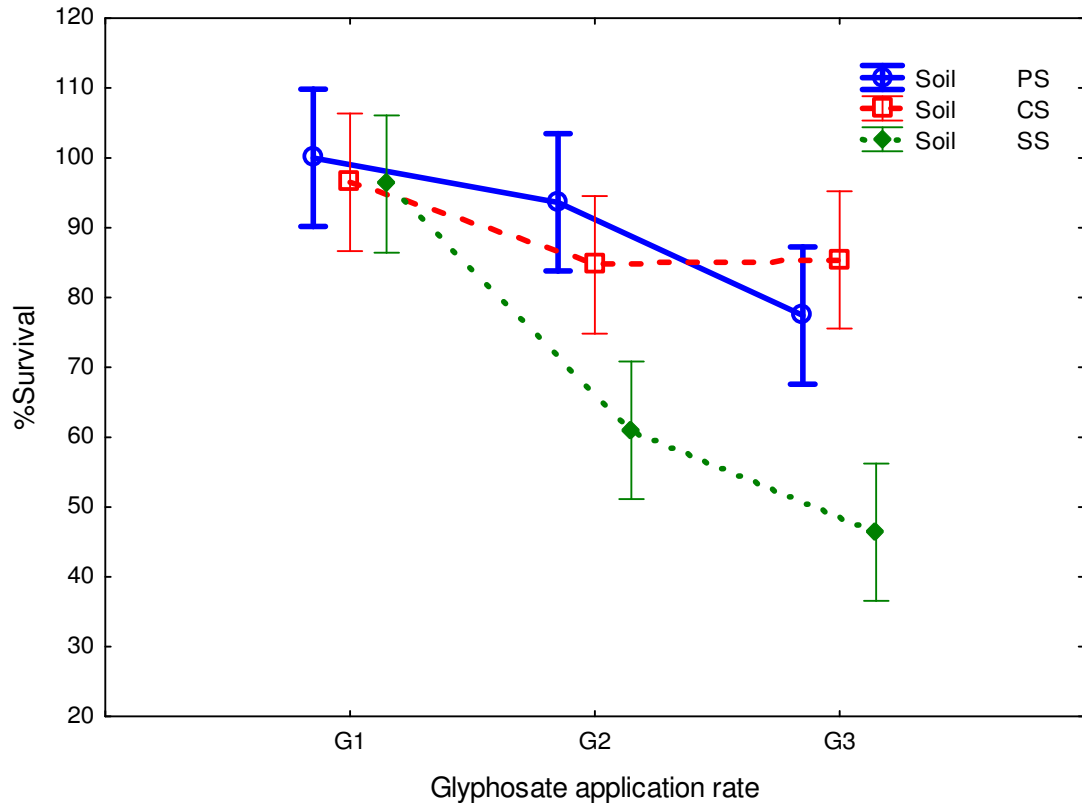


Figure 4.2 Effect of glyphosate application rate (G1 = 0 g a.i. ha⁻¹, G2 = 540 g a.i. ha⁻¹ and G3 = 3240 g a.i. ha⁻¹) and soil (PS = paddock soil, CS = crop field soil and SS = sandy soil) on the phytotoxicity of soil applied glyphosate to ryegrass seedlings. The vertical bars represent the standard error (1±SE) of the mean.

Figure 4.3 shows a significant two-way interaction between time after spraying (TAS) and GAR. There was time mediated increase in seedlings percentage survival. Non-treated seedlings at TAS 1 were significantly different (with 100% survival) to seedlings at G2 and G3. At TAS 2, only G3 GAR had significant effects on the percentage survival. At TAS 3 there was no significant difference between the percentage survival of the treated and the untreated seedlings. This is an indication of time mediated depletion of glyphosate from the soil. It also shows that the effect on the seedlings is dependent on the rate of glyphosate application.

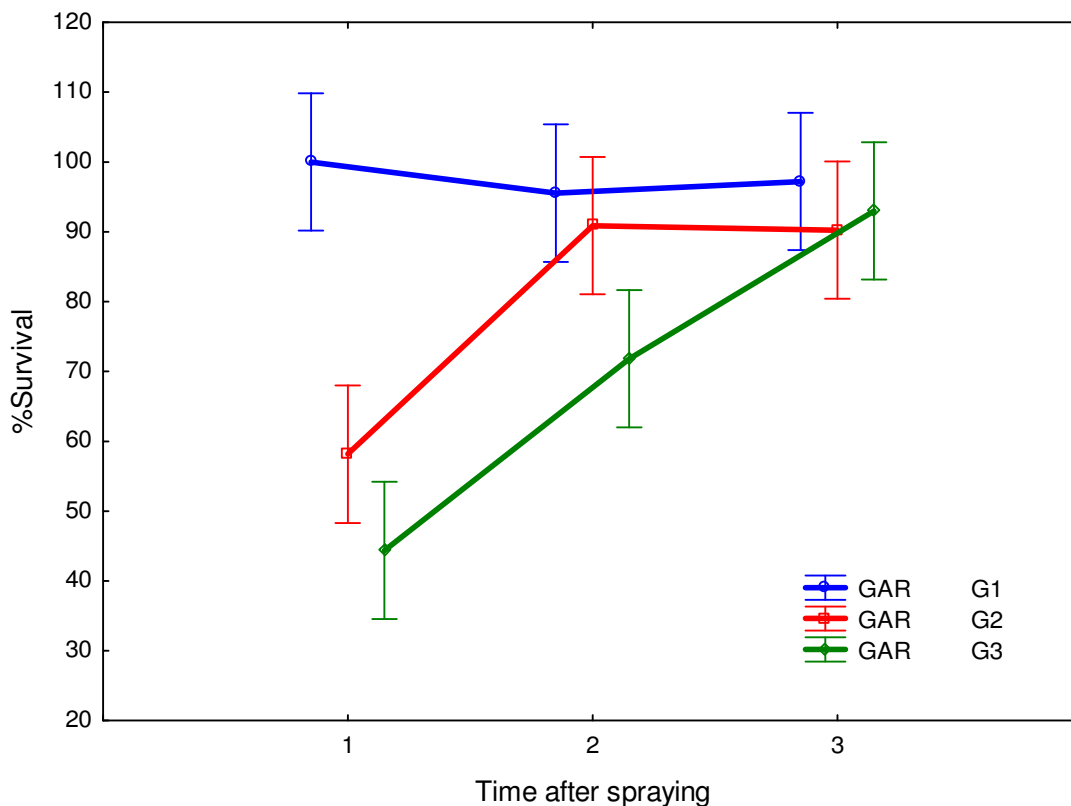


Figure 4.3 Effect of glyphosate application rate (GAR) (G1 = 0, G2 = 540 and G3 = 3240 g a.i. ha⁻¹) at three varying periods after glyphosate application (TAS 1 (two hours), TAS 2 (three weeks) and TAS 3 (four weeks after soil glyphosate treatment respectively)) on the phytotoxicity of glyphosate to ryegrass seedlings transplanted into the soil. The vertical bars represent the (1±SE) of the mean.

The abovementioned findings are depicted visually in Figure 4.4. Seedlings transferred two hours after spraying (TAS 1) showed that glyphosate impacted on the establishment of seedlings (Figure 4.4 A-C). This was marked by death of all seedlings in SS soil (Figure 4.4 A) at 540 (G2) and 3240 (G3) g a.i. ha⁻¹ rates of glyphosate. Also in PS (Figure 4.4 B) and CS soil (Figure 4.4 C) there was notable mortality at G3. With increasing time after spraying, there was a visual increase in percentage survival of ryegrass in PS and CS soils (Figure 4.4 E-F). This could have been as a result of increased degradation and adsorption to soil colloids or as a result of decay of glyphosate (half-life ranges from 3 days - 174 days). Seedlings in the SS soil still exhibited mortality at G3 GAR (Fig 4.4 D) though it improved compared to planting at TAS 1 (Figure 4.4 A).

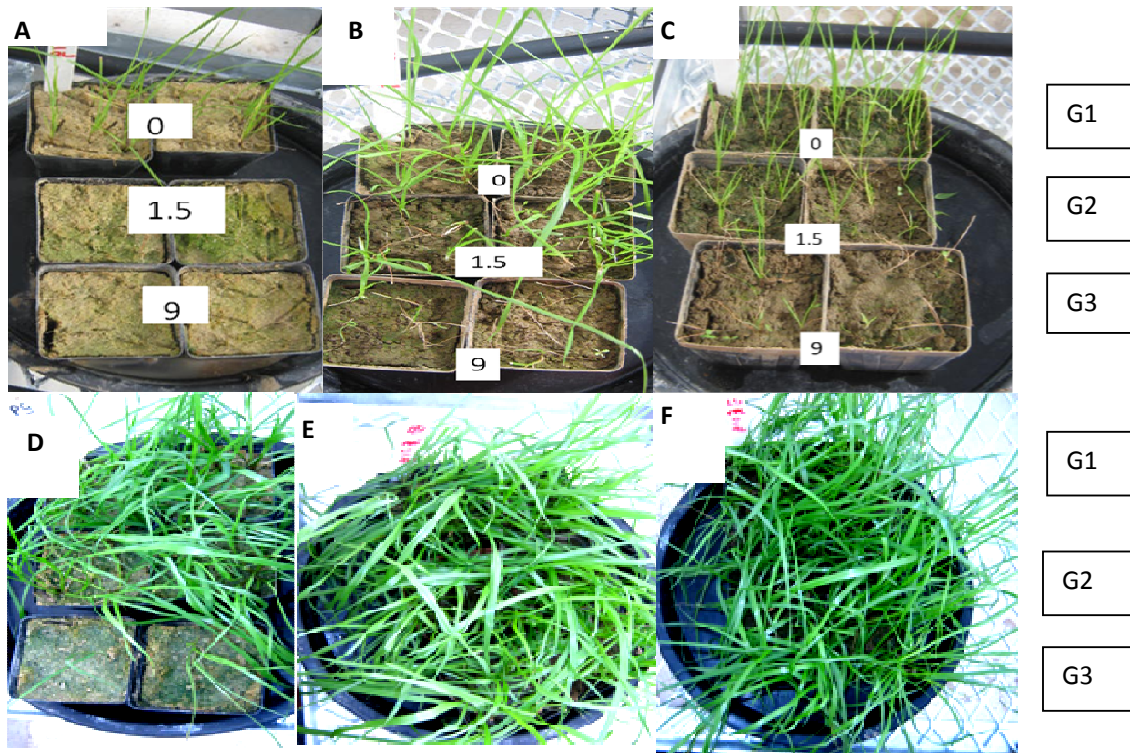


Figure 4.4 The phytotoxic effect of different glyphosate application rates (G1 = 0 g a.i. ha⁻¹, G2 = 540 g a.i. ha⁻¹ and G3 = 3240 g a.i. ha⁻¹) in different Soil types (A and D = SS (sandy soil); B and E = PS (paddock soil); C and F = CS (cropping soil) on the phytotoxic effect of glyphosate on ryegrass seedlings transferred two hours after treatment (TAS 1) (A-C) and ryegrass seedlings transferred three weeks after soil treatment (TAS 2) (D-F).

4.3.2 Dry mass

No significant two- or three-way interactions occurred in terms of dry mass production. Differential growth of ryegrass seedlings in different sandy soils significantly affected the dry mass yield per plant (Figure 4.5). It showed higher dry mass accumulation in PS soil grown seedlings followed by CS soil grown seedlings. There was no significant difference between dry mass production of the PS and CS soil grown seedlings. However, SS soil grown seedlings had significantly ($p < 0.05$) lower dry mass yield compared to PS soil grown seedlings. Due to soils being of similar texture (Table 4.2), largely sand, variation in survival and dry mass may have originated from the differences observed in the soil nutrient analysis. The nutrient analysis (Table 4.1) showed a higher nutrient content in the PS soil compared to the CS and SS soils. This could not only have contributed to better plant growth but also to

withholding (in antagonistic reaction) glyphosate from being in solution to cause harm in the plant.

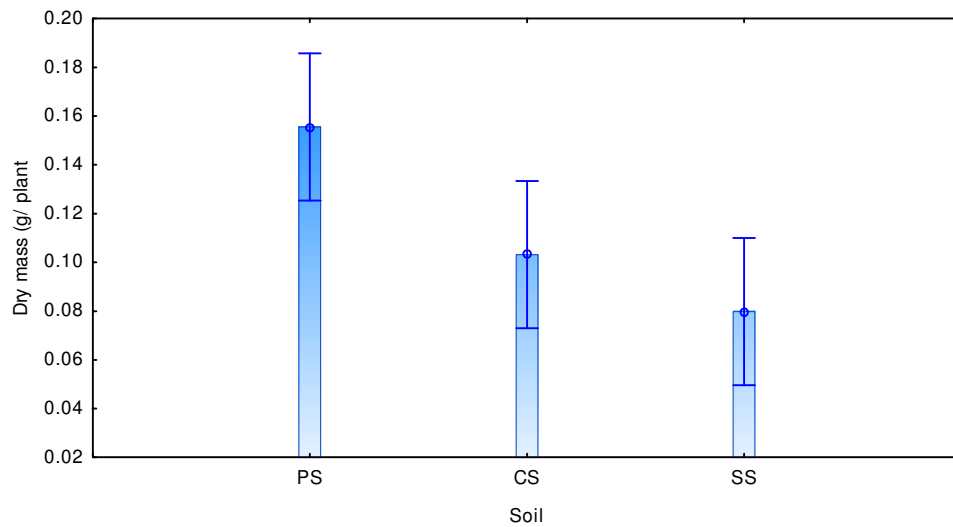


Figure 4.5 Effect of soil (PS = paddock; CS = cropping soil; and SS = sandy soil) on the dry mass of ryegrass seedlings grown in glyphosate treated soil. The vertical bars represent the ($1\pm SE$) of the mean.

Dry mass decreased proportionately to the increase in glyphosate application rate (Figure 4.6). At G3 GAR dry mass of the seedlings was significantly ($p < 0.05$) different from those of the untreated seedlings.

Figure 4.7 shows a significant ($p < 0.05$) effect of time after spraying on dry mass accumulation. This shows that as TAS increases dry mass also increase, due to depletion of glyphosate in the soil. The increase was significantly higher at TAS 3, similar to the findings in the percentage survival.

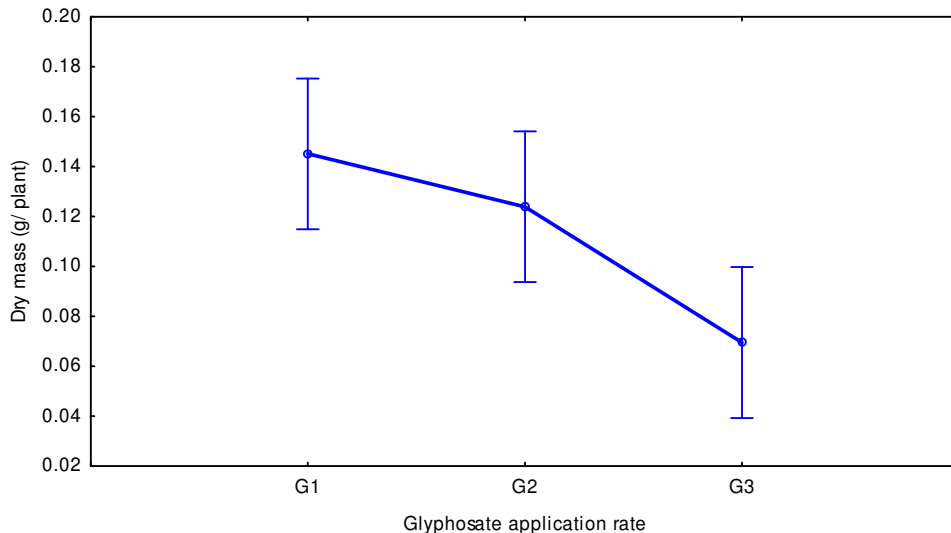


Figure 4.6 Effect of glyphosate application rate (G1= 0, G2 = 540 and G3 = 3240 g a.i. ha⁻¹) on the dry mass of ryegrass seedlings grown in glyphosate treated soil. The vertical bars represent the (1±SE) of the mean.

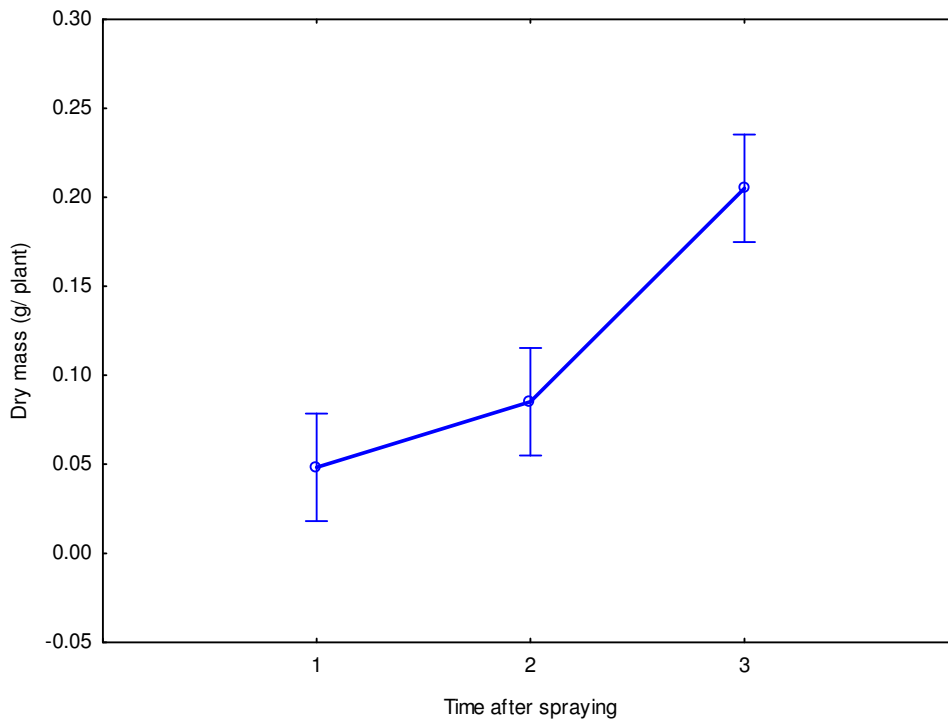


Figure 4.7 Increase in dry mass of ryegrass seedlings with increase in time after spraying (TAS); (TAS 1 (two hours), TAS 2 (three weeks) and TAS 3 (four weeks after soil glyphosate application respectively)). The vertical bars represent the (1±SE) of the mean.

4.3.3 Shoot length

No significant two- or three-way interactions occurred in terms of shoot length. Time after spraying significantly ($p < 0.05$) affected shoot length (Figure 4.8). There was a proportional increase in shoot length with increase in TAS by about 6 cm for each increase in TAS.

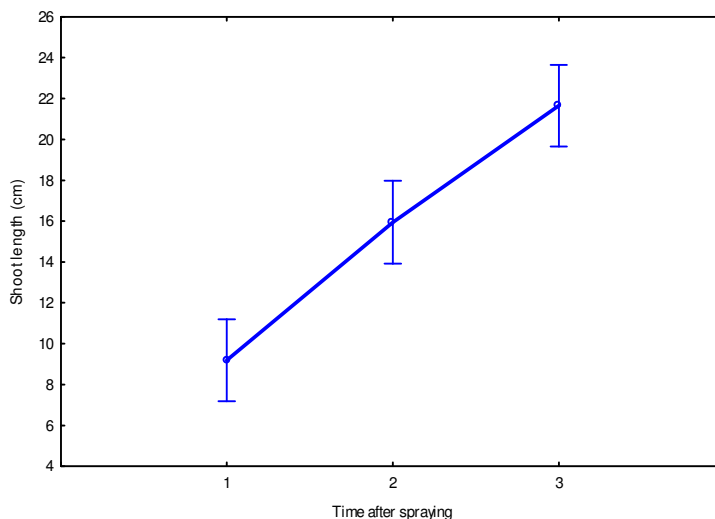


Figure 4.8 Effect of time after spraying on the shoot length of ryegrass seedlings grown in glyphosate treated soil. The vertical bars represent the standard error ($1\pm SE$) of the mean.

Analysis revealed significant ($p = 0.05$) GAR effect on shoot length with a decrease of about 4 cm with each increase in GAR (Figure 4.9).

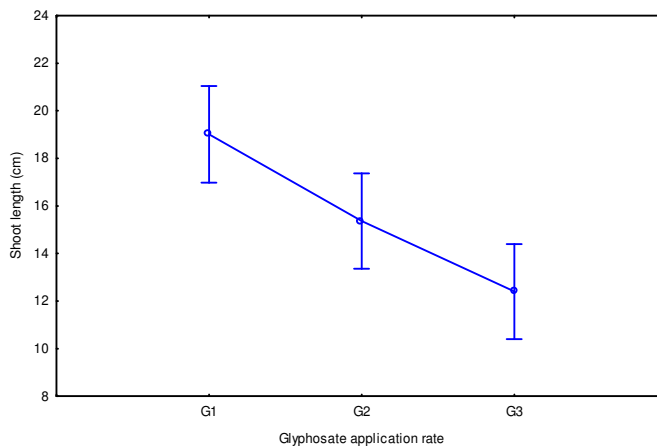


Figure 4.9 Effect of glyphosate application rate (G1= 0 g a.i. ha⁻¹, G2 = 450 g a.i. ha⁻¹ and G3=3240 g a.i. ha⁻¹) on the shoot length of ryegrass seedlings grown in glyphosate treated soil. The vertical bars represent the ($1\pm SE$) of the mean.

Soil type had a significant effect on the shoot length of ryegrass (Figure 4.10). The effect on the shoot length was similar to the observation on the percentage survival and dry mass.

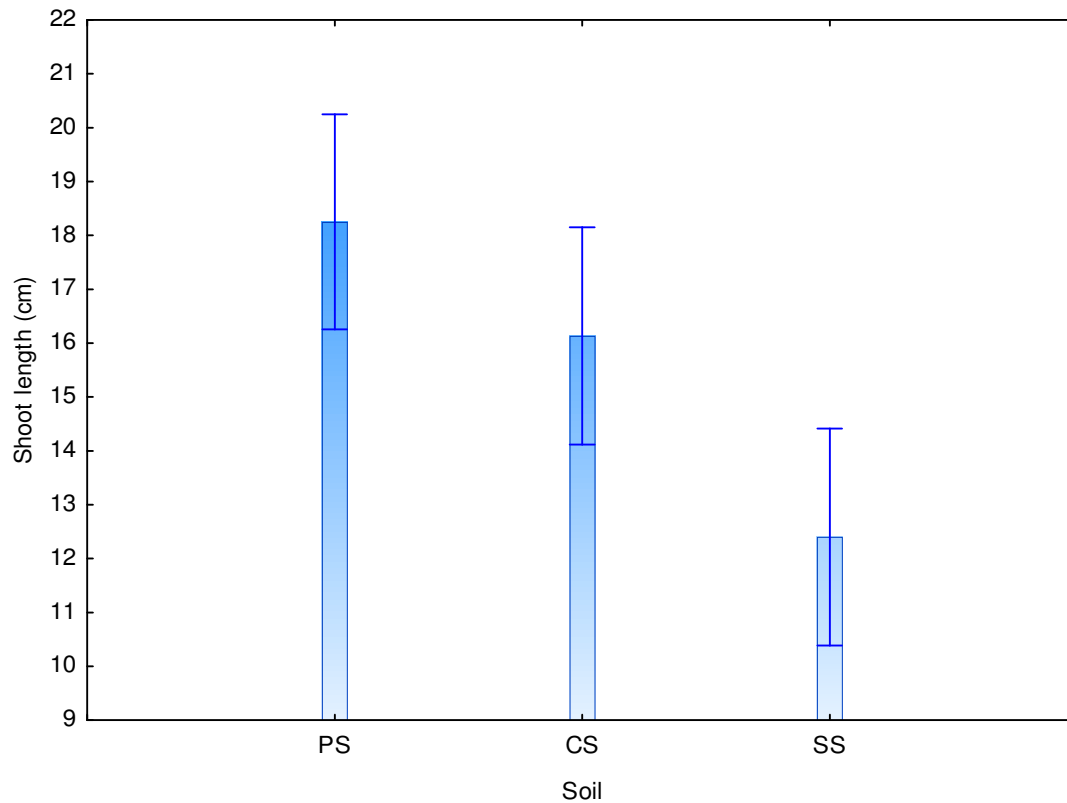


Figure 4.10 Effect of soil (PS=paddock; CS= cropping soil; and SS= sandy soil) on the shoot length of ryegrass seedlings grown in glyphosate treated soil. The vertical bars represent the $(1\pm SE)$ of the mean.

Under normal conditions, plant growth is vigorous and results in maximum biomass yield. Introduction of stress conditions may impede the maximum biomass yield of the plant. In this current study the objective was to investigate phytotoxic effects of soil applied glyphosate on plants (ryegrass in this case). The implication of the findings are the effects that glyphosate residues might have on the growth of subsequent crops and on exposure of weed seedlings to sub-lethal herbicide doses which might select for resistance genes. The results also substantiated previous findings that glyphosate persists in soil (Klingman & Murray, 1976; Baylis, 2000) contrary to the belief that glyphosate is a rapidly degrading herbicide (Laitinen *et al.*, 2007).

The decrease in percentage survival, shoot length and dry mass of ryegrass seedlings in this study is an indication of glyphosate residual activity in soil. Similar effects were observed for tomato plants planted after the application of glyphosate where the decrease in dry mass of tomatoes was soil dependent and greater effects was shown by tomato plants grown in sandy soils (Cornish, 1992; Cornish *et al.*, 1996). The results of the current study could have been impacted by the differences in nutrient content and the variation in gravel sand proportion that exists in each soil type (SS > PS > CS) (Table 4.2). As explained in Chapter 3, although the soils are largely sandy, texture of PS and CS soils appeared more like clay soil. The effect is reportedly due to the texture of sand that adsorbs less glyphosate residues compared to soil high in silt and clay content. Hence there was a warning by Salazar and Appleby (1982) to allow more time between pre-plant glyphosate weed control and subsequent planting in sandy soils. The interval could also contribute to depletion of glyphosate in soil, due to this being time dependent i.e. glyphosate effect on parameters measured was more at TAS 1 compared to TAS 3 on shoot length and dry mass. Increase in GAR was important to achieve a significant effect hence the effect at G3 was more compared to G2 GAR. As in any case of herbicide application, an adequate herbicide dose must reach the active site to effect phytotoxicity in plants.

4.4 Conclusions

These results show that glyphosate residues in soil are available for plant uptake depending on soil characteristics, time elapsed after application and dose of the herbicide applied. Sub-lethal doses of herbicide absorbed by plants may predispose the population to development of polygenic non-target site resistance (Moss, 2002).

References

- ALEDESANWA, R. D. & AKINBOBOLA, T.N., 2008. Effects of lime on herbicidal efficacy of atrazine and yield response of maize (*Zea mays* L.) under field conditions in south western Nigeria. *Crop Prot.* 27, 926-931.
- ANON, 2005. Glyphosate half-life in soils. www.monsanto.com (Accessed 20/04/10).
- BALTHAZOR, T.M. & HALLAS, L.E., 1986. Glyphosate degrading microorganisms from industrial activated sludge. *Appl. Environ. Microb.* 51, 432-434.
- BAYLIS, A.D., 2000. Why glyphosate is a global herbicide: strength, weaknesses and prospects. *Pest. Manag. Sci.* 56, 299-308.
- BORGGAARD, O.K. & GIMSIG, A.L., 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest. Manag. Sci.* 64, 441-456.
- CAMPBELL, M.H., 1974. Effects of glyphosate on the germination and establishment of surface sown pasture species. *Aust. J. Exp. Agric. Anim. Husb.* 14, 557-560.
- CERDEIRA, A.L. & DUKE, S.O., 2006. The current status and environmental impacts of glyphosate – resistant crops: a review. *J. Environ. Qual.* 35, 1633-1658.
- CHEN, Y., WU, Y., LIN, Y., DENG, N., BAZHIN, N. & GLEBOV, E., 2007. Photodegradation of glyphosate in ferrioxilate system. *J. Hazard. Mater.* 148, 360-365.
- CORNISH, P.S., 1992. Glyphosate residues in a sandy soil affect tomato transplants. *Aust. J. Exp. Agric.* 32, 395-9.
- CORNISH, P.S. & BURGIN, S., 2005. Residual effects of glyphosate herbicide in ecological restoration. *Restor. Ecol.* 13, 695-702.
- CORNISH, P.S., KHURSHID, A.A. & AGARWAL, K.N., 1996. Glyphosate: reappraisal of the threat to crop plants. In: D.L. MICHALK & J.E. PRATLEY (eds.). Proceedings of the 8th Australia Agronomy Conference, 30 January - 2 February, The University of Southern Queensland, Toowoomba, Queensland.
- DEVLIN, R.M., KARCZMARCZYK, S.J., ZBIEC, I.I. & KOSZANSKI, Z.K., 1986. Initial and residual activity of glyphosate and SC-0224 in sandy soil. *Crop Prot.* 8, 293-296.
- EGLEY, G.H. & WILLIAMS, R.D., 1978. Glyphosate and paraquat effects on weed germination and seedling emergence. *Weed Sci.* 26, 249-251.
- EKER, S., OZTURK, L., YAZICI, A., ERENOGLU, B., ROMHELD, V. & CAKMAK, I., 2006. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. *J. Agric. Food Chem.* 54, 10019-10025.

- FORLANI, G., MANGIAGALLI, A., NIELSEN, E. & SUARDI, C.M., 1999. Degradation of the phosphonate herbicide glyphosate in soil: evidence for a possible involvement of unculturable microorganisms. *Soil Biol. Biochem.* 31, 991-997.
- GRUNEWALD, K., SCHIMDT, W., UNGER, C. & HANSCHMANN, G., 2001. Behaviour of glyphosate and aminomethyl phosphonic acid (AMPA) in soils and water reservoir Radeburg II catchment (Saxony, Germany). *J. Plant Nutr. Soil Sci.* 164, 65-70.
- KJAER, J., OLSEN, P., ULLUM, M. & GRANT, R., 2005. Vadose-zone processes and chemical transport. *J. Environ. Qual.* 34, 608-620.
- KLINGMAN, D.L. & MURRAY, J.J., 1976. Germination of seeds of turfgrass as affected by glyphosate and paraquat. *Weed Sci.* 24, 191-193.
- KOLPIN, D.W., THURMAN, E.M., LEEB, E.A., MEYER, M.T., FURLONG, E.T. & GLASSMEYER, S.T., 2006. Urban contributions of glyphosate and its degradate AMPA to streams in the United States. *Sci. Total Environ.* 191-197.
- LAITINEN, P., RÄMÖ, S. & SIIMES, K., 2007. Glyphosate translocation from plants to soil- does this constitute a significant proportion. *Plant Soil* 300, 51-60.
- LIU, C.M., MCCLEAN, P.A., SOOKDOE, C.C. & CANNON, F.C., 1991. Degradation of the herbicide glyphosate by members of the *Rhizobaceae*. *Appl. Environ. Microbiol.* 57, 1799-1804.
- LUDVIGSEN, G.H., LODE, O. & SKJVDAL, R., 2003. Retrieval of glyphosate and AMPA in Norwegian streams, including studies on leaching due to heavy rainfall. Proceedings of the XII Symposium Pesticides Chemistry. Pesticides in air, plant soil and water system. 4-6 June 2003, Piacenza, Italy.
- MAMY, L. & BARRIUSO, E., 2005. Glyphosate adsorption in soils compared to herbicides replaced with the introduction of glyphosate resistant crops. *Chemosphere* 61, 844-855.
- MOSS, S.R., 2002. Herbicide-resistant weeds. In: R.E.L. Naylor (Ed.) *Weed management handbook*. John Wiley & Sons, U.K.
- NILSSON, T., 2009. Uptake of ¹³⁷Cs by fungi and plants due to its potassium fertilisation in Heby municipality in response to the Chernobyl nuclear accident. http://stud.epsilon.slu.se/786/1/nilsson_t_100121.pdf. (Accessed 01/08/2010).
- RUEPPEL, M.L., BRIGHTWELL, B.B., SCHAEFER, J. & MARVEL, J.T., 1977. Metabolism and degradation of glyphosate in soil and water. *J. Agric. Food Chem.* 25, 517-528.

- SALAZAR, L.C. & APPLEBY, A.P., 1982. Herbicidal activity of glyphosate in soil. *Weed Sci.* 30, 463-466.
- SEGURA, J., BINGHAM, S.W. & FOY, C.L., 1978. Phytotoxicity of glyphosate to Italian ryegrass (*Lolium multiflorum*) and Red clover (*Trifolium pretense*). *Weed Sci.* 26, 32-36.
- SIMONSEN, L., FOMSGAARD, I.S., SVENSMARK, B. & SPLIIT, N.H., 2008. Fate and availability of glyphosate and AMPA in soil. *J. Environ. Sci.* 43, 365-375.
- SMITH, A.E. & AUBIN, J.A., 1993. Degradation of ¹⁴C glyphosate in Saskatchewan soils. *B. Environ. Contam. Tox.* 50, 499-505.
- SPRANKLE, P., MEGGIT, W. F. & PENNER, D., 1975. Rapid inactivation of glyphosate in the soil. *Weed Sci.* 23, 224-228.
- STATSOFT, 2009. STATISTICA (data analyses software systems) version 9, StatSoft. Inc Tulsa, Oklahoma, USA.
- STRANGE-HANSEN, R., HOLM, P.E., JACOBSEN, O.S. & JACOBSEN, C.S., 2004. Sorption, mineralization and mobility of N-(phosphonomethyl) glycine (glyphosate) in five different types of gravel. *Pest Manag. Sci.* 60, 570-578.
- STRUGER, J., THOMPSON, D., STAZNIK, B., MARTIN, P., MCDANIEL, T. & MARVIN, C., 2008. Occurrence of glyphosate in surface waters of Southern Ontario. *B. Environ. Contam. Tox.* 80, 378-384.
- TESFAMARIAM, T., BOTTS, S., CAKMAK, I., RÖMHELD, V. & NEUMAN, G., 2009. Glyphosate in the rhizosphere – Role of waiting times and different glyphosate binding forms for phytotoxicity in non target plants. *Eur. J. Agron.* 31, 126-132.
- TORSTENSSON, L., BÖRJESSON, E. & STENSTRÖM, J., 2005. Efficacy and fate of glyphosate on Swedish railway embankments. *Pest Manag. Sci.* 61, 881-886.
- VEIGA, F., ZAPATA, J.M., FERNANDEZ MARCOS, M.L. & ALVAREZ, E., 2001. Dynamics of glyphosate and aminomethylphosphonic acid in a forest soil in Galicia, north-west Spain. *Sci. Total Environ.* 271, 135-144.
- VERECKEN, H., 2005. Mobility and leaching of glyphosate: a review. *Pest Manag. Sci.* 61, 1139-1151.

CHAPTER 5

EFFECT OF MOLYBDENUM LEVELS ON GLYPHOSATE EFFICACY ON RYEGRASS (*LOLIUM SPP.*)

Abstract

Trace metals reduce the efficacy of glyphosate in mixtures by the formation of glyphosate-trace metal complexes. This is due to glyphosate, initially, patented as a metal chelator before it was introduced as a herbicide. Manganese, zinc, iron and calcium were already found to be chemical antagonised by glyphosate. An interesting effect, may be due to antagonism at physiological level, of Mo on glyphosate was investigated. The assay was carried out on a susceptible commercial ryegrass cultivar (*Lolium multiflorum* cv. Agri Hilton) and a resistant ryegrass (*Lolium spp.*) biotype. A greenhouse study was conducted where 0, 135, 270, 540 and 1080 g a.i. ha⁻¹ of glyphosate was applied to seedlings grown at 0, 1x and 2x Mo application rates (where 1x = 0.05 mg L⁻¹ Na₂MoO₄(2H₂O)). The percentage survival of the ryegrass plants was determined. The Mo application significantly affected the efficacy of foliar-applied glyphosate. Molybdenum in the R biotype appeared to increase the efficacy of glyphosate whereas in the S biotype glyphosate application was not affected by increase in Mo application. This effect, although research to ascertain this is required, may be due to Mo being involved physiological and biochemical processes. Thus be termed glyphosate antagonism at physiological level. Although there were indications that Mo may influence glyphosate efficacy, more research is needed to obtain conclusive evidence on this aspect of physiological antagonism.

Keywords: biotype, glyphosate, molybdenum, ryegrass

5.1 Introduction

Glyphosate is a major herbicide in weed control. This is due to several positive attributes it has including systemicity, ability to control perennial weeds and the role it plays in modern agriculture (Baylis, 2000). The latter includes use in no-till cropping systems and widespread cultivation of glyphosate resistant crops (Eker *et al.*, 2006). The trace metal-glyphosate complex is as a result of glyphosate being initially patented as a metal chelator before it was introduced as herbicide. However, among other things, trace metals in mixture with

glyphosate reduces its efficacy. Weinberg *et al.* (2007) defines antagonism as “an interaction of two or more chemicals such that the effect, when combined, is less than the predicted effect based on the activity of each chemical applied separately”. Evidences of these antagonistic reactions were shown in many studies where glyphosate was used in tank mixtures (Bernards *et al.*, 2005).

The chemical structure of glyphosate, N-(phosphonomethyl) glycine, plays a significant role in these antagonistic reactions. The amino group, phosphonic and carboxylic moiety form stable complexes with metals (Sheals *et al.*, 2001). Antagonism may occur outside the leaf where glyphosate’s amine and carboxyl oxygen or phosphonate group are involved in the complex formation e.g. Mn - glyphosate complex (Gednalsdske, Undated; Eker *et al.*, 2006). The complex that is formed between glyphosate and trace metal is poorly absorbed by the plant leaf (Bott *et al.*, 2008). Trace metals that were implicated in antagonistic reaction include zinc (Zn), iron (Fe) and manganese (Mn) (Nalewaja & Metysiak, 1991; Eker *et al.*, 2006). In addition, monovalent and divalent cations found in hard water cause antagonistic reactions to glyphosate (Thelen *et al.*, 1995; Bernards *et al.*, 2005). In these reactions whilst glyphosate efficacy is reduced also the trace metal in the reaction becomes deficient. This was shown in Roundup Ready® soybeans, where mixtures of glyphosate and manganese resulted in yellowing which is the symptom of manganese deficiency (Cakmak *et al.*, 2009).

Glyphosate-trace metal complexation may also occur inside the leaf, and as such impede translocation of the herbicide within the leaf tissue (Hall *et al.*, 2000; Eker *et al.*, 2006). Occurrence of glyphosate reaction within the leaf was also reported by Cakmak *et al.* (2009) where glyphosate application resulted in reduced leaf concentration of Mn, magnesium (Mg) and calcium (Ca) in young leaves of soybeans. Similarly, Fe inside the leaf showed a tendency to be reduced (Cakmak *et al.*, 2009). This may reduce the amount of glyphosate reaching active sites and thus glyphosate efficacy. If this occurs it means there is an amount of glyphosate that gets involved in the complexation reaction during normal glyphosate application.

There are numerous reports on antagonism of glyphosate by above mentioned divalent cations. However this is not the only way glyphosate efficacy is reduced. Physiological antagonism occurs when two chemicals act at different sites counteract each other (Hoagland, 1989). Molybdenum is an essential element for higher plants and plays vital role in many

physiological and biochemical processes. In plants there are four Mo-enzymes. These are: nitrate reductase, aldehyde oxidase, xanthine dehydrogenase and sulphite oxidase which catalyse reaction in nitrate assimilation, phytohormone synthesis (indole-3-abscisic acid and abscisic acid (ABA), purine catabolism and sulphite detoxification in plants (Sun *et al.*, 2009). The involvement of glyphosate on the physiological reaction would indirectly affect the working of glyphosate. In order to understand this we investigated the effect of varying Mo levels on the efficacy of foliar-applied glyphosate on resistant and susceptible ryegrass (*Lolium* spp.) biotypes.

5.2 Materials and methods

5.2.1 Experimental procedure

Seeds from two ryegrass (*Lolium* spp.) accessions, one susceptible and one resistant to glyphosate, were obtained. The susceptible ryegrass biotype (Biotype S) was a commercial pasture crop (*Lolium multiflorum* L. cv. Agri Hilton) and the resistant biotype (Biotype R) was a weedy ryegrass (*Lolium* spp.) obtained from Groenkloof farm in the Tulbagh (33°20'S, 19°10'E) region. Seeds of the resistant and susceptible ryegrass species were sown into 21 cm long, 15 cm wide and 8 cm deep plastic trays containing pure sand. These were irrigated with six balanced nutrient solutions: without Mo, with normal Mo application or with double the amount of normal Mo equivalent to 0, 1x and 2x application rates respectively (where 1x = 0.05 mg L⁻¹ Na₂MoO₄(2H₂O) This relates to about 0.02 mg L⁻¹ Mo. The nutrient solution content is given in Appendix Table A1.1. Two weeks after germination, seedlings were cut to a uniform size and transplanted into 8 cm x 8 cm pots. Irrigation continued in the same way. One week after transplanting the seedlings glyphosate was applied to them. Glyphosate application was made by an automated cabinet sprayer equipped with a moving boom fitted with a flat fan nozzle operating at a pressure of 2 bar and water delivery was 100 L. ha⁻¹. The spray carrier was deionised water.

Experimental design was a 2 x 3 x 5 factorial with factors biotype (R and S), Mo (0, 1x and 2x) and glyphosate application rate (GAR) (0 (0x), 135 (1/4x), 270 (1/2x), 540 (1x) and 1080 (2x) g a.i. ha⁻¹) replicated three times.

5.2.2 Statistical analysis

The percentage survival and shoot length data were analysed using STATISTICA, software version 9 programme (Statsoft, 2009). This was performed using factorial ANOVA analyses in the PROC GLM command. Where the experiment showed significant interaction, the means were separated using Fisher's protected LSD test at $p = 0.05$.

5.3 Results and discussion

A significant three-way interaction ($p < 0.05$) between ryegrass biotype, molybdenum level and glyphosate application rate (GAR) was shown in terms of percentage survival of seedlings (Figure 5.1). Zero Mo application (Mo 0) showed a significant decrease in the percentage survival of the S biotype with glyphosate application compared to the control treatment that had no glyphosate. A 100% control level was achieved at 1/4 x GAR. In the R biotype a significant decrease by 61% was only exhibited at 2x GAR. The application of 1x of Mo gave similar results for the S biotype whereas the R biotype showed decreased survival at 1x and 2x GAR with 45% and 80% decrease respectively. At the 2x rate of Mo treatment there was approximately 85% survival in the S biotype at 1/4x GAR, while the R biotype showed a 0% and 5% survival at 1x and 2x GAR respectively.

The 2x Mo application to the S biotype resulted in an increase in the percentage survival at 1/4x glyphosate application rate compared to no survival at 0 and 1x Mo applications. This is similar to the findings by Abouzienna *et al.* (2009) where an increase in Zn application resulted in decreased glyphosate efficacy. In this case, however, this finding may very well be due to experimental error. Increase in the efficacy of glyphosate with increase in the application of Mo, in the R biotype, may be due to: the importance of Mo as a co-factor for the synthesis of the enzymes such as aldehyde oxidase and aldehyde dehydrogenase which both play a role in the biosynthesis of abscisic acid (Sun *et al.*, 2009). Abscisic acid is a plant growth regulator involved in the adaptation of plant crops to environmental stresses and seed maturation (Sagi *et al.*, 2002). It is also important in the use of nitrate assimilation due to nitrate reductase being a Mo-Co enzyme (Sun *et al.*, 2009). Assimilation of nitrate in plants represents a central point in the growth of plants (Viégas *et al.*, 2002).

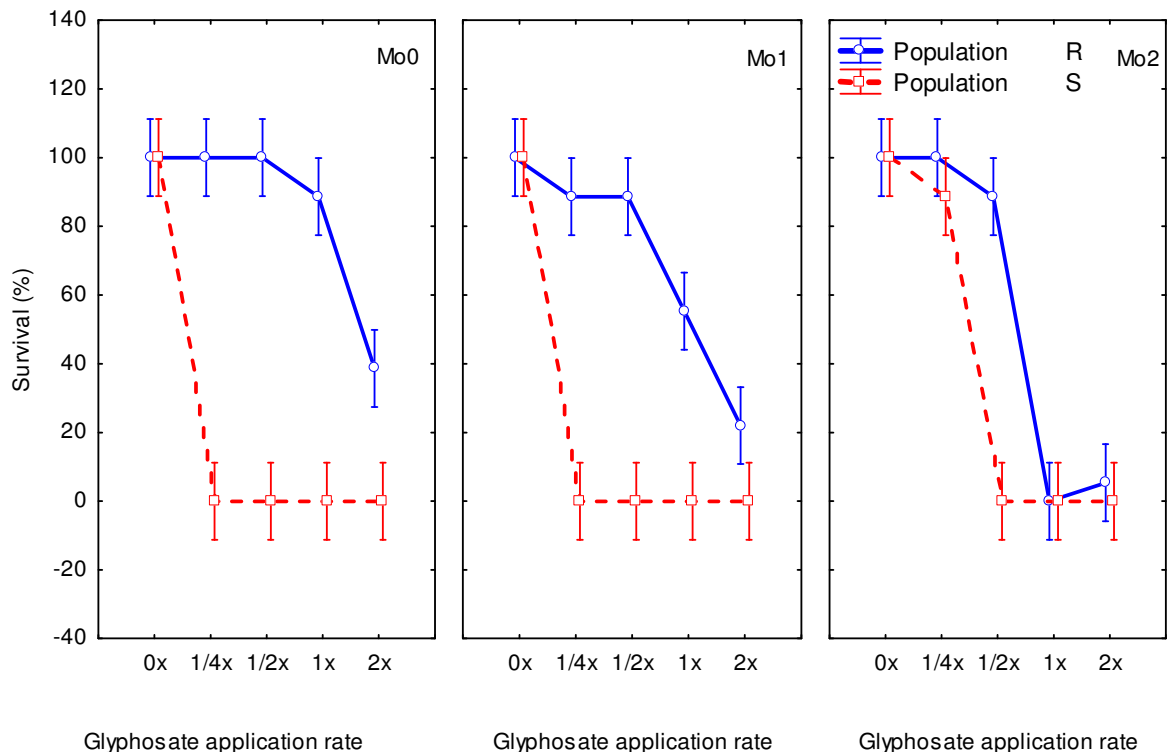


Figure 5.1 Effect of molybdenum applications and glyphosate application rates (GAR) on percentage survival of the resistant (R) and susceptible (S) ryegrass biotypes. Mo 0 = 0, Mo 1 = 0.05 and Mo 2 = 0.1 mg L⁻¹ Na₂MoO₄(H₂O) and GAR at 0x = 0; 1/4 x = 135; 1/2 x = 270; 1x = 570 and 2x = 1080 g a.i. ha⁻¹). The vertical bars represent the standard error (1±SE) of the mean.

5.4 Conclusions

This study shows that glyphosate efficacy in resistant biotypes can be influenced by the availability of molybdenum. The fact that the study was carried out at high pH in pure sand that was not acid washed makes it impossible to accurately quantify the exact amount of Mo available to the plants. In terms of Mo it is impossible to even estimate the Mo content relative to the other soils as none of three soil analysis laboratories contacted was willing to analyse for soil Mo content. Therefore the application of 1x and 2x Mo levels to the sand resulted in a qualitative increase in availability of the trace metals. To accurately quantify the amount of Mo available to the plants, the experiment should be repeated making use of acid washed sand. However, the Mo leaf content of susceptible seedlings grown in SS soil showed that Mo accumulation was 4213 µg/kg (Table 3.3). These were fed with balanced nutrient solution and no glyphosate was applied.

Molybdenum application has shown beneficial effect on glyphosate efficacy. This shows that weed control in soil which is deficient in Mo may indirectly select for resistance. The mechanism whereby molybdenum can improve the efficacy of glyphosate is unknown. Given the role of Mo in the biosynthetic pathway of abscisic acid it may at first seem surprising that this trace element can increase the activity of the herbicide in resistant plants. However, given the high level of Mo in the leaves of the susceptible plants growing in the sandy soil it is likely that similar levels were also present in the leaves of the resistant plants. This raises the question of whether supra-optimal levels of Mo in the plant can stimulate glyphosate activity in resistant plants. Further research will have to be conducted to determine the validity of this hypothesis.

References

- ABOUZINA, H.F., ELMERGAWI, R.A., SHARMA, S., OMAR, A.A. & SINGH, M., 2009. Zinc antagonizes glyphosate efficacy on a yellow nutsedge (*Cyperus esculentus*). *Weed Sci.* 57, 16-20.
- BAYLIS, A.D., 2000. Why glyphosate is a global herbicide: strength, weaknesses and prospects. *Pest Manag. Sci.* 56, 299-308.
- BERNARDS, M.L., THELEN, K.D. & PENNER, D., 2005. Glyphosate efficacy is antagonized by manganese. *Weed Technol.* 19, 27-34.
- BOTT, S., TESFAMARIAM, T., CANDAN, H., CAKMAK, I., RÖMHELD, V. & NEUMANN, G., 2008. Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate resistant soybean (*Glycine max* L.). *Plant Soil* 312, 184-194.
- CAKMAK, I., YAZICI, A., TUTUS, Y. & OZTURK, L., 2009. Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. *Europ. J. Agronomy* 31, 99-102.
- EKER, S., OZTURK, L., YAZICI, A., ERENOGLU, B., ROMHELD, V. & CAKMAK, I., 2006. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. *J. Agric. Food Chem.* 54, 10019-10025.
- GEDNALSKE, J.V., Undated. Herbicide antagonism and herbicide–fertiliser interactions. Online, Internet. www.agronomy.cfans.umn.edu (Accessed 13/11/ 2009).
- HALL, G.J., HART, C.A. & JONES, C.A., 2000. Plants as sources of cations antagonistic to glyphosate activity. *Pest. Manag. Sci.* 56, 351-358.

- HOAGLAND, R.E., 1989. Biochemical interaction of atrazine and glyphosate interaction in soybean (*Glycine Max*) seedlings. *Weed Sci.* 37, 491-497.
- NALEWAJA, J.D. & MATYSIAK, R., 1991. Salt antagonism of glyphosate. *Weed Sci.* 39, 622-628.
- SAGI, M., SZAZZUCHIO, C. & FLUHR, R., 2002. The absence of molybdenum cofactor sulfuration is the primary cause of the *flacca* phenotype in tomato plants. *The plant Journal* 31, 305-317.
- SHEALS, J., PERSSONS, P. & HEDMAN, B., 2001. IR and EXAFS spectroscopic studies of glyphosate protonation and copper (II) complexes of glyphosate in aqueous solutions. *Inorg. Chem.* 40, 4302-4309.
- STATSOFT, 2009. STATISTICA (data analyses software systems) version 9, StatSoft. Inc Tulsa, Oklahoma, USA.
- SUN, X., HU, C., TAN, Q., LIU, J. & LIU, H., 2009. Effects of molybdenum on expression of cold-responsive genes in abscisic acid (ABA)- dependent and ABA-independent pathways in winter wheat under low-temperature stress. *Annals of Botany*, 104, 345-356.
- THELEN, K.D., JACKSON, E.P. & PENNER, D., 1995. The basis of hard-water antagonism of glyphosate activity. *Weed Sci.* 43, 541-548.
- WEINBERG, T., STEPHENSON, G.R., MCLEAN, M.D., SATCHIVI, N.M. & HALL, C.J., 2007. Basis for antagonism by sodium bentazon of trisulfuron to white bean (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* 55, 2268-2275.
- VIÉGAS, R.A. & SILVERIA, J.A.G., 2002. Activation of nitrate reductase of cashew leaf by exogenous nitrite. *Braz. J. Plant Physiol.* 14, 39-44.

CHAPTER 6

GENERAL CONCLUSIONS

Glyphosate is an invaluable tool of weed control in modern agriculture. Thus, to maintain its sustainable use, it is important to investigate the factors affecting its efficacy and establishing factual knowledge on the phytotoxicity of glyphosate in soils as well as above ground.

Although glyphosate is a well researched herbicide, there is limited research regarding aspects contributing to its efficacy. These include importance of the soil in which weed control is carried out, nutritional status of the soil and the role played by glyphosate reaching the soil on the overall efficacy of glyphosate. Findings from this study showed that soil to which the weed control is carried out play a role in the control of weeds with a nutrient poor soil resulting in poor control (in this case SS soil). In addition, covering the soil surface at spraying has decreased efficacy of glyphosate in certain soils. This indicates that the amount of the herbicide reaching the soil is important in the overall efficacy of the herbicide. This is an indication that glyphosate activity is not solely dependent upon aspects that are directly related (such as spray components and foliar absorption) but also on indirect factors such as soil and nutrition of the weed during growth. This implies that factors such as soil type and nutrient content could influence the optimal glyphosate dose to be applied.

For a long time in the history of its usage, glyphosate has enjoyed appraisal as an 'environmentally friendly' herbicide (due to it being deemed non-reactive upon reaching the soil). Evidence of its herbicidal activity, however, has been reported by Salazar and Appleby (1982) among others. Findings from this study also established this, with mortality observed after seedlings were grown in glyphosate treated soil. This showed to be dependent on soil (and can be attributed to texture and soil nutrient status) time interval between planting and time of soil glyphosate application (in the soil depletion of glyphosate was concentration dependent). In this study persistence and phytotoxicity was higher in the SS soil (which was characterised by poor nutrient content and higher rough sand content than in the PS and CS soil). We recommend that where planting is to be carried out following glyphosate application, it has to be done after a period of time has lapsed (in this case complete degradation was shown after three weeks). Tesfamariam *et al.* (2009) emphasized the importance of waiting time to reduce the effect of glyphosate in soil. This has implications

for grain farmers in the South Western Cape. It is common practice to apply glyphosate pre-plant in the same operation as planting the crop. At the same time due to non-target site resistance, glyphosate doses in pre-plant operations are steadily increased. The combination of these two actions may cause poor crop establishment on nutrient poor, sandy soils.

Glyphosate, initially a metal chelator, has been reported to have antagonistic reaction with trace metals found in fertilisers or trace metals within the plant leaf (Hall *et al.*, 2000; Eker *et al.*, 2006). Previous studies by Bernards *et al.* (2005) and Abouzienna *et al.* (2009) have shown reduced efficacy of glyphosate upon co-application with Mn and Zn respectively. In this study Mo application had beneficial effect on the efficacy of glyphosate, shown by increase in efficacy with increase in Mo application. This is an indication that weed control in high Mo availability may be enhanced. In Mo deficient soil or where pH is low for Mo plant availability, Mo addition is important if best results are desired from glyphosate application.

References

- ABOUZINA, H.F., ELMERGAWI, R.A., SHARMA, S., OMAR, A.A. & SINGH, M., 2009. Zinc antagonizes glyphosate efficacy on a yellow nutsedge (*Cyperus esculentus*). *Weed Sci.* 57, 16-20.
- BERNARDS, M.L., THELEN, K.D. & PENNER, D., 2005. Glyphosate efficacy is antagonized by manganese. *Weed Technol.* 19, 27-34.
- EKER, S., OZTURK, L., YAZICI, A., ERENOGLU, B., ROMHELD, V. & CAKMAK, I., 2006. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. *J. Agric. Food Chem.* 54, 10019-10025.
- HALL, G.J., HART, C.A. & JONES, C.A., 2000. Plants as sources of cations antagonistic to glyphosate activity. *Pest. Manag. Sci.* 56, 351-358.
- SALAZAR, L.C. & APPLEBY, A.P., 1982. Herbicidal activity of glyphosate in soil. *Weed Sci.* 30, 463-466.
- TESFAMARIAM, T., BOTTS, S., CAKMAK, I., RÖMHELD, V. & NEUMAN, G., 2009. Glyphosate in the rhizosphere – Role of waiting times and different glyphosate binding forms for phytotoxicity in non target plants. *Eur. J. Agron.* 31, 126-132.

Appendices

APPENDIX A1: Nutrient solution used in the study

Table A1.1. Composition of nutrient solution used throughout the study.

Solution 1 High nutrients EC = 2.0						
ppm NH ₄ ⁺	K ⁺	Ca ²⁺	Mg ²⁺	NO ₃ ⁻	H ₂ PO ₄	SO ₄ ²⁻
	(g / 1000L)			mg/L	g/1000L	
KNO ₃	303	Fe: Libfer (Fe-EDTA)		0.85	6.54	
K ₂ SO ₄	261	Mn: Manganese sulphate		0.55	2.23	
Ca(NO ₃) ₂ ·2H ₂ O	900	Zn: Zink sulphate		0.30	1.33	
MgSO ₄ ·7H ₂ O	492	B: Solubor		0.30	1.46	
		Cu: Copper sulphate		0.05	0.20	
		Mo: Sodium molibdate		0.05	0.13	

APPENDIX A2: ANOVA'S for experiments done during the study

A 2.1: Chapter 3: The effect of three different sandy soils on the efficacy of glyphosate on *Lolium* spp.

Table A 2.1.1 ANOVA for the percentage survival *Lolium* seedlings grown in paddock soil (PS), crop soil (CS) and sand soil (SS) treated with glyphosate (Figure 3.1).

	Df	Percentage survival			
		SS	MS	F	p
Intercept	1	287041.7	287041.7	1653.360	0.000000
GAR	4	86604.2	21651.0	124.710	0.000000
Soil	2	1333.3	666.7	3.840	0.028857
GAR*Soil	8	3458.3	432.3	2.490	0.024961
Error	45	7812.5	173.6		
Total	59	99208.3			

Table A2.1.2 ANOVA for the percentage survival of susceptible and resistant *Lolium* seedlings grown in paddock soil (PS) and crop soil (CS) (Figure 3.2 & 3.3).

	Df	Percentage survival (%)			
		SS	MS	F	p
Intercept	1	173343.8	173343.8	1280.077	0.000000
GAR	4	34416.7	8604.2	63.538	0.000000
Soil	1	843.7	843.7	6.231	0.016777
Biotype	1	68343.8	68343.8	504.692	0.000000
GAR*Soil	4	875.0	218.8	1.615	0.189229
GAR*Biotype	4	19416.7	4854.2	35.846	0.000000
Soil*Biotype	1	843.7	843.7	6.231	0.016777
GAR*Soil*Biotype	4	875.0	218.8	1.615	0.189229
Error	40	5416.7	135.4		
Total	59	131031.3			

Table A2.1.3 ANOVA for the percentage survival of the susceptible rye grass grown in paddock soil (PS), cropping soil CS and sand soil (SS) and fed with nutrient solution or pure water (Figure 3.4)

	Percentage survival (%)				
	SS	Df	MS	f	p
Intercept	94737.8	1	94737.78	687.6129	0.000000
Soil	8648.9	2	4324.44	31.3871	0.000000
Dose	110373.3	4	27593.33	200.2742	0.000000
Nutrient	5137.8	1	5137.78	37.2903	0.000000
Soil*Dose	11573.3	8	1446.67	10.5000	0.000000
Soil*Nutrient	3795.6	2	1897.78	13.7742	0.000012
Dose*Nutrient	3528.9	4	882.22	6.4032	0.000233
Soil*Dose*Nutrient	3537.8	8	442.22	3.2097	0.004230
Error	8266.7	60	137.78		

Table A2.1.3 ANOVA for percentage survival of seedling grown in paddock soil (PS), cropping soil (CS) and sand soil (SS). The soils were either covered (with cotton wool) or uncovered when glyphosate was applied (Figure 3.5)

	SS	Degr. of	MS	F	p
Intercept	155417.8	1	155417.8	713.6531	0.000000
Soil	4275.6	2	2137.8	9.8163	0.000205
W/-W	40.0	1	40.0	0.1837	0.669770
Dose	104471.1	4	26117.8	119.9286	0.000000
Soil*W/-W	2906.7	2	1453.3	6.6735	0.002416
Soil*Dose	4968.9	8	621.1	2.8520	0.009379
W/-W*Dose	2337.8	4	584.4	2.6837	0.039818
Soil*W/-W*Dose	4915.6	8	614.4	2.8214	0.010041
Error	13066.7	60	217.8		

A 2.2: Chapter 4: Phytotoxicity of soil applied glyphosate on ryegrass (*Lolium multiflorum*) under varying soil conditions**Table A2.2.1** ANOVA for percentage survival of *Lolium* spp. seedlings transferred into glyphosate-treated soils (paddock soil (PS), crop soil (CS) and sand soil (SS)) (Figure 4.2 & 4.3).

	Df	%Survival			p
		SS	MS	F	
Intercept	1	1465138	1465138	2453.782	0.000000
TAS	2	25777	12889	21.586	0.000000
Dose	2	28655	14328	23.996	0.000000
Soil	2	22713	11356	19.019	0.000000
TAS*Dose	4	19792	4948	8.287	0.000004
TAS*Soil	4	4303	1076	1.802	0.130165

Dose*Soil	4	11492	2873	4.812	0.001020
TAS*Dose*Soil	8	3229	404	0.676	0.712361
Error	189	112851	597		
Total	215	228812			

Table A2.2.2 ANOVA for dry mass accumulation of *Lolium* spp. transferred into glyphosate-treated soils (paddock soil (PS), crop soil (CS) and sand soil (SS)) (Figure 4.5, 4.6 & 4.7).

	Df	Dry mass (g/ plant)			
		SS	MS	F	p
Intercept	1	0.687569	0.687569	176.2392	0.000000
TAS	2	0.241549	0.120774	30.9571	0.000000
dose	2	0.054674	0.027337	7.0071	0.003538
Soil	2	0.054166	0.027083	6.9420	0.003694
TAS*dose	4	0.006824	0.001706	0.4373	0.780483
TAS*Soil	4	0.007841	0.001960	0.5025	0.734175
dose*Soil	4	0.017112	0.004278	1.0965	0.378304
TAS*dose*Soil	8	0.019841	0.002480	0.6357	0.740856
Error	27	0.105336	0.003901		
Total	53	0.507342			

Table A2.2.3 ANOVA for shoot length of seedling transferred into glyphosate-treated soils (paddock soil (PS), crop soil (CS) and sand soil (SS)) (Figure 4.8, 4.9 & 4.10).

	Df	Shoot length (cm)			
		MS	SS	F	p
Intercept	1	51977.26	51977.26	703.3071	0.000000
TAS	2	5601.07	2800.53	37.8942	0.000000
Dose	2	1554.68	777.34	10.5182	0.000047
Soil	2	1253.41	626.70	8.4799	0.000298
TAS*Dose	4	627.17	156.79	2.1216	0.079779
TAS*Soil	4	323.16	80.79	1.0932	0.361279
Dose*Soil	4	239.95	59.99	0.8117	0.519134
TAS*Dose*Soil	8	441.98	55.25	0.7476	0.649406
Error	187	13820.06	73.90		
Total	213	23902.43			

A2.3 Chapter 5 Effect of manganese and molybdenum on efficacy of glyphosate on *Lolium* spp.**Table A2.3.1** ANOVA for percentage survival of seedlings grown at varying molybdenum (Mo) concentrations treated with glyphosate (Figure 5.1)

	Df	Survival (%)			p
		SS	MS	f	
Intercept	1	214524.8	214524.8	2275.187	0.000000
Biotype	1	47242.7	47242.7	501.042	0.000000
Dose	4	87668.3	21917.1	232.446	0.000000
Nutrient	2	806.0	403.0	4.274	0.018397
Biotype*Dose	4	23760.2	5940.0	62.998	0.000000
Biotype*Nutrient	2	7686.5	3843.2	40.760	0.000000
Dose*Nutrient	8	15168.3	1896.0	20.109	0.000000
Biotype*Dose*Nutrient	8	6278.0	784.7	8.323	0.000000
Error	60	5657.3	94.3		
Total	89	194267.2			